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TOPIC

Evaluation of α - amylase inhibitory activity and anti-diabetic effect of natural extracts from : *Asphodelus microcarpus, Bubonium graveolens and Haplophyllum tuberculatum*

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تقييم الفعالية التثبيطة على انزيم الفا اميلاز والنشاط المضاد لمرض السكري للمستخلصات الطبيعية للنباتات : البرواق، الطفس و الفيجل

الملخص

تمثل النباتات الطبية مصدرا أساسيا لاكتشاف جزيئات علاجية جديدة وفعالة ضد العديد من الأمراض، من هذا المنطلق قمنا بدراسة ثلاث نباتات محلية : البرواق، الطفس و الفيجل. و المشهورة بتأثيرها الايجابي على عدة أمراض، حيث قمنا بتقدير كمية المركبات الفينولية لمستخلصات هذه النباتات ، دراسة فعاليتها التثبيطية على نشاط انزيم الاميلاز وكذا سميتها و نشاطها المضاد لمرض السكري عند جرذان الويستار.

تراوحت كمية المركبات الفينولية ما بين 2,88 و11,97 ملغ / غ مكافئ لحمض الغاليك بينما تراوحت كمية الفلافونويدات مكافئ للروتين بين 0,48 و 1,5 ملغ/ غ . بينت نتائج الدراسة الحركية للتفاعلات الإنزيمية أن جميع مستخلصات النباتات المدروسة لها أثر تثبيطي لانزيم الاميلاز بتراكيز مثبطة عند % 50 تراوحت قيمتها مابين 9,78 ملغ / مل و82,01 ملغ / مل. لم تظهر اختبارات السمية الحادة للمستخلص المائي لنبتة البرواق ولمستخلص الزيوت العطرية لنبتة الطفس ، المقدمة إلى الفئران عن طريق الفم أي سمية قاتلة و ذلك حتى جرعة 5000 ملغ/كلغ.

تم استخدام اجرائين لدراسة النشاط المضاد للسكري للمستخلص المائي لنبات البرواق ومستخلص الزيت العطري لنبات الطفس، مقدمة عن طريق للجرذان العادية و الجرذان المصابة بداء السكري بعد حقنها بـ 150ملغ/كلغ من مادة الألوكسان . تمثل الإجراء الأول في اختبار تأثير المستخلصات على سكري الصائم و على المدى القصير (30 إلى 120 د) عند الفئران العادية .في حين أن الإجراء الثاني تمثل في اختبار المستخلصات على الفئران العادية و المصابة بداء السكري بعد علاجها اليومي بهذه المستخلصات لمدة 28 يوما . أظهرت النتائج تأثيرا خافضا لسكر الدم بارزا ، لوحظ في فترة زمنية قدرت ب 30 .إلى 60 دقيقة بعد جرع المستخلص وكان هذا التأثير واضحا بشكل خاص مع مستخلصات الزيوت العطرية، كما لوحظ تحسن في الاضطرابات التي تصاحب مرض السكري التجريبي ككثرة الأكل، العطش، وانخفاض وزن الجسم وتدهوربعض القياسات البيوكيميائية.

على ضوء هذه النتائج، يمكننا القول إن البرواق و الطفس هي نباتات طبية ذو تأثير فعال على خفض نسبة السكرالعالية في الدم و هذا ما تجلى من خلال تجاربنا على الجرذان العادية المشحونة عن طريق الفم .وتشيردراستنا أيضا أن الجزيئات الأكثر فعالية يُشتبه أنها تنتمي إلى أسرة الفلافونيدات.

الكلمات المفتاحية : ، داء السكري، برواق، طفس، فيجل، فلافونيدات، زيوت عطرية ، تثبيط الألفا أميلاز، سمية، السكري التجريبي.

Evaluation de l'activité inhibitrice de l'α-amylase et de l'effet antidiabétique d'extraits naturels de : *Asphodelus microcarpus, Bubonium graveolens* et *Haplophyllum tuberculatum*

Résumé

Les plantes médicinales représentent une source essentielle pour découvrir de nouvelles molécules thérapeutiques efficaces contre de nombreuses maladies. Dans ce contexte, nous avons étudié trois plantes locales : *Asphodelus microcarpus, Bubonium graveolens* et *Haplophyllum tuberculatum,* connues pour leur effet positif sur plusieurs maladies. Nous avons déterminé la teneur en composés phénolique des extraits de ces plantes, étudier leur effet inhibiteur sur l'amylase ainsi que leur toxicité et leur activité antidiabétique chez des rat Wistar.

Le contenu en phénols totaux est compris entre 2,88 et 11,97 mg en équivalent d'acide gallique / g de la matière sèche. Tandis que le contenu en flavonoïdes exprimé en équivalent de la rutine est compris entre 0,48 et 1,5 mg/g. L'analyse cinétique des réactions enzymatiques a montré que les composés phénoliques avaient des effets inhibiteurs sur l'enzyme amylase à des concentrations inhibitrices de 50%, avec des valeurs allant de 9,78 à 82,01 mg/ml. Des tests de toxicité aiguë des extraits aqueux d'*Asphodelus microcarpus* et d'huiles essentielles de *Bubonium graveolens,* administrées par voie orale à des rats Wistar n'ont montré aucune toxicité mortelle jusqu'à une dose de 5000 mg/kg.

L'étude de l'activité antidiabétique de l'extrait aqueux *d'Asphodelus microcarpus* et des huiles essentielles de *Bubonium graveolens,* administrés par voie orale, a été réalisée en utilisant trois procédures, chez des rats Wistar normaux et des rats rendus diabétiques par l'alloxane (150 mg / kg). La première procédure consiste à tester l'effet à court terme des extraits (30 à 120 min), sur la glycémie à jeun des rats normaux. Tandis que dans la deuxième l'effet des extraits a été testé chez des rats normaux et diabétiques, suite à un traitement quotidien de 28 jours. Les résultats ont montré un effet antihyperglycémiant significatif, observé dans un intervalle de temps de 30 à 60 min après administration de l'extrait. Cet effet a été évident, notamment avec les extraits d'huiles essentielles, comme il a été remarqué une amélioration dans les troubles qui accompagnent le diabète expérimental comme la polyphagie, la polydipsie, la chute du poids corporel et la détérioration de quelques paramètres sanguins.

A la lumière de ces résultats, nous pouvons dire que *Asphodelus microcarpus* et *Bubonium graveolens* sont des plantes médicinales à activité antihyperglycémiante remarquable, qui s'est manifesté dans nos expériences, par une diminution de l'hyperglycémie provoquée par une charge orale chez des rats normaux. L'étude présente indique aussi, que les molécules les plus soupçonnées appartiennent à la famille des flavonoïdes.

Mots clés: diabète, *Asphodelus microcarpus, Bubonium graveolens, Haplophyllum tuberculatum,* flavonoides, huiles essentielles, inhibtion de l'α-amylase, toxicité, diabète expérimental.

Evaluation of α – amylase inhibitory activity and anti-diabetic effect of natural extracts from : *Asphodelus microcarpus, Bubonium graveolens* and *Haplophyllum tuberculatum.*

Abstract

Medicinal plants represent an essential source for discovering new therapeutic molecules effective against many diseases. In this context, we studied three local plants: *Asphodelus microcarpus, Bubonium graveolens* et *Haplophyllum tuberculatum*, known for their positive effect on several diseases. We determined the content of phenolic coumpounds of extracts from these plants, study their inhibitory effect on amylase as well as evaluate their toxicity and their anti-diabetic activity in Wistar rats.

The amount of phenolic compounds ranged from 2.88 to 11.97 mg/g gallic acid equivalent, while the amount of flavonoids ranged from 0.48 to 1.5 mg/g quercetin equivalent. The kinetic analysis of the enzymatic reactions showed that phenolic compounds had inhibitory effects on the amylase enzyme at inhibitory concentrations of 50%, with values ranging from 19.78 to 82.01 mg/ml. *Asphodelus microcarpus* aqueous extract and *Bubonium graveolens* essential oils extract acute toxicity studies given orally to Wistar rats didn't exhibit lethal toxicity, even at the dose level of 5000 mg/kg.

For the antidiabetic study of aqueous extract of *Asphodelus microcarpus* and essential oils of *Bubonium graveolens*, the extract was administered orally to normal Wistar rats and diabetic rats induced by alloxan (150 mg/kg). The first procedure consisted of testing the acute effect of the extracts (30 to 120 min), on fasting glycemia of normal rats. While in the second procedure, plant extract effect was tested, in normal and diabetic rats, in daily treatment of 28 days. The results showed significant antihyperglycemic effect, observed in time interval of 30 to 60 min after the administration of the extract. The effect was evident mainly with essential oil extracts, an improvement was noted in the troubles that come with experimental diabetes, as polyphagia, polydipsia, body weight lost, and some deterioration blood parameters deterioration.

In the light of these results, we can say that *Asphodelus microcarpus* and *Bubonium graveolens H. scoparia are* a medicinal plants with remarkable antihyperglycemic activity, which appeared in our experiments with a reduction of hyperglycemia, induced by oral charge in normal rats. Our study indicates also that the most responsible molecules belong to the family of flavonoids

Keywords: diabetes, *Asphodelus microcarpus, Bubonium graveolens, Haplophyllum tuberculatum,* flavonoids, essential oils, α-amylase inhibition, toxicity, experimental diabetes.

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3. Seglab Fatiha, Khacheba Ihcen, **Chaoua Housseyn**, Bekhawa Abir, Yousfi Mohamed. Comparison of five evaluation antioxidant activity methods on *Cleome arabica* leaves extracts and there *in vitro* antidiabetic activity in different season. **Le 1er séminaire**

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ABREVIATIONS & SYMBOLS LISTE

WHO	The World Health Organization
AAD	The American Academy of Dermatology
TID	Type1 diabetes
TIID	Type II diabetes
DM	Dry mattre
IC_{50}	Inhibitory concentration of half-maximal response
BW	body weight
DNS	3,5-dinitrosalicylic acid
HDL	high-density lipoproteins
LDL	low-density lipoprotein.
TGO	Glutamate-oxaloacetate-transaminase
TGP	Glutamate-pyruvate-transaminase
ANOVA	Analysis of variance
GLUT2	Glucose transporter 2
C	Control group
UD	Untreated diabetic group
DTM	Diabetic treated by medicament
DT100	Diabetic treated with dose100mg/kg
DT200	Diabetic treated with dose200mg/kg
DT400	Diabetic treated with dose400mg/kg

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INTRODUCTION

Diabetes mellitus, which is a metabolic disease characterized by a persistent hyperglycemia, mainly type II diabetes, and has been considered as one of the epidemic diseases of third-millennium spreading globally (Whiting *et al.,* 2011). The number of diabetics patients is reached approximately 366 million in 2010, and 693 million are expected in 2045 (Malek *et al.*, 2019).

Algeria indicates more than 4 million patients with diabetes, nearly 15% of the total population. This disease has become a severe problem in Algeria from both sides: its high prevalence with a gradual increase and its economic and social consequences (Lamri *et al.,* 2014; Malek *et al.,* 2019).

Increasing evidence in both clinical and experimental ways has led to a conduct of various epidemiological studies which suggested that oxidative stress may have a relationship with diabetes because of its role in the development of complications. It also contributes to beta-cell pancreatic islets destruction in type 2 diabetes patients (King et Loeken, 2004). Hyperglycemia stimulates high oxidative stress and an adjustment of the antioxidant defense (Kim *et al.,* 2006). High blood glucose levels define the overproduction of reactive oxygen species (ROS), which leads to DNA and protein modification, and lipid peroxidation (Piconi *et al.,* 2003), that alteration can cause several serious complications affecting the heart, blood vessels, eyes, kidneys, and nerves. However, reasonable control of this disease can significantly reduce the risk of these complications (Fagot-Campagna *et al.,* 2010; Kim *et al.,* 2006).

For a long time, the type 2 diabetes treatment has been based on two ways: diet changes food and taking oral anti-diabetics; such as miglitol, voglibos, or acarbose which causes serious side adverse - effects like flatulence, diarrhea, and abdominal pain (Onsiyor *et al.,* 2019). Because of the high cost of these drugs, besides, their profound side effect; today, researchers are focusing on herbal medicines for several reasons: their ability to treat human diseases, their accessibility, low cost, and also they are an inexhaustible natural resource (Djeridane *et al.,* 2015; Onsiyor *et al.,* 2019).

Currently**,** more than 1200 species of plants are used globally in traditional medicine for their alleged hypoglycemic actions (Azzi *et al.,* 2012). These plants can produce a wide variety of natural substances that tend to be a very significant source of

molecules in the pharmacological field. (Benkhnigue *et al.,* 2014; Marouf, 2007). Several secondary metabolites synthesized by plants to shield themselves from biological and environmental constraints, have been considered as therapeutic agents for several diseases such as phenolic compounds in particular flavonoids (Nikavar *et al.,* 2008). In addition to their biological properties, flavonoids can also inhibit the activity of a large number of digestive enzymes such as α -amylase, α - glucosidase, pepsin, trypsin, and lipase (Andrade-Cetto *et al.,* 2008).

Due to the geographical location, Algeria contains different biological and climatic levels, leading to an increase in plant diversity and changes in secondary metabolites of the same plant species. The Algerian flora has more than 1,123 plants are traditionally used to treat diabetes (Tadjeddine *et al.,* 2013). The use of plants as a traditional medicine in Algeria is mainly inspired from the population's local practices. Still, the majority have not been scientifically evaluated for their biological activities.

The objective of this study, was to investigate the anti-oxidant and the anti-diabetic properties of various extracts from three local Saharan plants: *Haplophyllum tuberculatum, Asteriscus graveolens, and Asphodelus microcarpus*. The selection of these plants is related to the fact that considering their use on a very small scale, they are not recognized among herbalists as a treatment of diabetes, and no research has been performed to evaluate these plants. For a purpose to use these plants as a complement to synthetic medication and / or replace them.

This work, which is part of the activities of the fundamental sciences laboratory of Laghouat University, is a continuation of a work already carried out by us on other medicinal plant in 2017 (Seglab *et al.,* 2018) and which is part of the search for new plants grown in the Algerian steppe region that can find an anti-diabetic application.

The first chapter, is a bibliographic summary which includes an overview on phenolic compounds and their activities generalities on diabetes and their treatment as well as an overview on the methods for the evaluation of antidiabetic activity.

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The second chapter, is devoted to the description of the experimental protocols undertaken in this study, which are exposed as classic and simple methods for the preparation of aqueous and essential oil extracts, as well as the determination of total phenols and flavonoids contents, then the study of the anti-diabetic activity *in vitro* by an inhibitory effect on the enzymatic activity of α - amylase and *in vivo* on diabetic rats induced by alloxan.

In the last chapter, the various results obtained and their discussions are presented.

Finally the thesis ends with a conclusion summarizing the essentials of the work accomplished as well as prospects for further and complementary studies.

LITERATURE REVIEW

I.1.Diabetes

I.1.1.Definition of diabetes

Diabetes mellitus is an important endocrine metabolic disease (disorder) of multiple etiologies caused by alternating carbohydrate metabolism, which is characterized by abnormally high levels of plasma glucose which is termed hyperglycemia and glucose intolerance, with disruption of lipid and protein. The Diabetes mellitus is a chronic state of hyperglycemia with fasting glycemia greater than 1.26 g/l (Boucher *et al* , 2011). This disease is the consequence of an interaction of hereditary (acquired) and environmental factors. Diabetes is considered as a non-curable, but controllable disease; resulting from an absolute or relative deficit of insulin secretion by the Langerhans β cells of the pancreas, or the action of the latter which results in a decrease in the reactivity of the organs to secreted insulin, or a combination of the two leading to an inability to transport glucose from the bloodstream into cells, as well as insulin resistance of peripheral tissues, including the liver, muscles and fatty tissue (Calop *et al.*, 2012).

I.1.2. Main types of diabetes

The WHO and the AAD classify the diabetes mellitus according to clinical stages and etiological factors on: Type I diabetes (A: autoimmune and B: idiopathic), Type II diabetes (diabetes of other kinds) and Gestational diabetes (Care, 2014).

I.2.1.1.Type I diabetes (TID)

Type1 diabetes (TID) is represented by 5 - 10% of diabetics. Children and teenagers are frequently affected, but it can also occur at any age, including older people (Care, 2014). TID is characterized by the more or less rapid destruction of the β cells of the Langerhans and/or results from the autoimmune destruction in β cells of the islet of Langerhans. Anti-islet antibodies, anti-GAD (Glutamate Acid Decarboxylase) antibodies, anti-insulin antibodies, and anti-tyrosine phosphatase IA2 antibodies are responsible (Concannon *et al.*, 2009).

I.1.2.2.Type II diabetes (TIID)

Type II diabetes (TIID) is more prevalent, accounting for 90% to 95% of people with diabetes. TIID which is linked to genetic and environmental factors, is characterized by insulin resistance and a relative deficit in pancreatic β-cell insulin secretion (Stumvoll *et al.*, 2005). The experimental hyperglycemia is due to a decrease in glucose use by peripheral tissues, especially muscle (Shulman, 2000), and an increase in the production of hepatic

glucose by the liver (Virally *et al.,* 2007). Because of increased lipolysis, the amount of fatty acids is also high. (Kovacs et Stumvoll, 2005).

I.1.2.3. Gestational diabetes

Gestational diabetes is characterized as any degree of intolerance to carbohydrates that began or were first seen in pregnancy. Women with gestational diabetes may encounter no signs. The causes of gestational diabetes are pregnancy hormones or insulin deficiency. Like type II diabetes, gestational diabetes occurs more frequently in some ethnic groups and women with a family history of diabetes. This type of diabetes normally disappears after childbirth. Women who've had gestational diabetes have a 20 to 50 percent risk of developing type II diabetes within 5 to 10 years (Pirson *et al.,* .2016).

I.1.3. Diabetes treatment

I.1.3.1. Synthetic treatment

Treatment for diabetes mellitus depends on the type of diabetes.

A.Treatment of type I diabetes

Insulin remains to be the most effective way to achieve natural and well-regulated blood sugar. The function of the insulin administered for the patient is to replace the body's Insulin. The key expected effects are to enhance the use of peripheral glucose and to activate glycolysis, glycogenesis, lipogenesis, and protein synthesis. Also, Insulin attempts to prevent lipolysis and gluconeogenesis (Kelley and Goodpaster, 2001).

B.Treatment of type II diabetes

The Treatment depends on the stage the disease has reached. In the early stages, TIID can be managed by diet and physical activity. When average blood sugar is not achieved, it is imperative to prescribe oral antidiabetic medication. (Kelley and Goodpaster, 2001; Koski, 2006), which are classified into five categories: sulfonylureas, benzoic acid derivatives, biguanides, α-glucosidase inhibitors, and thiazolidinediones (antihyperglycemic agents) (Harrigan *et al.,* 2001).

However, the continued use of these synthetic agents should be reduced as these inhibitors are known to cause many side effects, such as unwanted gastrointestinal symptoms (gas, bloating, abdominal cramps, vomiting, abdominal distension and diarrhea). In addition to that, some of them may increase the incidence of kidney tumors, liver disorders (liver damage and acute hepatitis). These side effects may have been caused by excessive inhibition of pancreatic α-

amylase, leading to bacterial fermentation of undigested carbohydrates in the colon. These drugs have other drawbacks such as liver toxicity and increase symptoms and risk factors for heart disease (Arulselvan *et al.,* 2014).

The World Health Organization Committee Expert in diabetes recommended the traditional medicinal plants to be further investigated, as they are frequently considered to be free from toxic and side effects. Therefore, the search for safer and more effective bioactive agents has continued to be an important biomedical drug development research (Arulselvan *et al.,* 2014)

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I.1.3.2. Natural treatment

Herbal medicines have long been used effectively in treating diseases/disorders throughout the world.The use of medicinal plants to manage diabetes is one of the applications of herbal medicine. The community, therefore, uses various plants to keep the level of blood glucose within standards. This practice has attracted researchers to perform experiments to clarify the mechanism of action of these natural remedies (Arulselvan *et al.,* 2014).

Plants have three ways function to treat diabetes mellitus. The first way is decreasing the supply of glucose in the blood by inhibiting hepatic glucose production (endogenous sources of glucose). Or by reducing intestinal glucose absorption (exogenous origin of glucose). The second strategy is increasing glucose intake by the peripheral tissues, particularly muscles. The third strategy involves insulin secretion stimulation (Hui *et al.,* 2009).

The mechanism of most of the herbs used has not been scientifically determined. Many traditional plants and their derived bioactive compounds are used for treatments of diabetes through various mechanisms of actions (Figure I.)

- Stimulate pancreatic tissue function by increasing insulin production and inhibiting the insulin-degrading process (Arulselvan *et al.,* 2014).
- Prevent oxidative stress, which can be associated with diabetes beta-pancreatic cell dysfunction (Akhani *et al.,* 2004; Jarald *et al.,* 2008).
- Inhibition glucose absorption in the intestine by the Inhibition of α-glucosidase and α-amylase (Koga *et al.,* 2006).
- Increased hepatic glycogen and stimulated the transport of glucose in the adipocyte (Hannan *et al.,* 2007).
- Increase the peripheral uptake of glucose from blood to muscle/tissue (Arulselvan *et al.,* 2014).

- Decrease glucose production at liver (Arulselvan *et al.*, 2014).

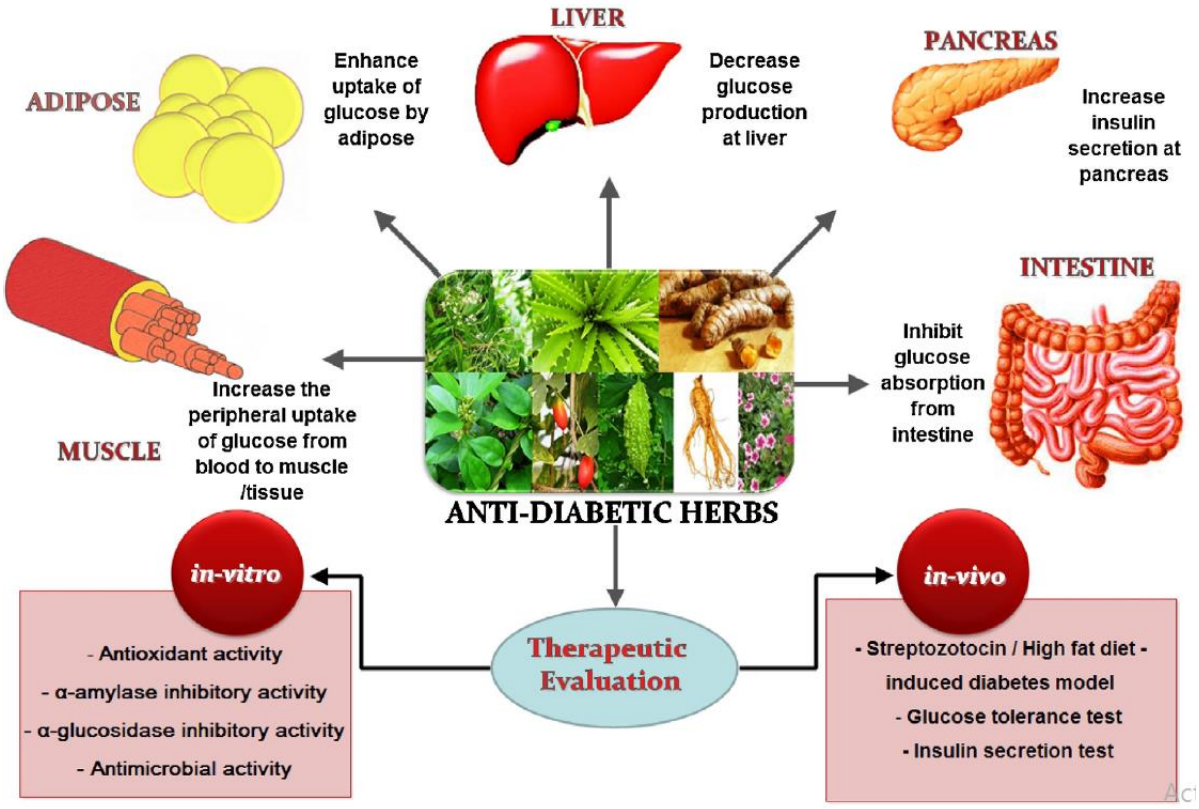


Figure I. 1. Antidiabetic herbs – various mechanisms of actions and persisting models of its therapeutic evaluation (Arulselvan *et al.*, 2014).

Recently, spices and other natural products have been used in the prevention and treatment of diabetes mellitus and its associated complications. In addition, they are also considered to be more natural, more economical and safer in the treatment of diabetes mellitus (Arulselvan *et al.*, 2014).

However, The World Health Organization has recommended a conduct of further studies on traditional herbal medicines due to the absence of science and clinical evidence confirming their efficacy. Therefore, appropriate biological assays should be used for biological standardization, drug and toxicological evaluation, and the development of different models for animal toxicology and safety evaluation. It is also essential to determine these plant extracts active ingredients to integrate herbal medicine into modern medical practice (Arulselvan *et al.*, 2014).

In Algeria, numerous experiments have shown and confirmed several plants hyperglycemic activity in experimental animal models; Table I.1 shows the biological activity of some medicinal plants used to treat diabetes mellitus.

Table I.1 The biological activity of some medicinal plants used to treat diabetes mellitus.

Scientific name	Family	Vernacular name	Part used	Extract (dose, duration of treatment)	The animals used	Possible mechanism of action	References
Zingiber officinale L.	*Ginger Zingiberaceae*	Zenjabil	Rhizomes	Juice (4ml / kg, PO 6 weeks)	Diabetic rats (STZ, 45m .i.v)	Increased insulin secretion	Akhani *et al.*, 2004.
Trigonella foenum graecum L.	*Leguminoseae*	Halba	Seeds	Methanol extract (0.5g / kg, PO, 28d)	Diabetic rats (STZ, 65mg / kg i.p)	Increased hepatic glycogen. -Stimulation of the transport of glucose in the adipocyte. -Decreased digestion of carbohydrates.	Hannan *et al.*, 2007.
Rosmarinus officinalis L.	*Lamiaceae*	Yazir	Leaves	Ethanol extract (20mg / mouse, 21days)	Normal mice non-diabetic	Inhibition of α-glucosidase.	Koga *et al.*, 2006.
Citrullus colocynthis (L.) Schrad.	*Cucurbitaceae*	Handal	Seeds	Aqueous extract (5ml / kg i.p, 14d)	Diabetic rats (STZ, 65mg / kg i.p)	Insulinotropic action	Benariba *et al.*, 2009 ; Benariba *et al.*, 2013.
Artemisia herba-alba Asso.	*Asteraceae*	Chih	Leaves	Water-ethanol extract (390mg / kg, PO, 60 days**)**	Diabetic rats (alloxan 120mg / kg.i.p	Prevention of insulin resistance**.**	Hamza *et al.*, 2010. Awad *et al.*, 2012
Nigella sativa L.	Ranunculaceae	Sanoudj	Seeds	Aqueous extract (2ml / kg, 5% i.p, 30 d) Thymoquinone (0.3mg / ml i.p, 30d)	Diabetic rats (STZ, 50mg / kg i.p)	Inhibition gluconeogenesis. - Improvement of the cellular and subcellular structure of β-pancreatic cells	Abdelmeguid *et al.*, 2010.

I.1.3.2. Bioactive phytoconstituents with antidiabetic potential

Several plants derived active principles representing numerous chemical compounds like alkaloids, glycosides, polysaccharides, galactomannan gum, guanidine, peptidoglycans, hypoglycans, hormones, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, and inorganic ions showed prominent antidiabetic activity. This affects various metabolic cascades, which affect the blood glucose level in the human body directly or indirectly (Prabhakar and Doble, 2011). Some of these substances are shown to be wholly hypoglycemic and may have therapeutic potential, while others may cause hypoglycemia as a side effect of their toxicity, such as hepatotoxicity (Jarald *et al,* 2008).

Many studies have been carried out on plants to isolate the antidiabetic compounds and determine the action mechanism. Hydroxyisoleucin is an amino acid that stimulates the secretion of insulin-induced by glucose (Avalos-Soriano *et la.,* 2016). Furthermore,4-hydroxy isoleucine derivatives showed an effect in stimulating glucose transport in L-6 skeletal muscle cells. Other modes of action have been suggested for other molecules isolated from plants. They found a triterpene isolated from *Rosmarinus officinalis* L that serves as an antidiabetic agent by stimulating insulin vesicle translocation, insulin secretion (Castro *et al.,* 2015). According to reports, Levophenol and metformine were isolated from *Aloe vera* L, *Galega officinalis*. These molecules can induce glucose catabolism and reduce glucose production by inhibiting gluconeogenesis enzymes (Dey *et al.,* 2002; Misawa *et al.,* 2012). Finally, we can cite the example of ferulic acid isolated from *syzyginum Camomile*. Which can play a role in pancreatic cell regeneration (Mandal *et al.*, 2008).

I.2. Evaluation of antidiabetic activity

Utilization of plant sources as a cure of diabetes has rich evidence in the history. Recently, the traditional medicinal plants have been investigated scientifically to understand the underlying mechanism behind antidiabetic potential. In this regard, a substantial number of *in vivo* and *in vitro* models have been introduced for investigating the bottom-line mechanism of the antidiabetic effect. A good number of methods have been reported to be used successfully to determine antidiabetic effects of plant extracts or isolated compounds (Mahmudul *et al.*,2018).

The present scenario of pharmacological screening involves testing of new chemical entities as extract from plant or any material (synthetic/semisynthetic) in isolated preparations followed by tests in whole animals. Rats, mice and sometimes higher animals like monkeys and dogs are used. Most drugs in use nowadays at therapy have been developed with these methods. Non-animal

alternatives are advisable wherever necessary. The challenge for pharmacologist always will be to correlate *in vitro* data with *in vivo* findings (Aher and Thete, 2020).

I.2.1. Methods for *in-vitro* evaluation of antidiabetic activity

The antidiabetic properties of synthetic or natural substances can be evaluated by *in vitro* methods such as study of glucose uptake; the inhibition of such glucose uptake by yeast cell is an important tool for the evaluation of antidiabetic property, effect on glycosylation of the hemoglobin; once a hemoglobin molecule is glycated, because of high blood glucose level, it remains that way. Hence the estimation of glycated hemoglobin is important tool in the antidiabetic screening procedure. (Aher and Thete, 2020) and inhibition of alpha amylase, alpha glucosidase and sucrase enzymes; the inhibitory nature of a test compound on alpha amylase enzyme reflects the antidiabetic activity because of unavailability of glucose from gastrointestinal tract. Alpha-glucose inhibitor reduces the impact of glucose on blood sugar and hence alpha glycosidase inhibitors can be considered as oral antidiabetic drugs. (Tundis *et al.*, 2010). Glucose diffusion assay is a simple diffusion method to evaluate the glucose movement *in vitro* and is expressed in terms of glucose diffusion retardation index (GDRI). *In vitro* technique like cell culture is one of the important methods to evaluate the activity. Level of insulin secretion in culture of HIT-T15 cells is also reported for evaluation of antidiabetic activity (Nair *et al.*, 2013).

I.2.2. Methods for *in-vivo* evaluation of antidiabetic activity

Animal models are one of the major tools to progress with establishing an effective model to investigate the mechanism of action as well as to explore the efficacy of the active principles and plants claimed to show antidiabetic potentials (Mahmudul *et al.*, 2018**).**

I.2.2.1. *In vivo* animal models of diabetes mellitus

Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals. The classical model employed by Banting and Best was pancreatectomy in dogs. It is also described prone strains to diabetes mellitus that have been employed in several researches. Currently, the murine model is one of the most used due to the availability of over 200 well-characterized inbred strains and the ability to delete or over-express specific genes through knockout and transgenic technologies (Frödea and Medeiros, 2008).

I.2.2.2. Induction of diabetes

A. Chemically induced diabetic model

Streptozotocin (STZ, 69%) and alloxan (ALX) (31%) (Figure I. 1) are widely used to induce diabetes mellitus in animals. Both chemicals can be administered through either intravenous (IV), intraperitoneal (IP) or subcutaneous (SC) ways (Mahmudul *et al.*, 2018). The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status. Both STZ and ALX are selective cytotoxic agents and consequently destroy the pancreatic beta cells selectively (Frödea and Medeiros, 2008).

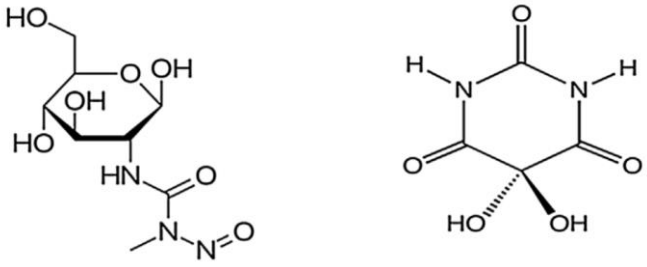


Figure I.2. Chemical structures of streptozotocin (STZ) and alloxan (ALX) (Mahmudul et al., 2018).

The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally (i.p.) or subcutaneously its effective dose must be higher. For instance, an intraperitoneal dose below 150 mg/kg may be insufficient for inducing diabetes in this animal species. In mice, doses vary among 100–200 mg/kg by intravenous route (Federiuk *et al.*, 2004).

In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single doses below 40 mg/kg may be ineffective (Patel *et al.*, 2006).

B. Surgically induction of diabetes

Another technique used to induce diabetes is the complete removal of the pancreas. Few researchers have employed this model in the last years to explore effects of natural products with animal species such as rats, pigs, dogs and primates (Masiello, 2006). The surgical removal of pancreas is an alternative in regard to reduce toxic side effects of chemically induced diabetes. It has been reported to develop diabetic animal model of some species viz., rats, dogs, pigs, etc. (Vedtofte *et al.*, 2010). However, the limitations of this technique have also been reported by many

published studies. The highly complex nature of the technique demands great expertise to perform the surgery. Additionally, the cost of the surgery room set up, large amount of analgesic and antibiotic, post-surgery pancreatic enzyme supplement and risk of animal infection are major drawback of this technique. Moreover, there have been many attempts to perform partial pancreatic surgery which is yet to be developed or acclaimed to achieve desired diabetogenic action (Müller ,2016).

C. Genetic models of diabetes

Several diabetic animal models have so far been developed and reported where no chemical or surgical methods have been used to induce diabetes. In these instances, diabetes is developed either spontaneously or by genetic alteration (Ro *et al.,* 2010).

D. Virus-induced diabetic model

It has been reported that the juvenile diabetes (Type I) has a link with virus infection as well as an autoimmune condition on specific beta cell. A virus named encephalomyocarditis which is a D-variant has been reported to destroy the pancreatic beta cells. Two of the virus susceptible animals are male ICR Swiss (adult) mice and male C3H/HeJ (adult) mice whereas the former was found more prone to the induction of diabetes than the later. The experiment was further confirmed by the pre-treatment of a potent immunosuppressant named cyclosporin. The consequences of the induction of immunosuppressant was found to increase in diabetic conditions in both cases (Mahmudul *et al.,* 2018).

I.2.2.3. *In vitro* animal models

In early studies, insulin-like activity had been measured by the glucose uptake into the fat cells isolated from epididymis of the animals. Adipose tissue of the rat epididymal fat pad had been widely used in this study. The epididymal rat adipose tissue was incubated in glucose containing media and the glucose uptake was measured by the glucose concentration of the media or the oxygen consumption in Warburg vessels. Glucose uptake by incubated rat epididymal adipose tissue was predominantly regulated by the rate of glucose transport across the cell membrane. Another type of experiment was done with the radio labelled glucose where $^{14}CO_2$ was captured and counted. The $^{14}CO_2$ produced from the Iodine labelled ^{14}C glucose was measured in this method. For the small amount of insulin quantification, a manometric assay was used to calculate the total gas exchange]. Lter on, some modification in this method was done in various studies. Among them, the isolated fat cells, 3T3-L1 adipocytes and primary

cultured adipocytes were found to be very effective in testing insulinlike activity through in vitro models (Morse and Soeldner, 2011).

I.3. Plants material

The choice of plants which interest our objectives is based on ethnopharmacological data indicating their use against certain diseases, in particular diabetes, as well as the novelty of their study.

The plants were collected in clean, ventilated places and far from any pollution impact Algeria (table II.1).

- ✓ ***Asphodelus microcarpus***: was collected in "Oued Morra", located about 89.5 km from the capital of the wilaya of Laghouat during the month of April 2019.
- ✓ ***Bubonium graveolens***: was collected in "Zalfana", located about 70 km from the capital of Ghardaïa, during the month of April 2019.
- ✓ ***Haplophyllum tuberculatum***: was collected at Bennasser Benchohra (or Bennacer Benchohra), formerly Mekhareg, located about 18 km from the capital of the wilaya of Laghouat during the month of April 2019.

The plants were identified by the director of the **L**aboratory de **S**ciences **F**undamental (LSF), University of Laghouat professor YOUSFI Mouhamed.

After cleaning the plant material, the samples were dried in a clean room away from humidity. After complete drying, the plant parts were grinded using a mortar and sieved to the same particle size and kept until analysis.

Table II.2. The investigated plant species and their properties

<table>
<tr><td colspan="2">Haplophyllum tuberculatum (Forssk.) A.Juss.</td></tr>
<tr><td>Systematic</td><td>Photo</td></tr>
<tr><td>Kingdom :Plantae</td><td rowspan="8"> 

(Chehma, 2006)</td></tr>
<tr><td>Phylum: Magnoliophyta</td></tr>
<tr><td>Class :Magnoliopsida</td></tr>
<tr><td>Order : Sapindales</td></tr>
<tr><td>Family :Rutaceae</td></tr>
<tr><td>Genus :Haplophyllum A.Juss.</td></tr>
<tr><td>Species: Haplophyllum tuberculatum (Forssk.) A.Juss.</td></tr>
<tr><td>Arabic name : الفيجل</td></tr>
<tr><td colspan="2">Botanical description</td></tr>
<tr><td colspan="2">Haplophyllum tuberculatum is a perennial herb belonging to the Rutaceae family, the height of the plant is around 60 cm, the leaves are alternately elliptical to obovate, the yellowish- green stem has a white and yellow flower of five petals and. This plant is covered by tiny elevated glands. The flowers are yellow with five petals and five free, ovate sepals. They provide the fruit of five carpels, each containing one or two black seeds. Flowering from June to August. The fruit is a capsule that carries a peduncle a little shorter than itself; it opens at maturity, producing various black or brownish seeds (Álvarez Cruz, 2011).</td></tr>
<tr><td colspan="2">Use in traditional medicine</td></tr>
<tr><td colspan="2">The aerial parts are used with different methods and preparations such as decoction for rheumatic pains and digestive problems including constipation and diarrhea. It can be also prepared as a juice extracted from the leaves for the treatment of skin infections and parasitic diseases as well. The plant was used also for gynecological problems and its essential oils have antimicrobial activity (Eissa, et al., 2014).</td></tr>
<tr><td colspan="2">Active substances</td></tr>
<tr><td colspan="2">Haplophyllum tuberculatum is rich in secondary metabolites such as alkaloids, polyphenols, flavonoids and essential oils. This, then, makes its critical uses in herbal medicine. It has been shown to have various pharmacological activities, including antioxidant activity, where ethanol extract and essential oils seem to have anti-free radical activity by inhibiting the production of toxic oxygenated derivatives (Eissa et al., 2014). Also, Ethanolic and The essential oils from aerial parts of Haplophyllum tuberculatum extract possesses antimicrobial activity against Gram+ and Gram-such as S. aureus, E. coli, and P. aeruginosa). Otherwise, Ibrahim et al., (2015) observed that the ethyl acetate extract has a high content of flavonoids and tannins, has an effective inhibitor of acetylcholinesterase. Besides, essential oils flowers of Haplophyllum tuberculatum impressive remarkable anti-inflammatory activity against edema induced by carragenine in rats, compared to indomethacin (Sabry et al., 2016).</td></tr>
</table>

Bubonium graveolens (Forssk.) Maire	
Systematic	**Photo**
Kingdom :Plantae	 (Chehma, 2006)
Phylum: Tracheophyta	
Class : Magnoliopsida	
Order : Asterales	
Family : Asteraceae	
Genus : Asteriscus	
Species: *Bubonium graveolens* (Forssk.) Maire	
Arabic name : الطفس	

Botanical description

Bubonium graveolens is a strong-smelling perennial and medicinal herb, growing to 60 cm and developing well-spaced tufts on the ground. The leaves are narrow pale green, and very canescenthairiness, with veins conspicuous on the underside. The inflorescences are elongated at the base, and the yellowish flowers are tiny in number (Benchelah *et al.*, 2004).

Use in traditional medicine

In Saharan folk medicine, *Bubonium graveolens* has been used to treat fever,gonorrhea, gastrointestinal tract disorders, Migraines, bronchitis, and anti-inflammatory agents(Cheriti *et al.*, 2007). *Bubonium graveolens* was also used for its antimicrobial and hypoglycemic properties. The essential oil of *Bubonium graveolens* has antibacterial activity against gram-positive and gram-negative bacteria and pathogenic fungi, such as candida Albicans and saccharomyces cerevisiae (Znini, *et al.*, 2011).

Active subtances

The aerial part of the plant indicates essential oils, saponoside alkaloids, terpenoids, and flavonoids such as Luteoline 7-o-glucoside, Luteoline 3-o-glucoside, Luteoline 7-o-galactoside, Kaempferol 7-o-glucoside, Quercetin 7-o-glucoside, 3, 6.3 'trimethyl, and herqueretagetine. The major essential oil's major components are 1,8- Cineole, B-Villandrin, M-Semin, Exo-2-Hydroxycinol, Trans-Chrysanthenyl, Menthyle Acetate(Ahmed *et al.*, 1991; Cheriti *et al.*, 2007; Haddouchi *et al.*, 2016; Chaib *et al.*, 2017).

<table>
<tr><th colspan="2">***Asphodelus microcarpus* Viv**</th></tr>
<tr><th>Systematic</th><th>Photo</th></tr>
<tr><td>Kingdom :Plantae</td><td rowspan="8"> 
http://atlas-sahara.org</td></tr>
<tr><td>Phylum: Tracheophyta</td></tr>
<tr><td>Class :Liliopsida</td></tr>
<tr><td>Order : Asparagales</td></tr>
<tr><td>Family :Asphodelaceae</td></tr>
<tr><td>Genus :Asphodelus L.</td></tr>
<tr><td>Species: Asphodelus microcarpus Viv</td></tr>
<tr><td>Arabic name : برواق</td></tr>
<tr><th colspan="2">Botanical description</th></tr>
<tr><td colspan="2">Asphodelus microcarpus is a perennial plant of a shrub about 1 meter long. Long, slender leaves with a width of 1 to 4 cm and a length of 50 to 60 cm, hollowed out in a triangular gutter and arranged in rosettes at the stem's base. The flowers are 35 mm in diameter, with six elongated white petals, consisting of a white filament and an orange anther, middle ovaries, and an upper one, surrounded by a salmon-orange capsule. Asphodelus microcarpus has fruits in small capsules, slightly narrowed at the base with thin valves and ellipticals with smooth edges. The roots are tightly bloated as cylindrical tubers (El-Ghaly, 2017).</td></tr>
<tr><th colspan="2">Use in traditional medicine</th></tr>
<tr><td colspan="2">Asphodelus microcarpusis mainly used in traditional Algerian medicine. The roots are used to treat rheumatism, fungal skin infections, abscesses, eczema, ectoderm (skin diseases), jaundice, psoriasis, and ear pain also Decoction of the leaves are used for their diuretic properties (tends to improve the production of urine) (Ali-Shtayeh et al 1999, Zellagui et al., 2013; Sarri et al., 2014; Chermat and Gharzouli 2015; El-Ghaly, 2017).</td></tr>
<tr><th colspan="2">Active substances</th></tr>
<tr><td colspan="2">The phytochemical analysis of many Asphodelus species showed many chemical compounds, including flavonoids, phenolic acids, terpenoids, anthraquinones, and alkaloids (Zellagui et al., 2013; Malmir et al., 2018). Roots have been reported to contain mostly anthraquinone derivatives such as chrysophanol and aloe-emodin, triterpenoids, and naphthalene derivatives. The aerial part includes flavonoids such as luteolin, isovitexin and isoorientine, phenolic acids, and few anthraquinones. The fatty acids, particularly myristic, palmitic, oleic, linoleic, and linolenic acids, have been found in seeds and roots. Recent analysis has demonstrated that the Asphodelus microcarpus extract has substantial efficacy against E. coli, S. aureus, S. haemolyticus, and B. clausii The natural substances derived from A. microcarpus are also has antioxidants effect. The ethanolic root extract of the plant has shown antioxidant efficacy in different in vitro tests, suggesting a potential use in pathological conditions of oxidative stress (Di Petrillo et al., 2017; Mayouf et al., 2019).</td></tr>
</table>

EXPERIMENTAL PART

II.1**. Preparation of plant extracts**

The choice of the suitable plant part for the extraction depends on the Species of plant used, and sometimes on the desired effect. For that, we have use the aerial parts of each plant where the active ingredients are concentrated.

II.1.1. Aqueous extract

Assuming the active ingredients are polar compounds, the extraction was performed with distilled water. The aqueous extract was prepared by two methods for appearing reasons: by decoction (herbal teas) thus mimicking the usual use of medicinal plants and by diffusion.

II.1.1.1. Decoction extraction

Five gram of powder of the air-dried plants (aerial parts) was macerated with 50mL distilled water at 75°C for 20 min the extract was filtered. After removing distilled water under reduced pressure in a rotary evaporator at 40° C, The dried residue was dissolved in 10 mL of water and kept at 4°C.

II.1.1.2. Infusion extraction

Five grams of powder of the air-dried plants (aerial parts) was macerated with 50mL distilled water at room temperature. After 24 hours, the extract was filtered, then the residue was extracted for the second time with 50 mL distilled water at room temperature for 24h and the extract was filtered again. After filtration, a rotary evaporator was used to remove distilled water under reduced pressure at 40°C. The residue was dissolved in 10 mL of distilled water and kept at 4°C.

II.1.2. Essential oil extraction

The essential oils from *Bubonium graveolens* and *Haplophyllum tuberculatum* who presented the existence of essential oils, were obtained by hydrodistillation using a Clevenger type apparatus; subsequently, the obtained EOs are measured (in mL per kg of plant) and dried over anhydrous sodium sulfate and stored in the dark at 4 °C.

- **Calculation of the essential oil content :** the content is defined as the quantity of essential oils extracted (in mL) from a kilogram of plant. This content is calculated by the following formula:

$$C = \frac{v}{m}$$

C : content expressed (in mL / kg);

V: volume of essential oils extracted (mL);

W: initial weight of the plant material (Kg).

II.2. Phenolic coumpounds quantification

II.2.1. Determination of total phenolics compounds

The concentration of total phenols in the extracts was determined by the Folin-Ciocalteu reagent using Singleton and Ross methods (1965). When 100 µL of each extract was combined with the Folin-Ciocalteu reagent (10 %), 2 mL of sodium carbonate (10 %) was

added after 2 min of incubation at room temperature. The samples were shaken and incubated at room temperature for 30min. The absorbance of both samples was measured at 760 nm using the Shimadzu 1601 visible spectrophotometer. The total phenolic content of each extract was expressed as gallic acid equivalents (GAE), which represented the phenolic content as the amount of gallic acid (mg) in 1 g of dry matter (Boussoussa *et al.*, 2018).

II.2.2.Quantification of flavonoids content

The total flavonoid content of the plant's dry aerial parts was measured spectrophotometrically by method of Laimaison and Carnat (1991). Changed by (Djeridan *et al.*, 2006, Floegel, 2011), this process is based on the combination of flavonoids with aluminum trichloride having complex flavonoids-aluminum with maximum absorption of 430 nm. The process shall be as follows: 500 µl of diluted sample was blended with 500 µl of aluminum trichloride methanol solution (2 %). After incubation at room temperature for 20 min, the reaction mixture's absorbance was measured at 430 nm with the Shimadzu 1601 visible spectrophotometer apparatus. The quality of flavonoids is expressed in mg quercetin equivalent (RE)/g dry matter (Bahorun *et al*,1997).

II.3. α-Amylase inhibitory activity

The analysis of our extracts' inhibitory effect on α - amylase is carried out by spectrophotometric determination of the product released into the reaction medium per unit of time, during hydrolysis of the substrate by a fungal α - amylase d *'Aspergillus oryzae*, under well-defined operating conditions. The increase expresses the enzymatic activity in maltose units released per unit of time during hydrolysis of the substrate (starch) under favorable temperature and pH conditions.

The enzymatic activity of α - amylase is assayed on its starch substrate (a mixture of 2 homopolymers, amylose, and amylopectin, composed of units of D-Glucose molecules).It catalyzes the hydrolysis of this substrate, releasing maltose and other products in the process. The amount of maltose emitted is measured using spectrophotometry.

Bernfeld's method is based on the dinitrosalicylic DNS, which stops the enzymatic reaction by changing the pH and forms a complex with the product. This method tests for the presence of afree carbonyl group (C = O) of the so-called reducing sugars, this involves the oxidation ofthe functional group present in glucose. Simultaneously 3,5-dinitrosalicylic acid (DNS) is reduced to 5-nitrosalicylic acid 3-amino under alkaline conditions.

Each glucose molecule (a hydrolyzed bond) released in the reaction medium interacts with the excess DNS, which is orange in color, reducing the latter while simultaneously causing a color change to brick red, which is measured at 530 nm after dilution. The anti-amylase activity is determined for each extract under the same reaction conditions and is expressed by the IC_{50} value, which reflects the concentration of inhibitor required to reduce the enzyme activity to 50% of its maximal uninhibited value. To do so, we used three different concentrations of extracts to vary the inhibitor concentrations.

II.3.1. Inhibition effect of aqueous extracts

The enzymatic activity of the fungal α - amylase of *Aspergillus oryzae* in the presence of different extracts is determined as follows (Bhandari *et al.*, 2008): In test tubes, the reaction media contained we add to each tube 200 µl of salt sodium phosphate buffer (pH 6, 8) was mixed with100 µl of soluble starch 1% as a substrate and 100 µl of plant extract. Test tubes incubated for 5min at 37, 100 µl of α-amylase (13 U/mL) were added to test tubes for started the reaction. After incubation for 5 min at 37°C, the reaction was stopped by the addition of 1 mL of basic solution DNS (dinitro-salicylic acid) 1% .the mixture was boiled for 5 min at 100°C, then the test tubes were cooled with tap water after dilution by adding 4 mL of distilled water. Enzyme activity was quantified by measuring optical density proportional to the maltose equivalents' quantity released from starch at 530 nm. The inhibitory activity of α-amylase inhibition was expressed as a percentage of inhibition.

For appearing reasons and with the same procedure, we have also measured the anti - amylase effect of the most prescribed drug for local diabetics « Gluconova » for reasons of appearing.

Inhibitory activity (%) was calculated by the following equation :

$$\text{Inhibitory activity (\%)} = [(A_0 - A_S)*100]/A0$$

A0 : Absorbance of control without inhibitor

As : Absorbance of test Sample with inhibitor

II.3.2. Inhibition effect of essential oils

In test tubes, the reaction media contained 100 µL of enzyme and 100 µL of each diluted essential oil extract. The tubes were then incubated for 5 minutes at 37 ° C, followed by the addition of a 1% starch solution, followed by incubation for 10 minutes at 37 ° C. The enzymatic reaction was stopped by adding DNS, the reaction medium was then boiled at 100 ° C for 5 min, after cooling the reaction medium was diluted by adding 2 mL of distilled water.Optical density was measured at 530 nm.

Inhibitory activity (%) was calculated by the following equation :

$$\text{Inhibitory activity (\%)} = [(A_0 - A_S)*100]/A0$$

A0 : Absorbance of control without inhibitor

As : Absorbance of test Sample with inhibitor

II.4. *In vivo* anti-diabetic effect

II.4.1. Animals used

The animals used in this section of the acute toxicity study and the part of the anti-diabetic activity are rats 'Rattusnorvegicus' of 'Wistar strain, 2 to 3 months of age, with body weight (BW) between 180 and 220 g. Animal keeping took place inside the Animal House of the Department of Biology, the Faculty of Nature and Life Sciences of the Earth and the Universe, Ferhat Abbas University, Setif.

Figure II.1. Wistar Albino Rats used for study

Albino Male Wistarrats were brought back from the Pasteur Institute (Breeding Center, Kouba, Algiers). They had been adapting to the conditions of the animal house for a week. The animals were fed ad libitum with a kibble diet (National Office for Cattle Feeding, Bejaia), the composition of which is shown in Table II.2. They were kept in ambient temperature and light conditions.

All animals are treated and handled according to the standards dictated in manuals for experimental animals' care and use (CCPA, 1984 and Festing, 2002).

Table II.1 Composition of the kibble diet (ONAB Bejaia).

Components	%
Mais	52.80
Son	10.80
Soy	32.10
Calcaire	01.50
Phosphate	00.80
Vitamin supplement	02.00

II.4.2. Evaluation of the toxicity of extracts

A toxicant is a substance destroying the normal functioning of a living organism, instantly or in the long term, leading to disrupt the normal functioning of a living organism, which can go as far as its complete suppression and lead to death. All substances are poisonous when

sufficient doses are administered. For a drug with pharmacological effects to possibly be used as a medicine, it is first necessary for the activity to appear at doses for which the toxicity is insignificant. Various methods can identify toxic effects; the Dragstedt and Lang method, the Karber and Behrens method; the Miller and Tainter method; the Trevanmethod; the Litchfield and Wilcoxon methods; Method according to the OECD Code 423 European Guideline.

The OECD Guideline 423 [OECD, 2002] is one of these methods. This approach is reproducible, uses very few animals, and differs from other acute toxicity approaches (Guidelines 420 and 425). It allows substances to be graded in the order of toxicity similarly. The specified dose of the drug is given orally to a group of animals. The approach uses fixed amounts and results in the classification of substances in the Globally Harmonized Classification System (SCGH) of substances that cause acute toxicity. This approach is not intended to measure the exact value for the LD_{50}. This method aims to assess the range of doses of the drug to be considered fatal. All available data on the test substance should be collected before testing. This knowledge includes the identity and chemical composition of the drug, its physicochemical properties, and the findings of any other in vitro and in vivo toxicity tests; it will be advantageous in choosing the optimum starting dose. The specified quantity of the drug is given orally to a group of animals. The substance is tested in a sequential process in which three single-sex animals are used at each level. The absence or presence of substance-related mortality in a group that received a dose at a given stage decides the next step: appropriate Stop-Test; allow the same quantity to be administered to three additional animals; enable the next higher or lower dose to be distributed to three other animals.

The acute toxicity test for *A. microcarpus* extracts was performed using the method defined by the Organization for Economic Cooperation and Development (Guideline, 2008). In short, male Albino rats weighting 180 to 220 g of body weight previously released for fasting for 16h were divided into groups of 5 rats per extract. Two doses of each extract (2g/kg) were given by mouth. Observation of behavioral modifications and signs of toxicity (resistance, convulsions, anorexia, asthenia, diarrhea, and death) was conducted within the first four hours after administering the extract, followed by 14 days.

II.4.3. Diabetes Induction

The most effective technique for inducing experimental diabetes usually is to eliminate the pancreas from the organism. However, to cause a detectable type of diabetes, at least 90-95 percent of the pancreas must be removed. Otherwise, Langerhans' islets in the remaining

pancreas can enlarge and secrete sufficient insulin to satisfy typical metabolic requirements. One of the most popular methods to cause diabetes is by destroying the pancreas using Alloxan and streptozotocin (STZ) (Szkudelski *et al* 2001).

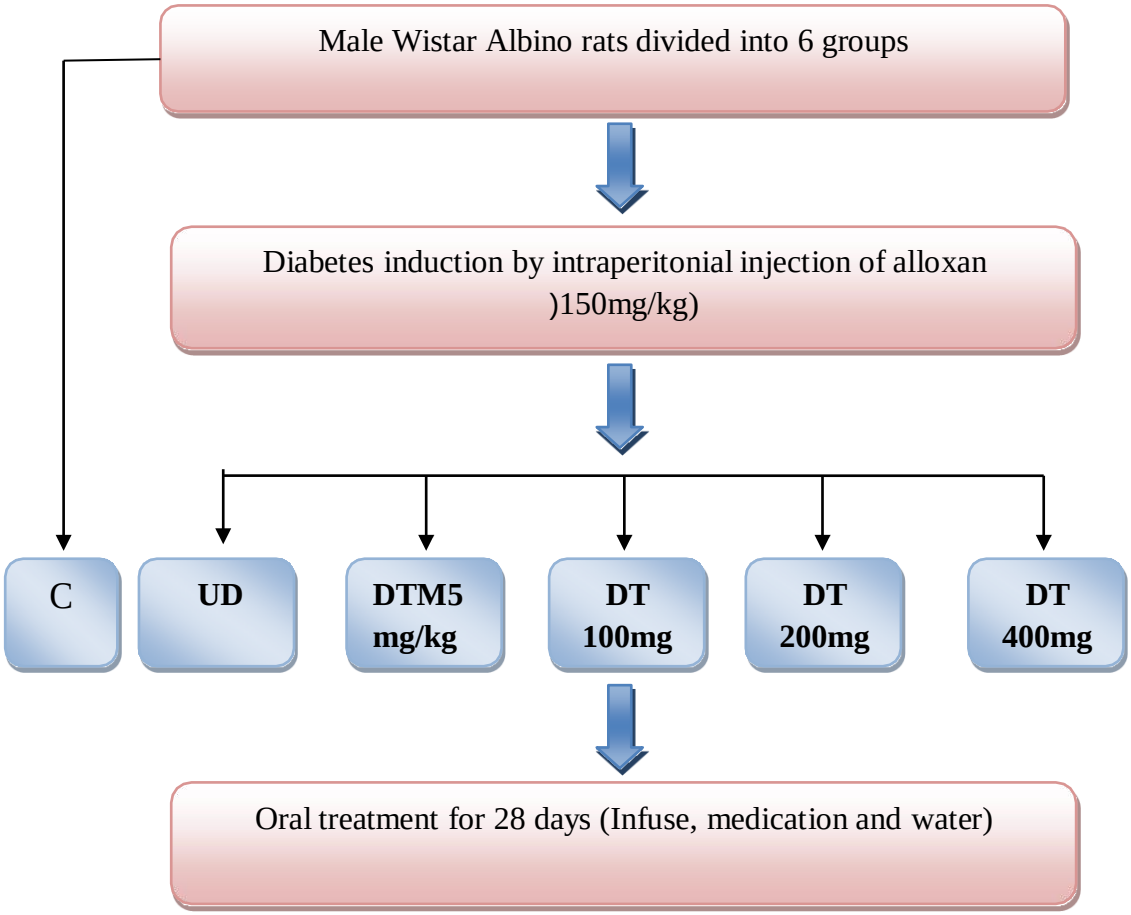
Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) is a hydrophilic compound that exists mainly in the monohydrate state, resulting from uric acid oxidation. It has a chemical structure similar to glucose because that Alloxan can pass the cellular membrane without additional action of specific protein transporters. Namely, it enters beta cells through GLUT2 transporters. Just after pancreatic beta cells uptake Alloxan, The reduction of Alloxan to dialuric acid occurs in the cytosol. This reduction is due to some agents, such as reduced cysteine, glutathione, ascorbic acid, and protein-bound sulfhydryl (-SH) groups. Alloxan interacts with two-SH groups at the glucose binding domain of glucokinase that leading to the disulfide bond formation and the enzyme's inactivation. (Lenzen *et al.*, 1988).

As a consequence of alloxan reduction, dialuric acid is produced, which is then re-oxidized back to Alloxan by forming a redox cycle for the generation of (ROS) and superoxide radicals. Superoxide radicals release ferric ions from ferritin and convert them to ferrous and ferric ions. Besides, superoxide radicals undergo dismutation to produce hydrogen peroxide (H_2O_2) in the existence of superoxide dismutase. As a consequence of the Fenton reaction, highly reactive hydroxyl radicals are composed principally of ferrous and H_2O_2. One other mechanism mentioned is the influence of ROS on the DNA of the pancreatic islets. DNA fragmentation occurs in beta-cells exposed to Alloxan that causes DNA damage, which activates poly-ADP-ribosylation, a mechanism that participates in DNA repair. (Rohilla *et al* 2012).

Disturbances in intracellular calcium homeostasis have also been observed to constitute a significant factor in the diabetic action of Alloxan. It has been reported that Alloxan increases the concentration of cytosolic free Ca^{2+} in pancreatic beta cells. Calcium inflow is the product of Alloxan's ability to depolarize pancreatic beta cells, which further opens voltage-dependent calcium channels and increases calcium concentration in the pancreatic cells. The increased concentration of Ca2+ ion also contributes to the supraphysiological release of insulin which, along with ROS, has been shown to cause damage to pancreatic beta cells ultimately(Rohilla *et al* 2012; Radenković *et al.*, 2016).

The rats were injected Intraperitoneally with Alloxane monohydrate (Sigma-Aldrich, USA) dissolved in sterile saline water at a dosage of 150 mg/kg body weight. Hyperglycemia was

confirmed after three days using the glucometer vital Check (Roche Diagnostic, Germany). Only rats with high blood glucose levels greater than 300 mg/dl were selected and used in this research. rats were randomly divided into six groups, each of which consisted of six rats (Orhan *et al.* 2005, Houcher *et al.* 2007).



C : control; **UD** : Untreated Diabetics ; **DTM** : DTM: Diabetics treated with medication 5 mg/kg; **DT 100 mg** : Diabetics treated with 100 mg / kg bw of plant; **DT 200mg** : Diabetics treated with 200 mg / kg bw of plant ; **DT400 mg** : Diabetics treated with 200 mg / kg bw of plant.

Figure II.2. Experimental protocol for treating diabetic rats with plant extracts**.**

To evaluate the anti-diabetic activity of the aqueous extract in laboratory rats, we used two procedures; short-term study, oral glucose tolerance test, and long-term study.

II.4.4. Study of the anti-diabetic activity of the mighty extracts on the normal rats and the diabetic rats

II.4.4.1. Short term study

Eighteen normal fasting rats were divided into three groups (n = 6) and were treated orally with the extracts at doses of 100, 200, and 400 mg/kg BW. The blood glucose was measured at 0 min (before force-feeding), at 30, 60, 90, and 120 min, on a drop of blood taken from the caudal end of the animals. A Vital Check Active glucometer was used for the measurements.

Five rats served as a control group and were force-fed with physiological saline solution (0.9% NaCl, 10 mL/kg BW) (Berrani *et al.* 2018).

II.4.4.2. Long term study (28 days)

Eighteen male diabetic rats have been divided into three groups (n = 6). The extracts was administered orally at a dosage of 200 mg/kg BW per day for 28 days to one (experimental) group, the other group being the control group, and was treated in parallel with the physiological serum (NaCl 0.9%, 10 mL/kg BW). In both groups, the fasting rats blood sugar was measured on a drop of blood taken from the caudal end. The weight of the rats, the amount of food, and the amount of water consumed were also measured (Houcher *et al.*, 2007).

II.4.5. Blood biochemical assays after sacrifice

At the end of the 28 days of therapy, the rats are sacrificed on an empty stomach in the morning. The blood is gathered directly in tubes to determine the different biochemical parameters such as glucose level, cholesterol, triglycerides, total lipids, HDL, LDL, complete protein, TGO, TGP, urea, and creatinine. All the parameters were measured in an automated spectrophotometer UV mindray BS 240.

II.5.Statistical analysis of the results

The results are represented as the mean ± standard deviation, and the differences were considered significant at $P \leq 0.05$. These calculations were carried out using the graph pad prism 09 for analysis and statistical processing of data. Group comparisons were performed by ANOVA analysis of variance. The significant difference between the control group and the Student's t-test assessed experimental groups.

The value found by the calculation of t can affirm that the populations are different with a risk of error p such that:

$p > 0.05$ = the difference is not significant (ns)

$0.05 > p > 0.01$ = the difference is significant *

$0.05 > p > 0.001$ = the difference is highly significant **

$p < 0.001$ = the difference is very highly significant ***.

RESULTS AND DISCUSSION

III.1. Preparation of Plants Extracts

Extraction is a crucial step for the isolation and identification of active ingredients with high added value from plant material, particularly the case of polyphenols, which are currently attracting a lot of interest due to their biological activities (Altemimi *et al*., 2017).

Two extraction methods were used in this study. The first one was an aqueous extraction with distilled water which contains polar and amphiphilic compounds in a preferential manner (Martins *et al.,* 2015). The second one, is that of essential oils which was carried out by hydrodistillation in a Clevenger type apparatus. Since the habit has always taken the path of aqueous extracts for *in vivo* tests, we wanted to choose a new path "why not try an *in vivo* test on essential oil extracts".

The aspect, the contents and the colors of the different extracts from the three plants investigated are shown in Tableau III.1. The yield results were expressed relative to the weight of the dry matter of the plants investigated. The aqueous extracts of *Asphodelus microcarpus* showed a better yield : 31.28 and 30.99% respectively.

In general, the extraction yield of different plant compounds depends on several factors, namely the extraction method, the maceration time (Tiwari, *et al*. 2011). The yields are proportional to the solution's polarity, which defines both the quantity and form of compounds extracted and temperature, sample-to-solution proportion, and chemical composition of the plant. It plays a critical role in the extraction process (Dai and Mumper, 2010).

Tableau III.1 Aspect, contents, and colors of aqueous and essential oils extracts from the three plants investigated.

Plant / Caracterestics	*Asphodelus microcarpus*	*Bubonium graveolens*	*Haplophyllum tuberculatum*
Aqueous decoction extracts			
Aspect	Viscous	Viscous	Viscous
Color	Dark brown	Yellow	Dark Yellow
Content (%)	31.28	15.56	17.57
Aqueous infusion extracts			
Aspect	Viscous	Viscous	Viscous
Color	Dark brown	Light yellow	Green
Content (%)	30.99	14.01	27.26
Essential oils extracts			
Aspect	/	Liquid	Liquid
Color	/	Yellow	Light green
Content (mL/kg)	/	5.33	3.33

We noticed that the aqueous extracts showed viscous aspects. A liquid appearance was observed for essential oil extracts. Different colors were recorded varying between Brown, Orange, Green and Yellow for the different extracts.

Two out of three plants have shown the existence of essential oils with lower contents than the aqueous extracts. Differences in essential oil yield from an organ to organ or a specie to specie have been reported. According to several authors, the origin of the species harvest, the harvest period, the plant organ, the drying time and the extraction method are the factors that can have a direct impact on the yields of secondary metabolites (Costa *et al.* 2014)

III.2. Quantification of phenolic and flavonoids compounds contents

Before any attempt to study biological activity, we performed an assay for total phenols on the various prepared extracts. In the present study, total phenolics and flavonoids contents among the different extracts are evaluated. The results obtained are presented in Table III.2.

There is no method which allows all the phenolic compounds present in an unpurified plant extract to be measured satisfactorily and simultaneously. However, a rapid and global estimateof the total phenol content can be obtained by different methods, in particular. by the use of a mixture of phosphomolybdate and phosphotungstate marketed under the name of the Folin - Ciocalteu reagent.

Each extracts phenolic content was measured using the gallic acid calibration curve and expressed in milligrams of gallic acid equivalent by gram of dry mater (mg GAE/ g DM).

Table III.2. Total phenols and flavonoids contents of aqueous extracts from the three investigated plants.

Plant / Contents	***Asphodelus microcarpus***	***Bubonium graveolens***	***Haplophyllum tuberculatum***
Aqueous decoction extracts			
Total phenolic content mg GAE/ g DM	6.35 ± 0.07	03.15± 0.10	3.69 ± 0.07
Flavonoids content mg QE/ g DM	0.95 ± 0.01	0.48± 0.02	0.83 ± 0.03
Aqueous infusion extracts			
Total phenolic content mg GAE/ g DM	8.47 ± 0.29	2.88 ± 0.05	11.97 ± 0.19
Flavonoids content mg QE/ g DM	1.49 ± 0.00	0.62 ±0.01	1.50 ± 0.04

The total polyphenols content varied in the different extracts and ranged from 2.88 to 11.97 mg GAE /g DM. The plant *Haplophyllum tuberculatum* had the highest average phenols values, while the plant *Bubonium graveolens* had the lowest total phenols values in the two aqueous extracts. The infusion extract, recorded the highest values of total phenols than that extracted by the decoction method, and there were significant differences between the two methods of extraction. These results confirm that a moderate temperature would favor the extraction; this fact has been approved by some authors who specify that the techniques using higher or lower temperatures and/or pressures would considerably increase the efficiency of the polyphenol extraction. The high content of phenols in infusion extract can be also explained by repeated the maceration more than three times in each extract. Time is also an important factor. It may explain the difference in the amount of phenols between the infusion and the decoction extract. For example, infusion extract take 72 hours while the decoction extract occurs in just 20 min.

The total phenolic contents of *Asphodelus microcarpus* and *Haplophyllum tuberculatum* reported for decoction extraction are lowest compared to maceration at room temperature. They were confirming that sustained exposure to heat renders this process inefficient for thermosensitive compounds. That heating can leading to compound degradation (Tiwari *et al.*, 2011). This is valid for polyphenols, which may have been degraded during the extraction process, minimizing their content.

When we compare the amount of total phenol compounds in both aqueous extracts, we find that the infusion extracts showed a higher total phenol than the decoction extracts. These results may explain can be explained by the fact that the extraction by infusion can extract other temperature-sensitive polar compounds that react with the Folin Cioncalteu reagent, such as sugars, proteins, alkaloids, and other compounds, resulting in increased phenolic compounds (Khacheba *et al.*, 2014).

The total phenolic content of three plants that less than other studies on the same plants species (Eissa *et al.*, 2014; Hamdi *et al.*,, 2018) for *Haplophyllum tuberculatum*,(Di Petrillo *et al.*, 2016) for *Asphodelus microcarpus* and (Haddouchi *et al.*, 2016) for *Bubonium graveolens*, This lowness among our plants may be related to the poverty of our plants in polyphenols or harsh climatic conditions (low temperatures in the winter, solar radiation, drought, and salinity), stimulating secondary metabolite biosynthesis, such as polyphenols. Furthermore, the plant being studied, the area and date of harvest, the extraction, and analytical method, and the solvents used. Indeed, a plants phenolic content is influenced by both endogenous (genetics) and external (environment) influences (cultural practices, maturity at harvest, and storage conditions). Besides that, the process of quantification may affect the overall phenol content determination.

The dosage of flavonoid content was determinated spectroscopically in our sample using the aluminum chloride process, which is specifically used to quantify flavones and flavonols. As a result, the obtained do not show the exact total flavonoid concentrations in the plants studied. The procedure was not practical for all types of plants, especially those with a height chlorophyll. The contents were measured using the quercetin calibration curve and expressed in milligrams of quercetin equivalent by gram of dry mater (mg QE/ g DM).

The amount of flavonoids content in aqueous extracts ranged from 0.48 to 1.50 mg EQ/g. The highest value of flavonoids found in the *Haplophyllum tuberculatum* infusion extract with value 1.50 mg EQ/g. The lowest was registered in the decoction extractof *Bubonium graveolens* with a value of 0.48 mg EQ/g.

Compared to the phenolic content, the three studied plants are rich in flavonoids. The rest could be other phenolic compounds with other chemical structures than those of the flavonoids (Phenolic acids, tannins, stilbenes...) present in minimal quantities. Thus, we can deduce that the analysis of polyphenols by spectroscopic methods gives us a good idea on qualitative identification of these compounds.

The amount of flavonoids varies proportionally with total polyphenol content from one plant to another.also confirming that flavonoids are the most important group of phenols in our plants.

III.3. Inhibitory effect of the different extracts from the three plants on α - amylase

Various pharmacological approaches are used to improve the treatment of diabetes by different modes of action such as stimulation of insulin secretion, increase in the number of glucose transporters, inhibition of gluconeogenesis and reduction of glucose. Absorption of glucose from the intestine (Vieira *et al.,* 2019). The best therapeutic approach for the treatment of diabetes and to decrease hyperglycemia especially after a meal is to delay and reduce the digestion of ingested carbohydrates via the inhibition of the main dietary carbohydrate hydrolyzing enzyme α-amylase in the digestive organs (Khacheba *et al.,* 2014).

In therapy, treatment for type II diabetics and to control blood sugar levels competitive α - amylase inhibitors, alone or in combination with other anti-diabetic agents are used. However, the continued use of these synthetic agents should be reduced, as these inhibitors are known to cause many side effects. Therefore, many efforts have been made to identify new sources of anti-diabetics from different traditional herbal medicines without side effects is still a challenge for the medical system and researchers (Salehi *et al.,* 2019).

In order to identify the plants having an inhibitory capacity on the *Aspergillus oryzae* α-amylase, *in vitro* inhibition tests were carried out for each extract. In our study, the enzymatic activity of α-amylase was measured on its substrate, starch. These inhibition tests performed at the same concentration of extracts allowed us to calculate the inhibition rates. The results are shown in the table III.3.

Table III.3. Percent inhibition of aqueous extracts of the three investigated plants.

Plant	***Asphodelus microcarpus***	***Bubonium graveolens***	***Haplophyllum tuberculatum***
Aqueous decoction extracts	94.08 ± 1.05	69.42 ± 0.99	71.20 ± 0.22
Aqueous infusion extracts	39.08 ± 2.36	50.83 ± 0.13	61.88 ± 0.53

After the analysis of the results obtained, we found that all the aqueous extracts showed a potential for inhibition on α – amylase, with inhibition rates greater than 50% except the infusion extract of *Asphodelus microcarpus* which recorded a value of 39.08. The plant

exhibited the best percentage for both extraction methods.

The aqueous decoction extracts have been shown to be better inhibitors compared to those of infusion. This may be explained by the difference between the active substances which present in the extracts due to the change in the extraction method; the heat used during the decoction method makes the effective principles more soluble and accelerates their extraction. (Tiwari *et al.,* 2011). The growth of bacteria and fungi in the extract, it may also affect the inhibitory activity, as we discovered in the lab that the infusion extract of *Asphodelus microcarpus* was the quickest to become infected with bacteria and fungi. Which proves that the use of plants in the form of herbal teas is more beneficial.

We have noticed that the results obtained for the two aqueous methods are close. They vary almost between 1.5 and 6.13 %, not exceeding 10 %, except for the extracts of *Asphodelus microcarpus,* who recorded the highest difference value between decoction and infusion extraction (63.1 %)**.** This for the extracts of *Asphodelus microcarpus,* who recorded the highest values**.** This similarity of values in the two forms of extraction may be due to active molecules with more or more minor identical structures or with the same inhibitory action mechanism in both.

A study carried out in the basic science laboratory on 18 local anti-diabetic plants, showed a majority of percent inhibition less than 20% (Khacheba *et al.*, 2017). It is clear that the percentages of inhibition differ between the extracts. This finding can be explained by the fact that the composition of active compounds and their chemical structures in our plant extracts vary with the species, which leads to different contacts with the active site of the enzyme,which proves that the composition and structure play an important role in inhibition (Khacheba *et al.,* 2014).

After analyzing the results of the different percentage inhibition values recorded for the aqueous extracts, we undertook the terpene compounds (essential oils); to get an idea of the metabolic class responsible for inhibitory activity on α-amylase.

The graphical representations of the variation in the percentages of inhibition as a function of the extract concentrations (see appendix), allowed us to determine the IC_{50} values for each extract that we have grouped together in Table III.4.

After reviewing the results, we noted that the aqueous and essential oils extracts prepared had an inhibition capacity against α – amylase. The Best IC_{50} values were shaown in both essential

oil extracts of *Bubonium graveolens* and decoction extracts of *Asphodelus microcarpus* (IC_{50} = 9.78 and 19.78 mg/mL) respectively.

Table III.4. IC_{50} values (mg / mL) of aqueous and essentials oils extracts on α -amylase.

Asphodelus microcarpus	***Bubonium graveolens***	***Haplophyllum tuberculatum***
Aqueous decoction extracts		
19.78 ± 0.09	56.29 ± 0.07	43.90 ± 0.00
Aqueous infusion extracts		
82.01 ± 0.70	57.79 ± 2.74	50.03 ± 0.10
Essential oils extracts		
/	9.78 ± 0.05	34.96 ± 2.56
Gluconova		
6.83 ± 0.45		

The aqueous decoction extracts showed the best value of IC_{50} compared to the infusion extracts which is in agreement with the percentage of inhibition determined previously. These findings prompted us to evaluate the *in vivo* effect of *Asphodelus microcarpus* decoction aqueous extract and *Bubonium graveolens* essential oil extracts.

Essential oil extracts showed the best activity against α-amylase compared with Aqueous extracts. The higher activity of essential oil than the aqueous extracts due to the decrease in the activity of aqueous extract because of the action of polyphenol oxidase enzyme, which degrades polyphenols in watter extracts (Khacheba *et al.*, 2014). Moreover, water is a better medium for the occurrence of micro-organisms (Acheuk *et al.*, 2012). The more useful explanation for the decrease in the activity of the aqueous extract can be attributed to polyphenol oxidase, an enzyme that degrades polyphenols extracted with water, while in methanol they are inactive. In addition, water is a better medium for the appearance of microorganisms (Tiwari *et al.*, 2011).

Jelenkovic *et al.*, 2014, has shown the existence of various substances such as β- pinene (6,6-dimethyl-2- methylenebicyclo[3.1.1.]heptane), Myrcene (7-methyl-3-methylene- 1,6-octadiene) in the essential oils which have varying inhibitory potency on the amylase enzyme.(Cheriti *et al.*, 2007),. It is possible that the essential oil of *Bubonium graveolens*

contains these compounds, this may clarify why the essential oil of *Bubonium graveolens* is the best inhibitor on α – amylase.

The inhibitory activity of the essential oil extracts can be attributed to the existence of terpene molecules of a polar nature making them less active towards the enzyme. This confirms that the active molecule and active site contact play an important role in the inhibition phenomenon (Jelenkovic *et al.,* 2014).

This result can be explained also by the fact that the chemical composition of in our plant extracts vary with different species, leading to varying bonds with the enzyme's active site, proving that the composition and the structure both play an essential role in inhibition. We have also observed that the extractor system is very important to screen (Rasouli *et al.,* 2017)

The majority of our aquous extracts recorded less value IC_{50} compared to the drug Gluconova, however the aqueous and essential oil extracts were less effective. We can deduce that the decrease in the activity of aqueous extracts and essential oils depends on the concentration as well as the number and position of the hydroxyl groups of the compounds. These inhibitory activities depend on the structure of the phenolic compounds reacting with proteins / enzymes and on various properties of the biopolymers such as molecular weight, solubility and digestibility in vitro. The mode of inhibition also depends on the specificity of the enzyme substrate. Acarbose, eg synthetic inhibitor exhibited mixed and non-competitive incompetitive type inhibitions when used with other substrates amylose, maltodextrin and maltoheptaose (Goncalves *et al* 2017).

If we compare between phenolic and flavonoid content and inhibition activity parameters, we can say that the inhibitory effect was not related to phenolic compound concentration but is related to the existence of certain individual active phenolic compounds rather than the phenolic content. As a result, an extracts inhibitory potency can not be clarified solely based on its phenolic content; careful characterization is also needed. Besides, there are many explanations for the unclear relation between inhibitory potency and total phenolics, including g the synergism among the inhibitors in the mixture accounted for the inhibition, which was dependent not only on the concentration of individual inhibitors but also on their structure and interaction (Djeridane *et al.,* 2015).

This anti-amylase activity may be due to the glucoside residue included in the flavonoid skeleton. Consequently, we can infer that the excellent inhibition of our phenolic extracts is

probably owed to the presence of the glycosidic groups in the phenolic skeletons (Djeridane *et al.,* 2015). Also, Previous phytochemical studies have indicated the presence of benzoic acids [vanillic acid], cinnamic acids [caffeic acid derivatives, ferulic acid, ferulic acid derivative, *p*-OH cinnamic acids], flavones [trihydroxy methoxy flavone methoxy luteolin glucoside, methoxy apigenin glucoside, methoxy luteolin, and other flavone derivatives] and flavonols [syringetin, quercetin glucuronide, quercetin derivatives in *Haplophyllum tuberculatum*. The presence of some types of flavonoids such as luteolin, quercetin shown inhibitory activity against digestive enzymes such as amylase by binding to the active site of the enzyme or close it (Martinez-Gonzalez *et al.,* 2019). The existence of these types of flavonoids in *Haplophyllum tuberculatum* has been established, which may explain why plant extracts have such strong inhibitory action against the enzyme amylase.

The synthesis of the various results clearly shows that the power of inhibition on α-amylase is a specific character for each extract which can vary according to its composition and the structure of the molecules responsible for the activity. Also For the same plant, the extraction methods and technical constraints lead to different types of extracts possible and of course to different biological effects. Also the drug / extract ratio depends on the quality of the plant used: if its content of active ingredients is high, the quantity of plant used is less. The plant title is therefore not the only quality criterion of the extracts.

III.4. *In vivo* anti-diabetic effect

III.4.1 Evaluation of the extracts toxicity

Medicinal plants have been used to treat many diseases since ancient times. Many people believe that herbal medicines aren't toxic. Many studies have shown that herbal remedies can have the same side effects as drugs. Therefore, for the safe use of these medicinal plants. The toxicity of plants must be studied to know the side effects and the nature of the toxic agent that allows its removal or dose adjustment to obtain active and non-toxic extracts, especially if this agent is responsible for the studied biological activity.

So before starting *in vivo* studies to evaluate the anti-diabetic activity of *Asphodelus microcarpus* aqueous extracts and *Bubonium graveolens* essential oils extracts, an evaluation of the toxicity of the latter was essential. These extracts were first tested orally for their acute toxicity in Wistar rats.

In rats receiving the *Asphodelus microcarpus* aqueous and *Bubonium graveolens* essential oilsextract orally at doses of 2000 or 5000 mg/kg. During the 14 days observation, there was

no mortality and no clinical signs of toxicity (change in behavior, breathing, water, food consumption, hair loss, diarrhea, vomiting, convulsion and difficulty in movement etc.) and no significant difference in the body weight in the treated groups compared to the control group. Changes in body weight have been used to indicate adverse effects of drugs and chemicals (El Hilaly, *et al.* 2004). Therefore, these different extracts appear to be no or moderately toxic up to 5000 mg / Kg. These extracts can consequently be used with minimal risk of toxicity**.** Because there were no significant differences in body weight between the treatment and control groups, it was concluded that *Asphodelus microcarpus* aqueous and *Bubonium graveolens* essential oils extract had no effect on rat growth at the oral dosage used.

In general, changes in body weight growth and internal organ weights in rats after exposure to toxic chemicals would indicate toxicity. In animals, organ weight is an important indicator of physiological and pathological condition. The relative organ weight is important in determining whether or not an organ was damaged (Dybing, *et al.* 2002). After 14 days, no significant changes in internal organ weight were detected in this investigation.

During the 28 day observation duration, we noted that the *Bubonium graveolens* essential oil extract had a hypnotic and sedative effect after administering it to rats. These effects appeared after about 20 minutes of administering. This effect was repeated for the duration of the experiments. We may conclude that the essential oil of *Bubonium graveolens* has a soothing effect based on this finding. This might be due to the chemicals such as □-cadinene which have an effect on the nerve system (Chen, *et al*. 2015), which would support its usage infolk medicine as a sedative for headaches (Cheriti *et al.,* 2007).

Our findings align with several human and animal studies that have shown that several essential oil produce diverse pharmacological responses in the nervous system, including anxiolytic, analgesic, antidepressant, anticonvulsant, and sedative effects. As a result, it has been suggested that essential oil may help alleviate the symptoms of various mental diseases, such as depression, anxiety, and dementia. (Lizarraga-Valderrama *et al.*,, 2021).

In fact the toxicity of medicinal plants can be of two origins, the presence of extremely toxic substances in the plant itself for example pyrrolizidine alkaloids, or the poor maintenance of the quality of the plant from its habitat to its formulation as a treatment

Many factors can influence the quality of plants, such as their toxicity; growth factors, harvest time (summer, winter, etc.), the age of the plant at harvest and the part of the plant used .Other

agents are also responsible, such as errors in the identification of the plant, an overdose and an interaction with other active ingredients that are natural (another plant, for example) or synthetic (drugs).

III.4.2. Anti-diabetic effect of extracts on the normal and diabetic rats

In several laboratories worldwide, work on medicinal plants represents a field of research of great importance, and this thanks to the richness of these natural remedies in therapeutic molecules, which can be at the origin of new drugs for many diseases.

Thus, several biological activities have been proven for medicinal plants, such as anticancer activity, antifungal activity, and antibacterial activity. We became interested in anti-diabetic activity and because of the extent of diabetes mellitus as a disease worldwide. This scourge requires the accentuation of research work to discover new effective and less expensive therapeutic solutions.

Algeria has a wealth of natural resources. Thousands of species of varied interests make up this potential of plant medical products, and they serve as an axis of scientific study, particularly in the field of active natural products. Among them, phenolic chemicals, and essential oils, have an important role in the treatment of a variety of diseases, including diabetes mellitus (Hamza *et al.*, 2010).

According to herbal medicine research conducted in the Laghouat region (west of Algeria), the herb *Asphodelus microcarpus and Bubonium graveolens* is widely utilized by the local community to cure otitis and diabetes respectively and which scientists have been widely interested in, and several of its biological activities have been studied. This, therefore, prompted us to experimentally demonstrate the hypoglycemic effect *Asphodelus microcarpus* extracts of the and *Bubonium graveolens* essential oils extracts. The present work investigated the anti-diabetic activity of these extracts from *Asphodelus microcarpus* and *Bubonium graveolens*, which according to our current knowledge, there are no experiments conducted on this activity of these extracts.

The anti-diabetic activity was evaluated in vivo in normal Wistar rats rendered diabetic by Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil), which is a hydrophilic compound that exists mainly in the monohydrate state, resulting from uric acid oxidation, long used for the induction of experimental diabetes in animals. It has a chemical structure similar to glucose because that Alloxan can pass the cellular membrane without additional action of specific protein transporters. Namely, it enters beta cells through GLUT2 transporters. Just after

pancreatic beta cells uptake Alloxan, The reduction of Alloxan to dialuric acid occurs in the cytosol. This reduction is due to some agents, such as reduced cysteine, glutathione, ascorbic acid, and protein-bound sulfhydryl (-SH) groups. Alloxan interacts with two-SH groups at the glucose binding domain of glucokinase that leading to the disulfide bond formation and the enzyme's inactivation. (Lenzen *et al.*,1988).

As a consequence of alloxan reduction, dialuric acid is produced, which is then re-oxidized back to Alloxan by forming a redox cycle for the generation of (ROS) and superoxide radicals. Superoxide radicals release ferric ions from ferritin and convert them to ferrous and ferric ions. Besides, superoxide radicals undergo dismutation to produce hydrogen peroxide (H_2O_2) in the existence of superoxide dismutase. As a consequence of the Fenton reaction, highly reactive hydroxyl radicals are composed principally of ferrous and H_2O_2. One other mechanism mentioned is the influence of ROS on the DNA of the pancreatic islets. DNA fragmentation occurs in beta-cells exposed to Alloxan that cause DNA damage, which activates poly-ADP-ribosylation, a mechanism that participates in DNA repair. (Rohilla *et al.*,2012).

Disturbances in intracellular calcium homeostasis have also been observed to constitute a significant factor in the diabetic action of Alloxan. It has been reported that Alloxan increases the concentration of cytosolic free Ca^{2+} in pancreatic beta cells. Calcium inflow is the product of Alloxan's ability to depolarize pancreatic beta cells, which further opens voltage-dependent calcium channels and increases calcium concentration in the pancreatic cells. The increased concentration of Ca2+ ion also contributes to the supraphysiological release of insulin which, along with ROS, has been shown to cause damage to pancreatic beta cells ultimately(Rohilla *et al.*,2012; Radenković *et al.*,, 2016). Moreover the degree and the severity of the diabetes depend mainly on the injected dose.

The procedure involves testing the effect of the extracts on the fasting blood sugar levels of normal and diabetic rats. After a 10-hour fast, the animal is in a post-absorptive state, any extract or molecule likely to lower blood sugar under these conditions must act by inhibiting hepatic and renal glucose production either directly or indirectly by release of insulin.

In diabetic rats, the significant absence of insulin, creates a permanent hyperglycemia, in this case any anti-diabetic molecule, must mimic the effect of insulin, acting by increasing the absorption of glucose at the level muscle and fatty tissue, and by inhibiting hepatic glucose production and the release of free fatty acids.

Extraction processes, made it possible to obtain extracts with a composition of secondary metabolites and different biological activities. In the following we will discuss the effect of each extract prepared from each plant on the blood glucose levels of rats according to the experimental procedure used.

To determine the effect of plant extracts on blood glucose in diabetic rats, we used two methods Short and long-term effects.

III.4.2.1.Anti-diabetic effect of *Bubonium graveolen*s essential oils extracts

III.4.2.1.1. Effect of the essential oils extract on blood glucose

*Bubonium graveolen*s essential oils extracts has been tested in normal rats and diabetic rats, with the first aim of confirming its anti-diabetic effect claimed in ethnobotanical studies.

A. Short term effect

The effect of essential oils extracts of *Bubonium graveolens* have been realised at different doses (100, 200 and 400 mg / kg bw) administered orally on the fasting blood glucose level of diabetic rats after 2 h.

For simplicity and in order to better comparison between the control group and the diabetic treated groups with essential oils extract , the histograms (figure III.1 et III.2) illustrates the results of the short-term effect of the essential oils of *Bubonium gravolens* on blood glucose.

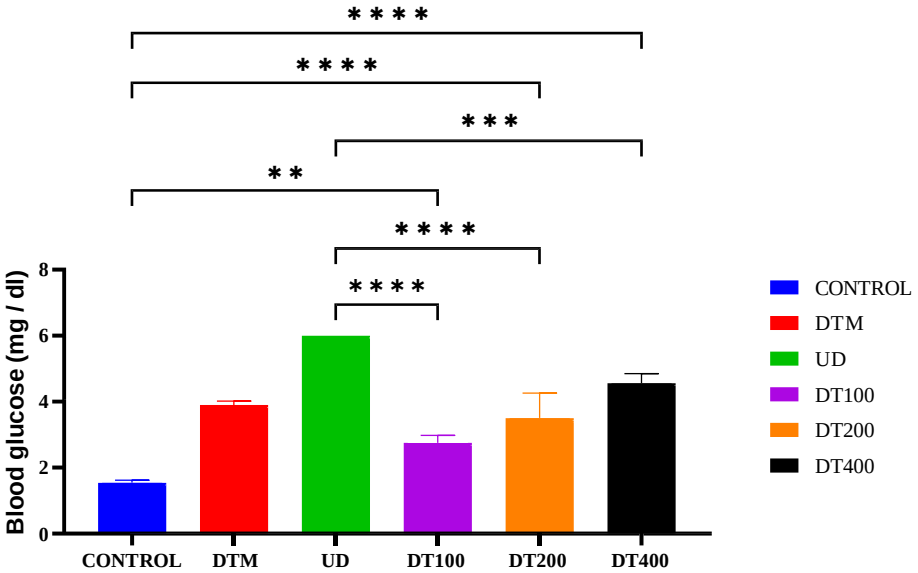


Figure III.1. Blood glucose variations in normal rats treated with *Bubonium graveolens* essential oils extracts. (Mean ± ESM, n = 6****.: p <0.0001.)

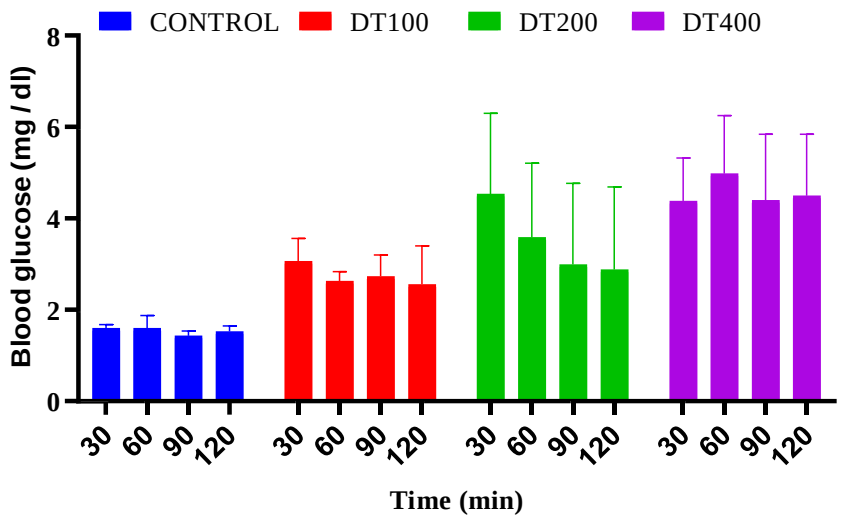


Figure III.2. Blood glucose variations as a function of time in normal rats treated with *Bubonium graveolens* essential oils extracts. (Mean ± ESM, n = 6***.: p <0.001.).

The study results, which represent the difference in glucose levels during fasting as a function of time, show that rats given doses of 100 - 200 mg/kg of essential oil extracts from *Bobonium graveolens* extracts had no significant reduction blood glucose, this value remained stable for 120 minutes after gavage. However, the oral intake of essential oils at a concentration of 200 mg/ kg bw resulted in a significant decrease in blood glucose levels in diabetic rats after 60 minutes of gavage compared to that in the controls receiving physiological water.

Natural chemicals and medicinal plants have been studied for their anti-diabetic properties in previous reviews. Extensive research screened *in silico* a library of over 2,300 compounds extracted from 30 common herb species, a rich source of anti-diabetic compounds such as flavonoids and alkaloids, *in silico* (Perreira *et al*., 2019). Another research found flavonoids, glycosides, and oligosaccharides to be the main active compounds in 111 medicinal plants that were documented to positively affect diabetes mellitus (Eddouks *et al*., 2014).

Our results corroborated some of the findings from these studies, demonstrating that α-amylase is an important molecular target not only for the phytochemical groups identified but also for essential oils, both of which are capable of lowering blood glucose levels in several experimental models.

According to the results of the studies described above, the majority of laboratory models used for assessing the anti-diabetic activity of essential oils were in vivo. Several essential oils were used to lower blood glucose levels and protect pancreatic beta cells from chemical aggression. Other methods used genetically modified laboratory rats, such as db/db mice, which may reproduce some of the pathological characteristics of diabetes mellitus. The administration of lemon balm essential oil partly corrected insulin resistance and obesity in these mice, which had a mutation in their leptin receptor (Chung *et al*., 2010).

This research led us to conclude that the hypoglycemic effect of essential oils extracts of *Bubonium graveolens* can be explained by an improvement in glucose tolerance in peripheral tissues in rats pretreated with these active substances. They were suggesting that these tissues use glucose very efficiently.

B. Long term effect

To better exploit the anti-diabetic effect of the essential oil extract from *bobonium graveolens* demonstrated in this first part, we undertook further experiments with the essential oil. Daily oral gavage for ten days of essential oil at 100, 200 and 400 mg/kg in diabetic rats was accompanied by a noticeable effect on fasting blood glucose levels, compared to that of control rats. Blood glucose was monitored for 28 days after oral administration of the essential oils extract from *Bubonium graveolens.*

For simplicity and in order to better comparison between the control group and the diabetic treated groups with essential oils extract , the histograms (figure III.3 and III.4) illustrates the results of the long-term effect of the essential oils of *Bubonium Gravolens* on blood glucose.

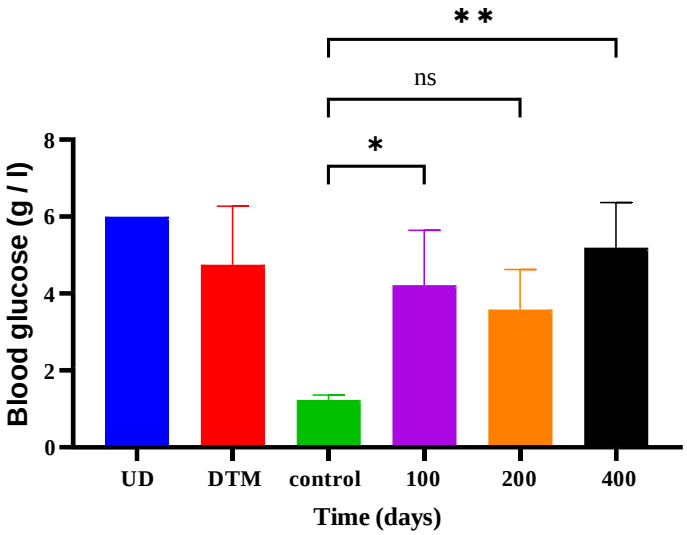


Figure III.3. The rate of change of blood glucose level during 10 days in diabetic rats treated by *Bubonium graveolens* essential oils extracts. The values are given as Mean ± E.S.M (n = 6). Student test: ** p <0.01 the difference is highly significant

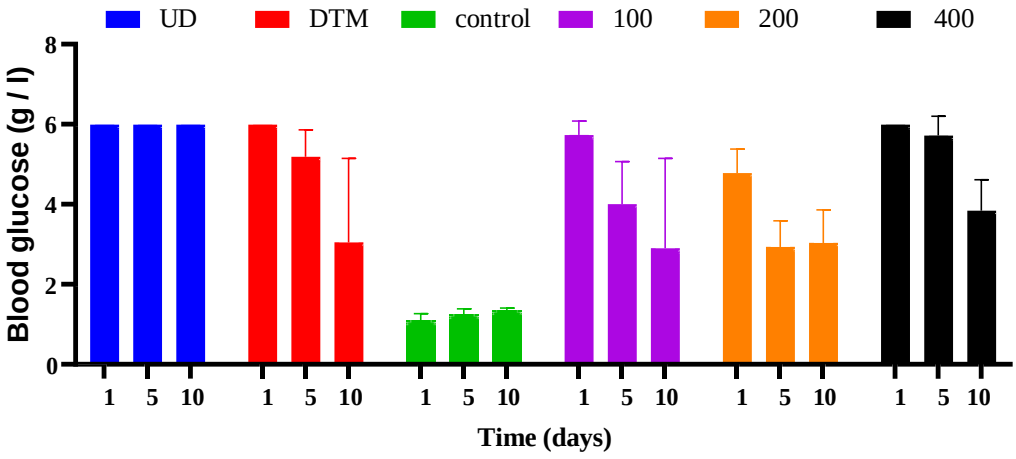


Figure III.4. Change in blood glucose concentration in diabetic rats during 10 days in diabetic rats treated by *Bubonium graveolens* essential oils extracts. The values are given as Mean ± E.S.M(n = 6).

Statistical analyzes showed a significant difference ($p < 0.01$) in control rats and the three treated groups (100, 200 and 400) which the blood glucose levels were ranged from 1.09 to 1.4 g/l during the 28-day- trial. No significant difference ($p < 0.01$) between the three diabetic treated groups with100, 400 doses and the untreated group (UD). On the other hand, the results showed a significant difference ($p < 0.01$) between in control rats and the treated groups with dose 200 and the untreated group (UD). Also, we noted that no significant difference ($p < 0.01$) was recorded between the three treated groups (100, 200, and 400).

The results show that in the untreated diabetic group, glucose levels are four times higher than those of the healthy control group. All concentrations of the essential oil extract of *Bubonium graveolens* showed varying effects on the blood glucose in diabetic rats. A decrease in blood glucose level started from the fifth day of gavage with essential oil in the groups treated with 100,200 mg/kg BW concentrations, while the treatment group with a 400 mg/kg BW did not show any change in blood glucose level until after ten days of treatment with essential oil. A drop in blood glucose level was also observed from the 5th day in the treated group with the reference medicine gluconova. The values obtained with the treatment with essential oil extract of *Bubonium graveolens* and gluconova are highly and / or very highly significant compared to the saline-treated control group ($p < 0.01$and $p < 0.001$).

The Treatment with the extract in 100 mg/kg BW showed a more effective drop in blood glucose (-50%) than the reference drug (-43.66%). Whereas treatment with the extract at 200 and 400 mg / kg of body weight showed a less effective reduction in blood glucose (-36.40%) (-36.00%) than the reference drug (-43.66%).

These results show the significant efficacy of *Bubonium graveolens* essential oils in the decrease in blood glucose level, which confirms that this medicinal plant can be considered as a potent agent in the treatment of diabetes (Telli *et al*. 2016). Previous studies have also proven the effectiveness of the aqueous extracts of this plant on anti-diabetic activity using an animal model of diabetes (El-Ouady *et al*. 2020). Moreover, in recent years, studies with other combinations of essential oils have shown more effective anti-diabetic activity. This potential effect was attributed to different mechanisms such as blockage of glucose absorption, enhanced insulin sensitivity, and lowered circulating glucose levels (Talpur, *et al*. 2005).In addition to the effect of essential oils on blood glucose levels, studies indicate that using of the essential oils in Aromatherapy reducing nerve pain and improves the quality of life in patients suffering from painful diabetic neuropathy (Gok Metin, *et al*. 2017).

III.4.2.1.2 Effect of the essential oils extract on body weight

In this experiment, we also followed the effect of the EA extract, administered doses of 100,200 and 400 mg/kg/day, on the evolution of body weight and the quantities of food and water consumed by diabetic rats. The results are shown in Table III.11.

The results showed the average body weight gain observed in the C and DTM groups, where the percentage of overweight was estimated at + 39.17% and +28.78%. In addition, untreated group had undergone during the same period a regular stability of body weight. In groups diabetics, administration by gavage of the essential oils extract from *Bubonium graveolens* at the dose 400 mg/kg daily for one week induced a very significant change in body weight(-8.54%) compared to the diabetic control group ($p < 0.001$).According to Table **V.2**, a decrease in weight is observed also in the group diabetic treated with DT100 and DT200 with percentage of weight loss -11.00% and -5.35% during the 10 days of experimentation. the reduction in weight in these groups can be explained by the catabolic processes such as glycogenolysis, lipolysis, and proteolysis (Prabhakar *et al*., 2008).

Table III.5. Effect of *Bubonium graveolens* essential oils extract on body weight.

Lots	**Dose /kg mg**	**Body weight g (Growth of rate in%)**		
		Days		
		1	**5**	**10**
C		149.76 ± 8.85	180.2 ± 14.80 + 20.32 %	208.43 ±16.30 + 39.17 %
UD		192.8 ± 5.63	207 ± 17.53 + 7.36 %	198.6 ± 12.89 + 12.89 %
DTM	**5**	185.08 ± 35.63	201.4 ± 21.55 + 8.81 %	238.36 ±35.49 + 28.78 %
DT *Bubonium graveolens*	**100**	287.66 ± 66.00	269.33 ± 14.57 - 6.37 %	256 ±18.35 - 11.00 %
	200	261.66 ± 7.37	256 ± 3.00 - 2.16 %	247.66 ±4.61 - 5.35 %
	400	288.66 ± 9.45	288 ± 8.18 - 0.2 %	264 ± 10.53 - 8.85 %

III.4.2.1.3.Effect of the essential oils extract on the various biochemical parameters

A biochemical analysis was carried out to see if the essential oils extract had any impact on the liver, kidneys, or glucose metabolism. A number of factors were tested, including aspartate aminotransferase, alanine aminotransferase, creatinine, uric acid, glucose, triglycerides, and cholesterol. The liver and kidneys are known to play important roles in a variety of metabolic processes. The liver is engaged in glucose metabolism, and the kidneys are the principal organs engaged in medication clearance, hence they are particularly vulnerable to exogenous chemical toxicity (Bidhe *et al*., 2004).

The results of the influence of essential oils extract from *Bubonium graveolens* on the lipid profile in healthy rats and rats made diabetic with alloxan for 10 days are summarized in Table III.10. In groups of rats diabetics (untreated and treated with essential oils and Gluconova), we found that the injection of alloxan caused a very highly increase in concentration serum of total cholesterol and triglycerides compared to the healthy control group. However, daily administration of essential oils at doses of 100, 200,400 mg/kg, Was not reduced serum concentration of total cholesterol and triglycerides. On the other hand, the results showed an affinity with the group treated with Gluconova. These results agree with those published by (Ravi *et al.*, 2005; Sharma *et al.*, 2008), where they suggest that the high abnormal concentration of lipids serum observed in diabetic subjects is mainly due to the increase in mobilization of fatty acids from adipose tissue.

The results obtained also show an apparent increase in the concentration serum in urea and creatinine which are important markers of renal failure (Jarald *et al*. 2008). This is because proteins can be divided into amino acids and then into urea and creatinine. Our results indicate a decrease in creatinine and urea concentration after taking essential oil extracts, similar to the drug-treated and control groups, and this is indicative of renal dysfunction induced by the hyperglycemia that accompanies diabetes

Table III.6. Measurement results for the various biochemical parameters assayed after the sacrifice of the rats administered with *Bubonium graveolens* essential oils extract.

Settings	**Lots**					
	C	**UD**	**DTM**	**DT 100**	**DT 200**	**DT 400**
Glucose (mg/ dl)	1.2 ± 0.07	2.59 ± 1.84	2.69 ± 1.84	5.25 ± 1.12	1.14 ± 2.77	2.5 ± 0.71
Cholesterol (mg/ dl)	0.46 ± 0.07	0.63 ± 0.17	0.61 ± 0.03	0.85 ± 0.20	0.69 ± 0.04	0.63 ± 0.11
Triglycerides (mg/ dl)	0.85 ± 0.07	0.86 ± 0.40	1.06 ± 0.82	1.44 ± 0.20	1.14 ± 0.64	1.38 ± 0.47
TGO (Ul/L)	150.6 ± 16.5	227 ± 36.94	189.2 ± 63.66	159 ± 90.30	116.66 ± 30.17	140 ± 3.46
TGP (Ul/L)	39.6 ± 7.03	64.33 ± 2.80	91.5 ± 45.62	104.25 ± 64.79	219.66 ± 69.61	62.33 ± 132.34
Urée (mg/dl)	0.53 ± 0.04	2.39 ± 0.00	ND	0.76 ± 0.03	0.77 ± 0.37	0.59 ± 0.11
Creatinine (mg/dl)	4.06 ± 0.85	8.58 ± 0.50	3.91 ± 1.07	5.30 ± 0.48	6.82 ± 2.89	5.04 ± 0.72
Uric Acid	14.64 ± 2.28	21.47 ± 7.21	14.25 ± 3.70	13.02 ± 3.87	25.30 ± 23.03	16.46 ± 5.48

In terms of enzymatic parameters, we found that untreated diabetic rats had higher activity of transaminases (TGO and TGP) than the healthy rat's group, which explains the accumulation of amino acids such as alanine and glutamate in serum from the degradation of compounds protein in the body. As a result, these amino acids can be transformed under the action of serum transaminases to carboxylic compounds such as α-ketoglutarate and pyruvate. This means that TGO and TGP have high enzymatic activity. The hepatotoxic effect of alloxane can also explain this, where the liver cells of diabetic rats, are irreversibly destroyed, causing the TGO and TGP enzymes to be released into the blood. In contrast, the treatment of rats diabetics with essential oils extract from *Bubonium graveolens* restored values close to normal.

III.4.2.2. Effect of the *Asphodelus microcarpus* aqueous extracts

III.4.2.2.1. Effect of the aqueous extract on blood glucose

A. Short term effect

In this experimental procedure, we performed oral gavages of aqueous extract of *Asphodelus microcarpus* at various doses (100, 200, and 400 mg/kg BW) in diabetic rats and blood glucose changes were tracked every half hour until two hours after oral gavage.

The effect of aqueous extract of *Asphodelus microcarpus* at various doses (100, 200, and 400 mg/kg BW) administered orally on the fasting blood glucose level of normal rats are shown in Figures III.09 and III.10.

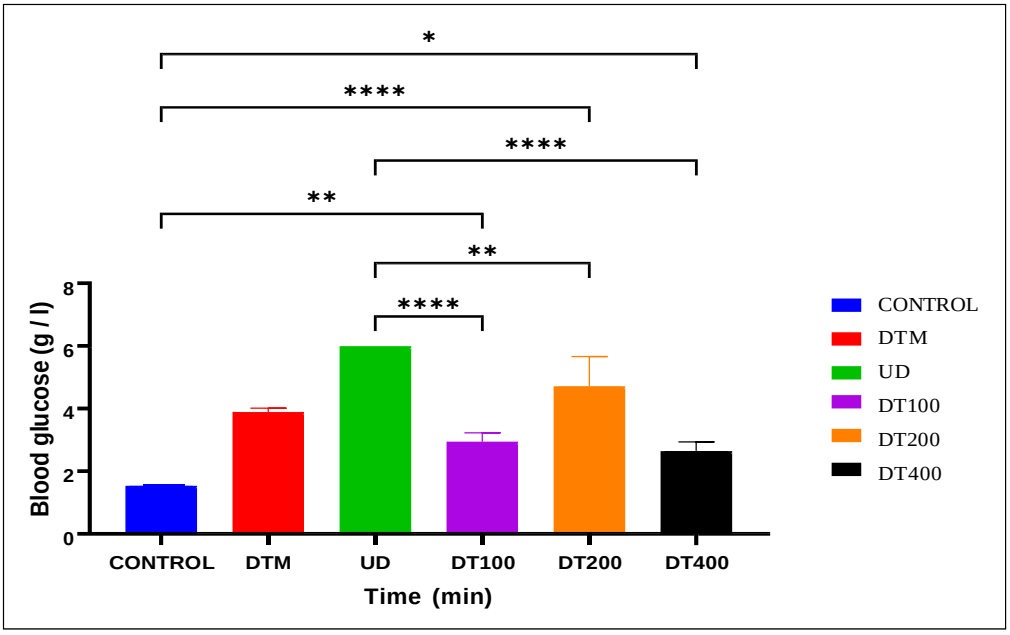


Figure III.5. Blood glucose variations in normal rats treated with *Asphodelus microcarpus* aqueous extracts. (Mean ± ESM, n = 6***.: p <0. 001.)

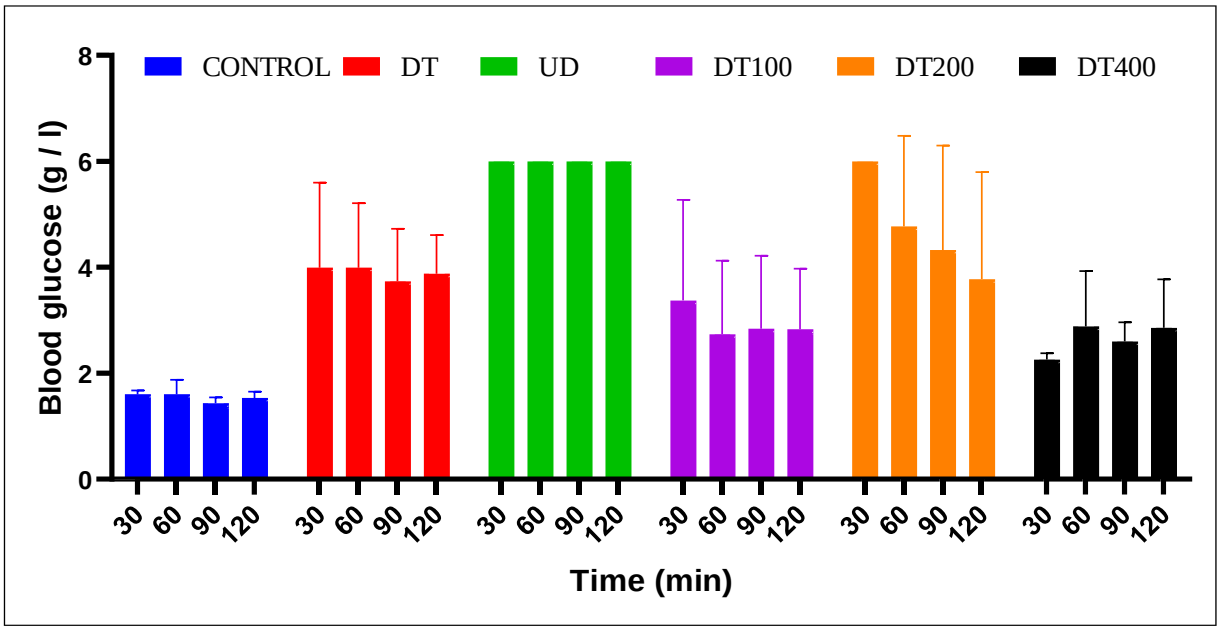


Figure III.6. Blood glucose variations as a function of time in normal rats treated with *Asphodelus microcarpus* aqueous extracts. (Mean ± ESM, n = 6***.: p <0.001.).

The experimental results showed significant changes in blood sugar levels in treated rats with aqueous extract of *Asphodelus microcarpus*. Concentration 200 was shown to effectively lower blood sugar levels over time, with the impact beginning 60 minutes after oral gavage and lasting up to two hours. The treated groups at concentrations of 100 and 400 showed stability in blood sugar for the duration of the experiment.

On the other hand, in the treated rats with *Asphodelus microcarpus* aqueous extract, the blood glucose level is low with significant values compared to the blood glucose level in the untreated rats or those treated with the Gluconova. The effectiveness of the aqueous extract on blood glucose may be due to secondary metabolites such as include alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycan, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. These affect various metabolic cascades, which directly or indirectly affect the level of glucose in the human body which studies have proven its high ability to reduce blood sugar levels through many mechanisms.

Studies have shown, for example, flavonoids, terpenoids, which increase insulin secretion, regulating the gluconeogenolysis pathway. improve tissue absorption of glucose, regulate the Krebs Cycle, which is very important in glucose metabolism (Prabhakar *et al*, 2008).and since the aqueous extract contains most of these secondary metabolites, one of the mechanisms mentioned above may be responsible for lowering the glucose level in Blood in treated rats.

B. Long term effect

Daily oral gavage for ten days of aqueous extracts at 100, 200 and 400 mg/kg in diabetic rats was accompanied by a noticeable effect on fasting blood glucose levels, compared to that of control rats. Blood glucose was monitored for 28 days after oral administration of *Asphodelus microcarpus* aqueous extract.

For simplicity and in order to better compare between the control group and the diabetic treated groups with essential oils extract , the histograms (figure III.11 and III.12) illustrates the results of the short-term effect of aqueous extract of *Asphodelus microcarpus* on blood glucose.

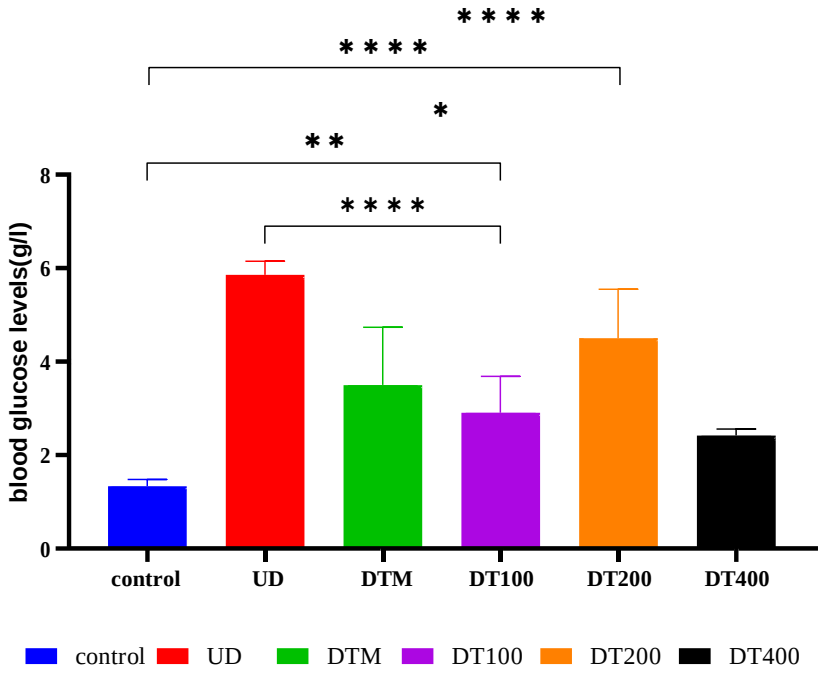


Figure III.7. The rate of change of blood glucose level during 28 days in diabetic rats treated by *Asphodelus microcarpus* aqueous extract. The values are given as Mean ± E.S.M (n = 6). Student test: *** p <0.001 the difference is highly significant

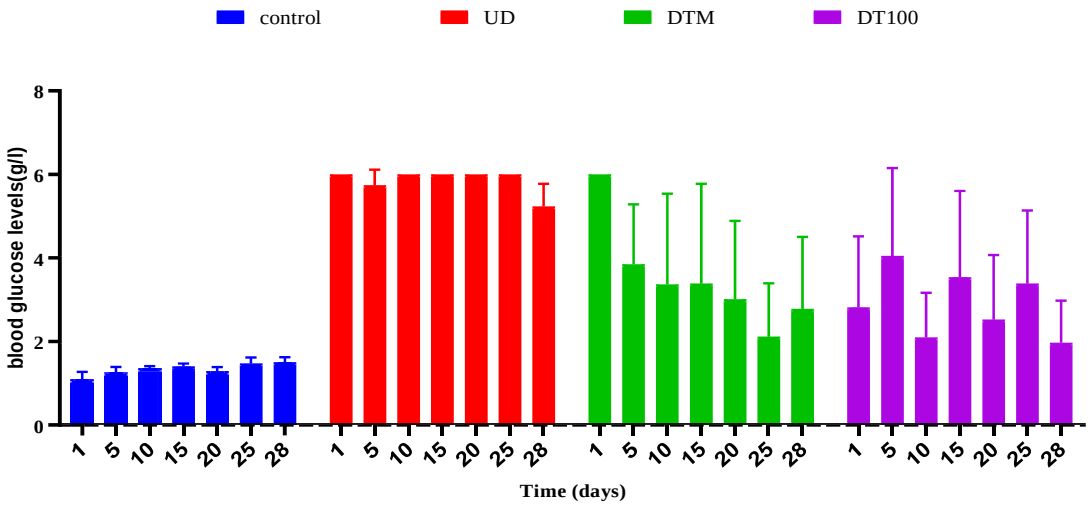


Figure III.8. Change in blood glucose concentration in diabetic rats treated with dose of 100 mg/kg of *Asphodelus microcarpus* aqueous extract during 28 days. The values are given as Mean ± E.S.M (n = 6).

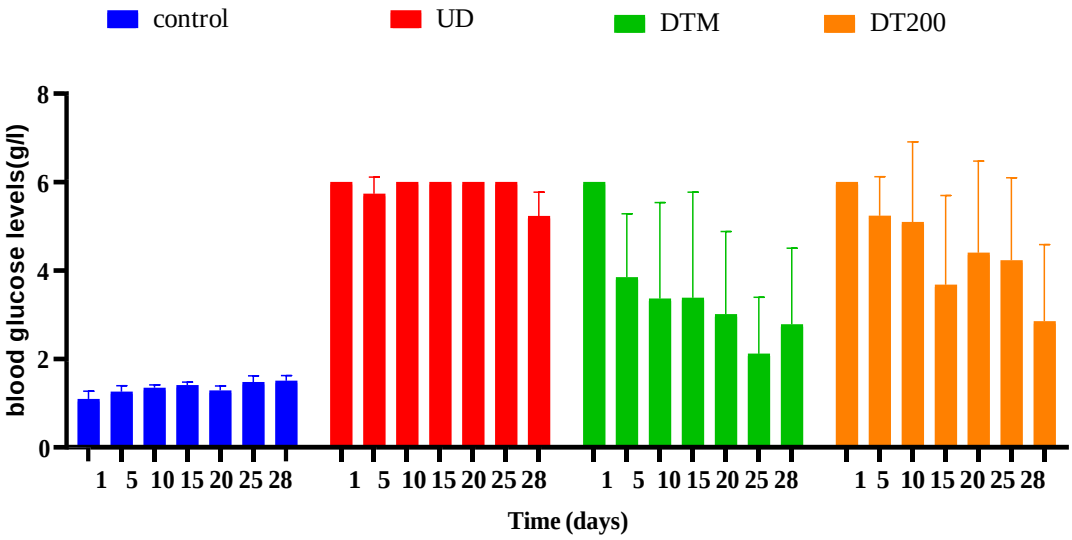


Figure III.9. Change in blood glucose concentration in diabetic rats treated with dose of 200 mg/kg of *Asphodelus microcarpus* aqueous extract during 28 days. Thevalues are given as Mean ± E.S.M (n = 6).

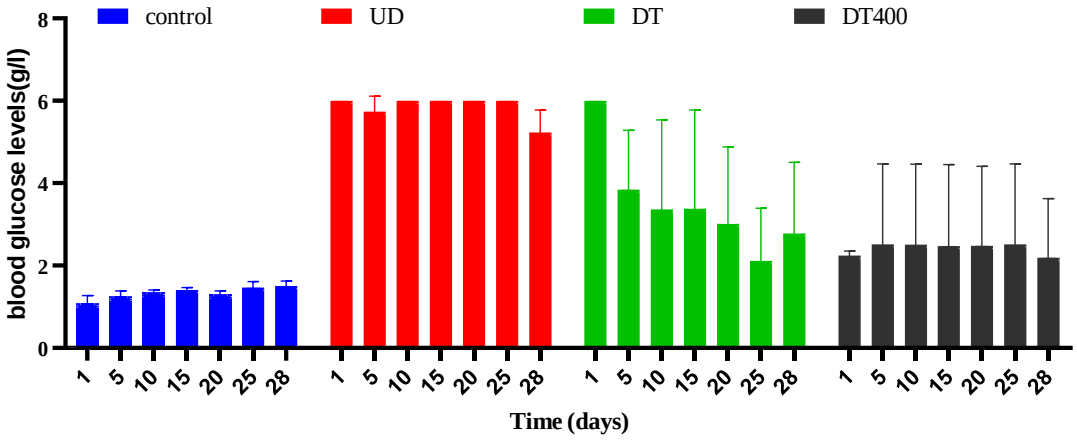


Figure III.10. Change in blood glucose concentration in diabetic rats treatment with dose of 400 mg/kg of *Asphodelus microcarpus* aqueous extract during 28 days. The values are given as Mean ± E.S.M (n = 6).

In diabetic rats, regular oral administration of *Asphodelus microcarpus* extract at doses of 100, 200, and 300 mg/kg for 28 days resulted in a significant reduction in fasting blood glucosein diabetic rats. The results show that glucose levels in the chronic diabetic group are

2 to 3 times higher than in the control group. Blood glucose levels in diabetic rats began to stabilize on the fifth day after treatment with *Asphodelus microcarpus* at dose of 400 mg/kg BW and in the treated group with the reference medication gluconova. These results indicate the efficacy of infused with *Asphodelus microcarpus* in reducing blood glucose levels, suggestingthat this herbal medication may be considered as a good agent in the treatment of diabetes.

This effect is probably due to the variety in composition of the species. Significant variety of secondary metabolites responsible for the biological activities were produces by *A. microcarpus*, including essential oils, flavonoids, phenolic acids, terpenoids, anthraquinones (in free form or as a glycoside), and alkaloids (Zellagui *et al.*, 2013; Malmir *et al.*, 2018).

Also, the aerial part of this species includes flavonoids like luteolein isovitexine, and isoorientine, phenolic acids, and a few anthraquinones (Malmir *et al*. 2018). All these families of secondary metabolites are well classified among the natural molecules with interesting anti-diabetic power (Chen *et al.*, 2015).

Luteolin, a flavone type of flavonoid isolated from *Asphodelus microcarpus*, possesses various pharmacological activities and has been proved to exert its beneficial effects in several experimental disease models. Luteolin exhibits appreciable anti-diabetic potential, which has been well studied. Several mechanisms of action have been proposed, such as improving insulin sensitivity by influencing the Akt2 kinase.31 Akt2 prevents the dephosphorylation of the insulin receptor and thus prevents attenuation of the insulin-signaling process. Akt2 is also responsible for the regulation of uptake of glucose, and the translocation of GLUT4 glucose transporter mediates this effect to the surface of the cell (Sangeetha, 2019).

In addition, a study has shown that isovitexine has a high inhibitory potential against certain enzymes that contribute to the development of complications in diabetic patients. For example, ddose reductase (AR) in the polyol pathway is abnormally activated during chronic hyperglycemia, resulting in excessive sorbitol formation in various tissues, including lenses and nerves, leading to diabetic complications such as neuropathy, retinopathy, and cataracts (Kawanishi *et al*., 2003; Santiago *et al*, 1993; Feldman *et al*., 1997).

In addition to the increased flow of the polyol pathway, the concentration of AGE in prolonged hyperglycemia has been considered to play a major role in the pathogenic mechanism of diabetes and diabetic complications (Ahmed, 2005). On the other hand,PTP1B,

a non-transmembrane enzyme found in human tissues, is a prototype of the family of protein tyrosine phosphatases. It has been considered to be a key regulator of intracellular insulin signaling, which dephosphorylates both activated insulin receptors and insulin receptor substrates (Asante *et al*, 2003; Johnson *et al*., 2002).

The inhibition of AR, AGEs formation and PTP1B is involved as important therapeutic approaches for the treatment and prevention of diabetes. isovitexine, has shown promising results antidiabetic potential by inhibiting the formation of RLAR, HRAR, AGE and PTP1B. *Asphodelus microcarpus* extracts contains flavonoids such as quercetin and kaempferol, which showed an anti-diabetic effect explained by the stimulation of glucose transport induced by insulin in mature adipocytes, and by their effect as agonists of PPARs (Fang *et al.*, 2008).

The anti-diabetic action of *Asphodelus microcarpus* aqueous extract, is due to the abundance of alkaloids, it showed improved glucose tolerance in DB / DB mice and increased insulin action in rats (Lee *et al.*, 2006). On the other hand, trigonelline, which is the principal alkaloid isolated from *Trigonella foenum-graecum*, its anti-diabetic effect is explained by regeneration of β-pancreatic cells and an increase in insulin secretion (Zhou *et al.*, 2012). Steroid alkaloids isolated from *Veratrum nigrum* have been studied in vitro and have improved peripheral glucose transport in skeletal muscle cells (Kang *et al.*, 2015).

III.4.2.2.2. Effect of the aqueous extract on body weight

Energy is required by all tissues, which is normally supplied by metabolizing glucose. Insulin promotes the absorption of glucose from the blood into cells, the liver, skeletal muscle, and adipose tissue (Prabhakar *et al.*, 2008).

In diabetic patients, insufficient insulin prevents the body from getting glucose from the blood into the body's cells to use as energy. When this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight. a good indicator for detecting side effects of drug abuse (Raza *et al*, 2002). It also plays an important role in knowing the extent and development of diabetes.

The results presented in Table III.13 show changes in weight body among diabetic and normal rats**.**

The results obtained in our study showed that the injection of alloxan-induced diabetes was characterized by a significant loss of body weight in the untreated group of diabetic control

rats. This decrease is in the order of +8.07%, 3.68+%, -0.28%, and -7.69% after each week of treatment. In addition, the healthy control group had undergone during the same periods a regular increase of 43.42%, +65.88%, +77.08%, and +93.28%.

Table III.7. Effect of *Asphodelus microcarpus* aqueous extract on body weight.

Lots	**Dose /kg mg**	**Body weight g (Growth of rate in%)**				
		days				
		1	**5**	**10**	**20**	**28**
C		149.76 ± 8.85	214.80 ± 14.86	248.43 ± 15.85	265.20 ± 15.2	288.10 ± 12.08
UD		191.54 ± 5.98	207.00 ±17.53	198.60 ± 12.89	191.00 ± 10.90	176.80 ± 21.94
DTM	**5**	185.00 ± 20.03	201.40 ± 19.28	240.36 ± 29.97	253.00 ± 38.60	258.00 ± 41.04
DT *Bubonium graveolens*	**100**	165.45 ± 4.87	168.75 ± 33.42	199.25 ± 29.48	219.75 ± 39.15	225.75 ± 44.45
	200	173.20 ± 12.91	221.00 ± 35.40	222.80 ± 37.96	236.80 ± 48.32	243.40 ± 46.18
	400	164.62 ± 6.59	211.20 ± 10.00	223.60 ± 7.98	244.60 ±8.50	258.80 ± 12.04

However, in the diabetic group treated, gavage of at daily doses of 100, 200 and400 mg/kg for four weeks allowed improving the change in body weight compared to the diabetic group control. Significant decrease in the untreated diabetic group because, during diabetes, the weight reduction is due to catabolic processes such as glycogenolysis, lipolysis, and proteolysis (Prabhakar *et al.,* 2008).

As we know Diabetes mellitus is associated with various disorders of the locomotor system including the decline in mass and function of skeletal muscle. The mechanism underlying this association has remained ambiguous, however. Now many studies show that the abundance of the expression of genes related to muscle atrophy are increased in skeletal muscle of diabetic model mice, and that mice with low expression of this genes are protected from the diabetes-induced decline of skeletal muscle mass. hyperglycemia, a central disorder in diabetes, promotes muscle atrophy via this pathway. (Hirata, *et al*. 2019). The efficacy of our

extract may be related to improving the weight of mice through this pathway because this pathway may serve as a therapeutic target for decline in skeletal muscle mass accompanied by diabetes mellitus. These results also lead us to conclude the absence of any harmful effects of our extract.

III.4.2.2.3 Effect of the aqueous extract on the various biochemical parameters

In diabetic rats, a biochemical analysis was done to evaluate the effect of our extract on liver, kidney, and glucose metabolism. Several factors were investigated, including aspartate aminotransferase, alanine aminotransferase, creatinine, uric acid, glucose, triglycerides, and cholesterol. The liver and kidneys are well-known for their functions in several metabolic processes. The liver is engaged in a xenobiotic role, and the kidneys are the principal organs involved in drug elimination. Hence they are particularly vulnerable to exogenous chemical toxicity (Bidhe *et al.*,2004) TGO and TGP are well-known serum enzymes used as biomarkers to predict potential toxicity and as indicators of liver function (Rahman *et al.*, 2001). When hepatocytes or their cell membranes are damaged, the transaminases TGO and TGP seep into the bloodstream. TGP is a more specific marker of hepatocellular injury because it occurs exclusively in the liver, whereas TGO occurs to some extent also in the heart, skeletal muscle, kidney, brain, pancreas, and blood cells (Kew *et al.*, 2000).

After oral and daily administration of the *Asphodelus microcarpus* aqueous extract at 100, 200 and 400 mg/kg for 28 days, we observed an increase in the activity of transaminases (TGO and TGP) in the serum of untreated diabetic rats to that of the control. This explains the build-up of amino acids like alanine and glutamate in serum from the breakdown of protein compounds in the body. As a result, these amino acids can be transformed under the action of serum transaminases into carboxylic compounds such as α-ketoglutarate and pyruvate. This then implies a strong enzymatic activity of TGO and TGP. The hepatotoxic effect of Alloxane can also explain this (Abd Eldaim, *et al.*, 2017).

Therefore, no significant changes in TGO and TGP activities in diabetic mice treated with the plant extract suggest that the *Asphodelus microcarpus* aqueous extract administration did not alter hepatocyte function.

The liver is where cholesterol is disposed of or degraded, as well as where it is synthesized. Similarly, the liver regulates glucose production and produces free glucose from hepatic glycogen reserves (Li *et al.*, 2010).Diabetes mellitus is also associated with hyperlipidemia

and causes profound abnormalities in the concentration and composition of plasma lipids(Sirois *et al.,* 2015). These abnormalities are an important risk factor for cardiovascular disease and coronaries. And for that, we try to find out the effect of the plant extract on the cholesterol and triglyceride values. We measured them in diabetic mice treated with the aqueous extract.

The effects of the *Asphodelus microcarpus* aqueous extract on the lipid profile in control and diabetic rats treated for 28 days are summarized in Table III.14.. We observed that injecting alloxan-induced a very highly significant rise in blood total cholesterol content in diabetic rats untreated, treated with dose 100 mg/kg BW and gluconova but no significant change in controls and the treated goups with dose 200,400 mg/kg BW. However, daily administration of the the aqueous extract Asphodelus microcarpus has not reduced the concentration of triglycerides in the blood compared to the control group.

It remained high in the treated groups with the extract compared to the control group. These results indicate that the *Asphodelus microcarpus* aqueous extract can lower cholesterol in the blood, which may be due to the extract's content of flavonoids, which many studies have proven to be great at lowering cholesterol in the blood. (Rigelsky and Sweet 2002). There was also a significant change in uric acid and creatinine between the treated and control groups. Indeed, uric acid and creatinine are considered important markers for kidney dysfunction (Mukinda *et al.*,2007; Gnanamani *et al.,* 2008).

Creatinine is a nitrogenous end product of metabolism removed from the blood by the kidneys. It is the most commonly used clinical serum biomarkers of renal damage (Hayes, 2001). Any rise in creatinine levels is only observed if there is marked damage to functional nephrons (Van Biesen *et al*., 2006). Thus, the results recorded in this study suggest that the *Asphodelus microcarpus* aqueous extract with 400 alters the values of uree and uric acid compared to control and untreated groups.

Table III.8. Measurement results for the various biochemical parameters assayed after the sacrifice of the rats treated with the aqueous extract of *Asphodelus microcarpus*.

Settings	Lots					
	C	**UD**	**DTM**	**DT 100**	**DT 200**	**DT 400**
Glucose (mg/ dl)	1.2 ± 0.07	2.59 ± 1.28	2.69 ± 1.84	4.18 ± 2.69	3.39 ± 2.5	2.03 ± 1.84
Cholesterol (mg/ dl)	0.46 ± 0.07	0.63 ± 0.17	0.61 ± 0.03	0.66 ± 0.12	0.55 ± 0.07	0.59 ± 0.04
Triglycerides (mg/ dl)	0.85 ± 0.07	0.86 ± 0.4	1.06 ± 0.82	1.26 ± 1.00	0.81 ± 0.26	ND
TGO (Ul/L)	150.6 ± 16.5	227 ± 36.94	189.2 ± 63.66	195.5 ± 87.88	154 ± 44.95	132.2 ± 68.43
TGP (Ul/L)	39.6 ± 7.03	64.33 ± 2.8	91.5 ± 45.62	97.25 ± 51.56	78.6 ± 20.05	58 ± 35
Urée (mg/dl)	0.53 ± 0.04	2.39 ± 0	ND	ND	0.6 ±0	0.44 ± 0.065
Creatinine (mg/dl)	4.06 ± 0.85	8.58 ± 0.5	3.91 ± 1.07	5.87 ± 0.81	6.98 ± 3.28	8.64 ± 0.45
Uric acid	14.64 ± 2.28	21.47 ± 7.21	14.25 ± 3.7	17.48 ± 4.64	13.88 ± 5.72	12.21 ± 1.82

III.4.2.2.4. Effect of the aqueous extract on the amounts of food and water consumed.

Diabetic's tissues are unable to absorb glucose properly, causing it to accumulate in the blood. Osmotic forces are activated as blood glucose concentrations rise, increasing blood volume and urine output (polyuria). When blood glucose levels rise above the renal threshold (i.e., 180 mg/dl), glucose is excreted in the urine (glucouria). This results in increased water loss from the body, which initiates a compensatory adjustment that increases thirst (polydipsia) (Prabhakar *et al.*,2008).

The management of diabetes without any side effect is still a challenge to the medical system. To better understand the effect of the aqueous extract on water consumption by diabetic rats, we measured the volume of water consumed during the experiment. The results are shown in the table III.15.

During the 28 days of the treatment of normal and diabetic rats with the *Asphodelus microcarpus* extract, we made daily measurements of the animals quantities of food and water consumed. No change was observed in the consumption of food in diabetic rats and the

control group. Whereas, the volume of water consumed was variable from one group to another.

In diabetic rats treated with the *Asphodelus microcarpus* extract or treated with gluconova, water consumption became almost two times greater than that recorded in normal rats. We observed that a group of diabetics treated with doses 100, 200 and 400 mg/kg has no difference with the control group. Also, Rats treated with the *Asphodelus microcarpus* extract were observed to urinate more frequently than in untreated rats, confirming the ability of this plant to increase urine flow (Sarri *et al.*, 2014).

Table III.9. The amounts of water consumed by normal and diabetic rats treated by the *Asphodelus microcarpus* aqueous extract

Lots	**Dose kg/mg**	**The amounts of water consumed mL/100g/24h**			
		Days			
		1	7	14	21
C		150	190	259	280
UD		350	550	1030	880
DTM	**5**	150	170	600	360
DT *Asphodelus microcarpus*	**100**	400	250	280	310
	200	450	350	250	250
	400	320	220	300	295

In conclusion, the assay of blood parameters for the two extracts showed an elevated level of TGO, TGP in diabetic rats. This correlates well with the complications that result from the action of alloxan. Studies have shown that liver cells in diabetic rats treated with alloxan are irreversibly destroyed, causing the TGO and TGP enzymes to be released into the blood. Likewise, urea and creatinine levels are elevated in, and this is indicative of renal dysfunction induced by the hyperglycemia that accompanies diabetes.

It is quite clear that our extracts have not succeeded in correcting the blood disorders induced by alloxan.

Based on the results of the experiments performed and their discussion, we suggest that three classes of secondary metabolites may be responsible for the anti-hypoglycemic effect observed in our extracts; alkaloids, flavonoids, and essential oils and most likely work synergistically.

On the other hand, these molecules can act by inhibiting the intestinal transport of glucose, or by stimulating the secretion of insulin, or by stimulating the peripheral use of glucose.

From what has been studied above, we can conclude that the aqueous extract of *Asphodelus microcarpus* showed significant effectiveness in reducing the blood glucose level as a function of time compared to the essential oil extract *Bubonium graveolens*. Which also showed reduced efficacy of the blood glucose level after about 60 minutes of oral gavage. No significant effect on the glycemic level was observed in the long term in mice treated with essential oil extract for ten days. On the contrary, rats treated with aqueous extract of *Asphodelus microcarpus* showed stability in blood sugar during 28 days of the experiment. So the aqueous extract of *Asphodelus microcarpus* may be used to regulate blood glucose levels.

This study proved for the first time the anti-diabetic effect of the plant *Asphodelus microcarpus* and *Bubonium graveolen*s essential oils.

CONCLUSION

Although, the great strides that have been made in the understanding the pathophysiology of diabetes and management of diabetes, the disease associated complications are increasing. Despite the presence of known anti-diabetic medicine on the pharmaceutical market, therapeutic remedies from medicinal plants are used with success to treat this disorder and its ramifications. Medicinal plants derived drugs and herbal preparations are often considered to be less toxic and free from side effects than synthetic ones. Most of the worldwide available medicinal plants, the effective treatment of diabetes with bioactive phytochemicals has not been scientifically validated which may support their substitution for the current therapeutics. Based on the WHO recommendations, anti-hyperglycemic agents of natural plant origin used in traditional medicine are important. The attributed anti-diabetic potential of herbal plants is due to their ability to restore the function of pancreatic tissues by causing an increase in concentration of insulin. The research for alternate remedies (from the plant kingdom) for diabetes mellitus will continue all over the world as the disease poses many challenges not only to the physician, but also to the researcher.

In this context, the main objective of this study was the biological activities evaluation of three medicinal plants. The selection of these plants was based on how the local population used them. The ethnobotanical survey results were used to perform the pharmacological examination.

The current study was carried out to evaluate *in vitro* and *in vivo* anti-diabetic effects of aqueous, methanolic, chloroformic, and ethyl acetate extracts prepared from *Asphodelus microcarpus*, *Bubonium graveolens* and *Haplophyllum tuberculatum*. Antioxidant activity of aqueous and organic extracts from the selected plants was also studied *in vitro* by the main of different methods.

This study showed that extracts from the selected plants contained varying amounts of total phenolic compounds. Aqueous extract obtained with diffusion method had the highest amount of polyphenols and flavonoids. The abundance of active ingredients makes the three plants with extraordinary pharmacological properties, which could justify their various medicinal indications and traditional therapeutic use.

The phytochemical tests showed the presence of phenols and flavonoids in all of the plants' aerial parts. We estimated the quantities of total phenols and flavonoids in the extracts of these plants, results showed various values ranged between 0. 2 and 11.97 mg gallic acid

equivalent per gram of dry matter and 0.0015 to 10.96 mg quercetin eq per gram of dry matter, respectively.

The evaluation of the antioxidant power of the extracts and several commercial antioxidants taken as references (gallic acid, Quercetin, and Vitamin C) by four chemical tests in vitro: DPPH, ABTS, FRAP, and CUPRAC tests, detected exciting antioxidant activities in the studied plants with IC_{50} values varying between 0.02 to 6.47 mg/ml for the DPPH test and 0.11to 21.52 mg/ml for the ABTS test, CUPRAC values ranging from 0.0013 to 0.046 µmol equivalent vitamin C/g Dry weight. While the FRAP values ranged from 0.05 to 236.95 µmol equivalent vitamin C/g Dry weight.

Most of the extracts inhibited α- amylase activity with the highest percentages reported for essential oils of *Bubonium graveolens* and aqueous extract of *Asphodelus microcarpus*. The values of IC_{50} vary between 0.05 and 82.01 mg / ml.

The toxicity analysis showed that the aqueous and essential oils extracts of *Asphodelus microcarpus* and *Bubonium graveolens* did not cause any fatalities or other toxicity symptoms. Acute toxicity testing revealed that the plants aqueous extract is safe up to a dosage of 5 g/kg of rats body weight when administered orally.

In vivo test indicate that aqueous extracts of *Asphodelus microcarpus* have significant anti-diabetic efficacy, with blood glucose decreases varying from -1.8 percent to -45.50 percent. This reduction is less potent than gluconova (Acarbose) (-79.66 percent). As compared to the results of control diabetic rats, specific biochemical parameters suggestive of metabolic anomalies have been virtually returned to typical values. Our results have also shown the ability of the essential oils of *Bubonium graveolens* to reduce the level of blood glucose by a ratio between -36 to-50 percent. This decrease is more potent than or equal than gluconova (Acarbose) (-43.66 percent).

This research added new ethnopharmacological and phytochemical knowledge about local plants in the Laghouat area, highlighting the importance of natural polyphenols in managing oxidative stress and glycemic normalization disorders.

This study opens up experimental perspectives in the future that should to allow to identify the active molecules that could be responsible for the antioxidant and antidiabetic effects of the studied plant by the combination of chromatographic and spectroscopic techniques which

will allow us to specify the metabolic functions and to make a good study of the chemical structure relationship of the inhibitor and function of enzymes. An attempt to study the antioxidant efficacy of plant extracts *in vivo* and to determine how they affect the organism. Determine the clinical importance of bioactive molecules in the treatment of diseases affected by oxidative stress.

Several studies can be envisaged in this field of research. First of all, it should be noted that there are still many useful local plants which have not been studied yet. Therefore, it is recommended to determine their toxicity, antioxidant and anti-diabetic effect in vivo for the safe use of these plants.

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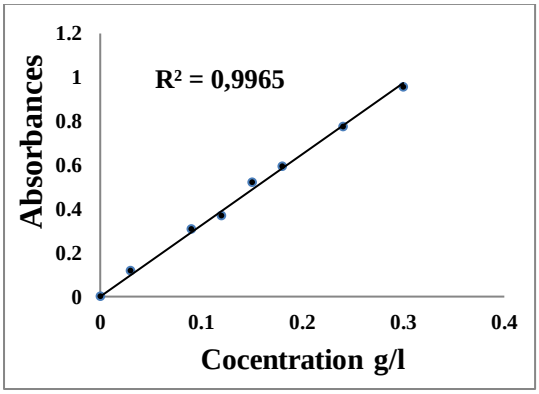
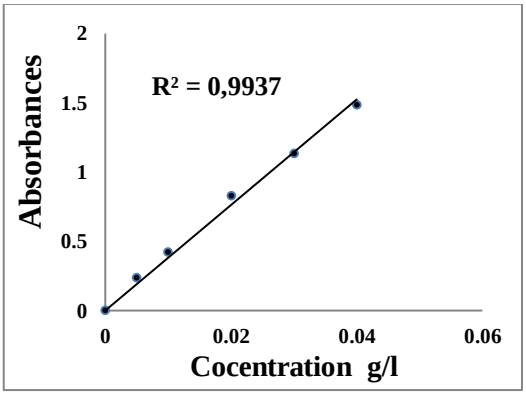
APPENDIX

Calibration curves



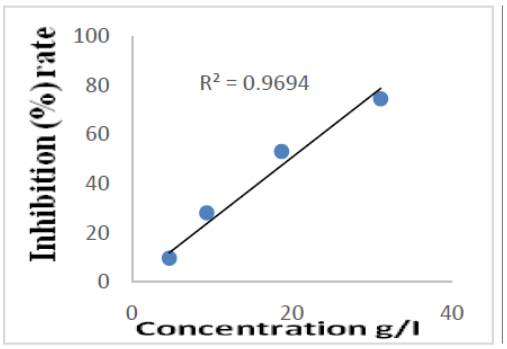
Gallic acid



Quercetin

Variation in the percentage of inhibition as a function of aqueous extracts concentration on α - amylase

Decoction aqueous extracts



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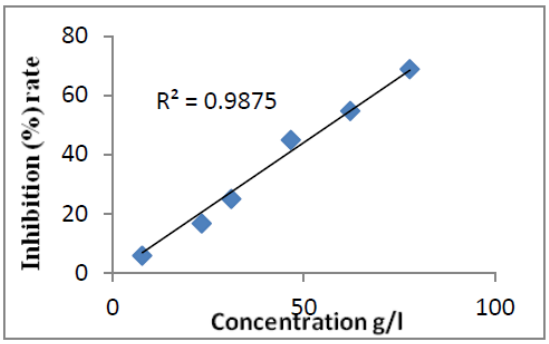
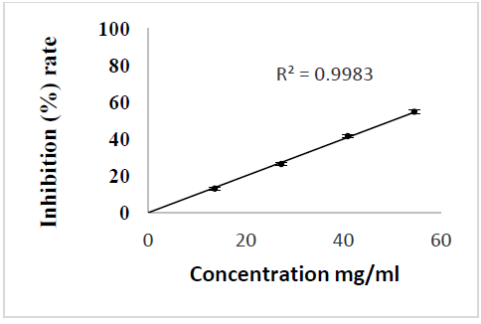
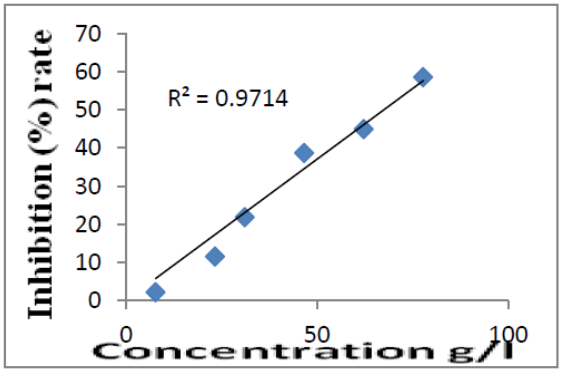


Bubonium graveolens



Haplophyllum tuberculatum

Difusion aqueous extracts



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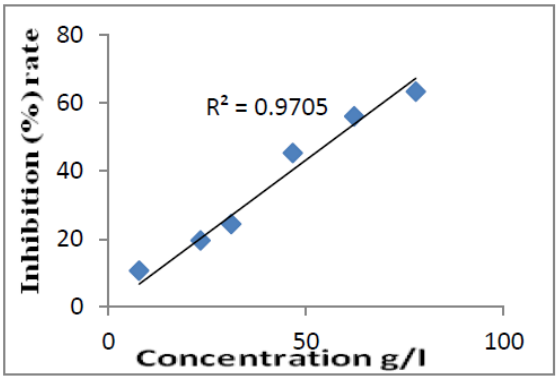

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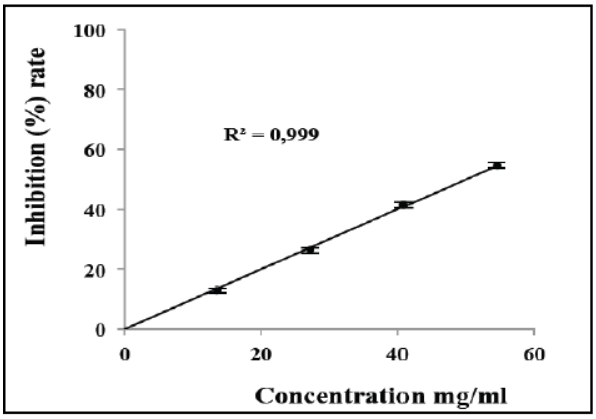
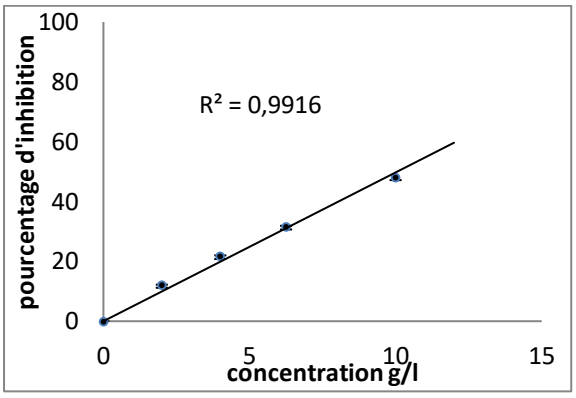


Haplophyllum tuberculatum

Variation in the percentage of inhibition as a function of essential oils extracts concentration on α – amylase



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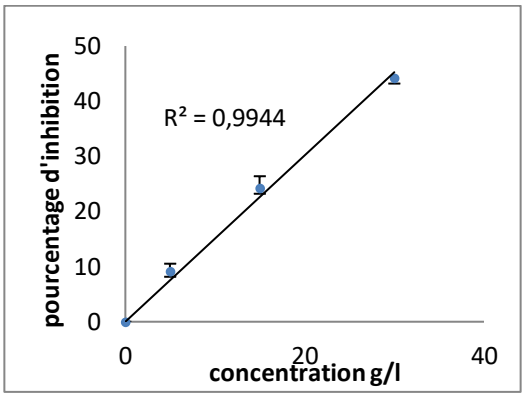
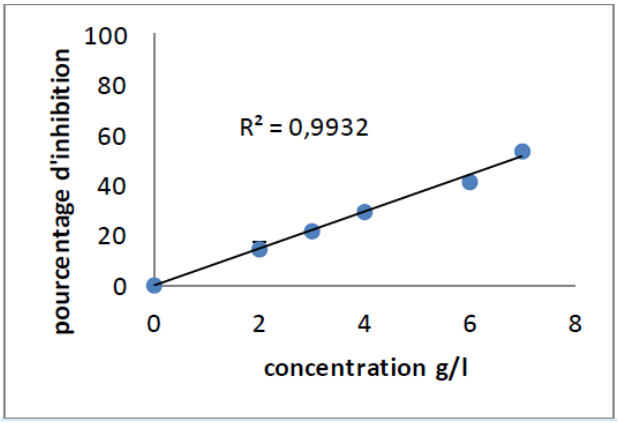


Haplophyllum tuberculatum

Variation in the percentage of inhibition as a function of gluconova medicament concentration on α – amylase



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RESEARCH ARTICLE

A Potent *In Vitro* α-Amylase Inhibitory Action of *Haplophyllum tuberculatum* Extracts

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Abstract: ***Background*:** Natural plant active compounds were found to inhibit the activity of several enzymes that may be related to several diseases.

***Objective*:** This study aimed at testing the antidiabetic activity related to the phenol content by *in vitro* α-Amylase inhibitory action effect of aqueous, organic and essential oil extracts of *Haplophyllum tuberculatum*, collected in the town of Laghouat in the steppe region of Algeria.

***Methods*:** Two types of aqueous extracts were prepared: Decoction and Diffusion extracts. The organic extracts were prepared with successful maceration in hexane, dichloromethane, ethyl acetate, ethanol and methanol. Also, essential oils were obtained by hydrodistillation. The analysis of the total Phenol content of our extracts was done with Folin-Ciocalteu reagent, as the flavonoid content was obtained in mixture with aluminum trichloride. The effects of the plant extracts on the catalytic efficiency of α-amylase enzyme were represented by the enzymatic inhibitory percentage of each extract in which the inhibitory activity was expressed as IC_{50}.

***Results*:** The total phenol content showed values ranging between 0.27 and 11.97 mg gallic acid equivalent / g dry matter. The flavonoid contents vary from 0.05 to 1.50 mg equivalent of rutin /g of dry matter. All the extracts showed good inhibitory activity against α- amylase of IC_{50}, values ranged from 0.05 to 50.03 mg/ml.

***Conclusion*:** This study reports for the first time the inhibitory capacity of Algerian *Haplophyllum tuberculatum* species against α-amylase which could provides natural biologically active agents to be used in the management of diabetes.

Keywords: Diabetes, enzyme inhibition, essential oils, *Haplophyllum tuberculatum*, phenolic compounds, α amylase.

1. INTRODUCTION

The inhibition of α-amylase and α-glucosidase enzymes retards the absorption of glucose in the intestines, this is one of the therapeutic approaches for decreasing postprandial hyperglycemia and managing diabetes mellitus [1, 2].

Using natural medicines, more particularly plants, to inhibit α-amylase is definitely safer than using drugs such as acarbose, miglitol, or voglibos that cause serious side adverse-effects such as flatulence, diarrhea, and abdominal pain [3-5]. Actually, there is an increasing interest to report evidence about the efficiency and safety of specific herbs and natural dietary supplements that have been used for treating diabetes in traditional medicine. In fact, many of the currently available drugs have been directly or indirectly derived from plants [6].

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Haplophyllum tuberculatum is a perennial herb which belongs to the Rutaceae family, the height of the plant is about 60 cm, the leaves are alternately elliptic to obovate, the yellowish-green stem color tends to white and yellow flowers of five petals and five free ovate sepals. This plant is covered with tiny raised glands [7, 8]. These species are distributed from North Africa to Southwest Asia [7]. The Algerian named this plant "Fidjel".

Haplophyllum tuberculatum is rich in secondary metabolites such as alkaloids, polyphenols, flavonoids and essential oils. Therefore, this gives it vital applications in traditional medicine [7, 9]. The aerial parts of *Haplophyllum tuberculatum* are used with different methods and preparations such as decoction for rheumatic pains and digestive problems including constipation and diarrhea [9]. It can be also prepared as a juice extracted from the leaves for the treatment of skin infections and parasitic diseases as well [7, 10]. The plant was used also for gynecological problems [7, 9] and its essential oils have antimicrobial activity [11].

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To our knowledge, no research focused on the antidiabetic activity of *Haplophyllum tuberculatum* extracts; due tothis fact, and for the first time we deemed essential to report the *Haplophyllum tuberculatum* extracts evaluation of α-amylase inhibitory effect in this study.

2. MATERIAL AND METHODS

2.1. Plant Material

The aerial parts of *Haplophyllum tuberculatum* were collected in April 2019 from Laghouat city in the steppe region of Algeria. A voucher specimen of the sample was placed in the herbarium of the laboratory of Fundamental Sciences University of Laghouat. The aerial parts were dried, ground and kept for test and analysis.

2.2. Chemical Reagents

All chemical reagents were from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland), and Merck (Germany). The used solvents were of analytical grade.

2.3. Preparation of Plant Extracts

2.3.1. Aqueous Extract

2.3.1.1. Decoction Extraction

5 grams of aerial part powder of plant was heated in 50ml of distilled water at 75°C for 20min. The extract was filtered. The distilled water was evaporated under reduced pressure in a rotary evaporator at 40°C. The dried residue was dissolved in 10 ml of distilled water and kept at 4°C [12].

2.3.1.2. Diffusion Extraction

5 grams of aerial part powder of plant was macerated in 50 ml of distilled water at room temperature, after 24 hours the extract was filtered then the residue was extracted for the second time in 50 ml distilled water at room temperature for 24h, the extract was filtered again. This process was repeated five times to ensure that all the possible compounds could be extracted. After filtration, a rotary evaporator was used to remove the distilled water under reduced pressure at 40°C. The residue was dissolved in 10 ml of distilled water and kept at 4°C [13].

2.3.2. Organic Extracts

The organic extracts were prepared in a successive maceration process; we put 5grams of the aerial part of plant powder in 50ml of hexane at room temperature until exhaustion. After filtration, the remaining residue was sequentially extracted with 50 ml of increasing polarity solvents for 72 hours such us: dichloromethane, Ethyl Acetate, Ethanol, and Methanol. The organic fraction was evaporated by a rotary evaporator at 40°C. The dried residue was dissolved in 10 ml of methanol and kept at 4°C until analysis [2, 4].

2.3.3. Essential Oils Extraction

The different samples of the essential oils were obtained by hydrodistillation using a Clevenger type apparatus; subsequently, the obtained essential oils were dried over anhydrous sodium sulfate and stored in a dark at 4°C [14].

2.4. Determination of Total Phenolics Compound

The concentration of total Phenolics Compound was estimated by the Folin-Ciocalteu reagent using the method of Singleton and Ross (1965) [15]. Where 100 µL of each extract was mixed with 500 µL of the Folin-Ciocalteu reagent (10%). After 2 min of incubation at room temperature, 2 ml of sodium carbonate (2%) was added. The samples were incubated at room temperature for 30 min, the absorbance of samples was measured at 760 nm using the Shimadzu 1601 visible spectrophotometer apparatus. The total phenolic content of each extract was expressed as gallic acid equivalents (GAE) [5].

2.5. Quantification of Flavonoids Content

The total flavonoid content in dried aerial parts of the plant was determined spectrophotometrically by method of Laimaison and Carnat (1991); modified by [16, 17]. The principle of this method is based on combining flavonoids to aluminum trichloride having mixed flavonoids-aluminum with absorption maximum at 430 nm. The procedure is as follows: 500 µl of the diluted sample was mixed with 500 µl of aluminum trichloride methanolic solution (2%). After incubation at room temperature for 20 min, the absorbance of the reaction mixture was measured at 430 nm with a Shimadzu 1601 visible spectrophotometer apparatus. The flavonoids content is expressed in mg rutin equivalent (QE) / g of dry matter [16].

2.6. α-Amylase Inhibitory Activity

In this study, the inhibitory activity of *Haplophyllum tuberculatum* extracts was carried on an *Aspergillus oryzae* α-amylase.

Structural studies on a limited number of α-amylase enzymes have shown that greater homology exists between them with respect to polypeptide chain folding. These results also show that these α-amylases have similar active site regions that are centered on three highly conserved carboxylate groups. On the other hand, the use of this enzyme is based on the fact that α -amylase from Aspergillus oryzae has four homologous regions compared to the human enzyme. It was inferred that these four homologous regions were likely to be the active and/or substrate-binding sites comparisons with related enzymes [18, 19].

2.6.1. Aqueous and Organic Extracts

The inhibitory activity was determined according to the literature method [2, 20]. Firstly, 200 µl of salt sodium phosphate buffer (pH 6,8) was mixed with 100 µl of soluble starch 1% as a substrate and 100 µl of plant extract. The test tubes were incubated for 5 min at 37°C, after that 100 µl of α-amylase (13 U/ml) was added to start the reaction, after an incubation for 5 min at 37°C the reaction was terminated by the addition of 1 ml of basic solution DNS (dinitro-salicylic acid) [5]. Finally, the mixture was boiled for 5 min at 100°C

Table 1. Total Amount of Phenolics Compound and Flavonoids of *Haplophyllum tuberculatum extracts.*

Extracts		Total Phenolics (mg GAE/g dw)[a]	Flavonoids Content (mg RE/g dw)[b]
Aqueous	Decoction	3.69 ± 0.07	0.83 ± 0.03
	Diffusion	11.97 ± 0.19	1.50 ±0,04
Organic	Dichloromethane	0.30 ± 0.00	0.05 ± 0.01
	Ethyl acetate	0.27 ± 0.01	0.07 ± 0.01
	Ethanol	1.31 ± 0.40	0.15 ± 0.05
	Methanol	0.93 ± 0.07	0.51 ± 0.34
Essential oil		-	-

[a.] milligrams of gallic acid equivalent per gram of dry weight of the plant.
[b.] milligrams of rutin equivalent per gram of dry weight of the plant

and then the test tubes were cooled using tap water, then their content was diluted by adding 4 ml of distilled water. Enzyme activity was quantified by measuring the optical density which is proportional to the quantity of the maltose equivalents released from starch at 530 nm [3, 4]. The experiments were performed in triplicates. The percentage of inhibition was calculated using the following equation:

Inhibitory activity (%) = $[(A_0-A_S)x100]/A_0$ where:

A_0 : Absorbance of control without inhibitor

A_S : Absorbance of test Sample with inhibitor

The inhibitory activity of α-amylase inhibition is expressed as IC_{50} value which represents the concentration of the extract needed to inhibit 50% of α -amylase activity.

2.6.2. Essential Oil Extract

In vitro α-amylase inhibitory assay was carried out according to Murugan *et al.* [21] with a slight modification. Starch solution (1% w/v) was prepared using distilled water. Various concentrations of essential oil solutions were prepared using ethanol. Initially, 100 µl of essential oil sample of various concentrations and 100 µl of α-amylase solution ((13 U/ml on salt sodium phosphate buffer-pH 6.9) were incubated at 37°C for 5 min. Then 100 µl of 1% starch solution was added to each tube and the mixtures were incubated at 37°C for 10 min. The reaction was stopped by adding 200 µl of dinitrosalicylic acid color reagent and tubes were incubated in a boiling water bath for 5 min. The mixtures were cooled under tap water and diluted with 4 ml of distilled water. The absorbance of each mixture was measured at 530 nm. The experiments were performed in triplets. The percentage of inhibition was calculated using the below-mentioned formula. The activity was also expressed in IC_{50} value as the concentration essential oil required to inhibit 50% of α-amylase.

% inhibition = [Ac-As / Ac] x 100

where, Ac is for the absorbance of the control and As is for the absorbance of the sample.

3. RESULTS AND DISCUSSION

3.1. Total Phenolic and Flavonoid Content

The amount of total phenol compound which was measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent ranged in the different extracts from 0.27 to 11.97mg/ g (Table **1**). The highest amounts of the total phenolic compound were detected in Diffusion extract 11.97mg/ g followed by Decoction extract 3.69 mg/ g and Ethanol extract 1.31 mg/g. The lowest total phenolic compound levels were detected in Ethyl acetate extract. If we compare the amount of total phenol compounds in both aqueous and organic extracts, we find that the aqueous extract has a higher amount of total phenols compared to the organic extracts. These results may be explained as follows: firstly, the majority of polyphenols in this plant are in a glycosidic form which is soluble in water contradictory to aglycones [22]. Secondly, the water can extract other polar compounds that react with Folin-Ciocalteu reagents such as sugars, proteins, alkaloids, and other compounds resulting in increased levels of phenolic compound [3].

The total phenolic content of *Haplophyllum tuberculatum* is less than found in other studies on the same plant species [9, 23]. This tiny quantity may be related to deficiency of polyphenols in the plant and probably to unfavorable biotic conditions or this plant grew up in a region where the biosynthesis of these molecules was not stimulated due to the fact that the polyphenols biosynthesis is related to several internal and external conditions, such as genetic factors, infections, temperature and UV rays [22].

The content of flavonoids measured by aluminum chloride method varies from 0.05 to1.50 mg/g Quercetin equivalent. The highest amounts *i.e.* 1.50 mg/g of flavonoids were found in diffusion aqueous extract. However, the lower amounts of flavonoids were found in Dichloromethane extract which is around 0.05 mg/g. The outcome values are significantly less than those found in previous studies on the same plant species [23]. A correlation analysis between the total phenolic content and the total flavonoid content of *Haplophyllum tuberculatum* is equal to r= 0.961.

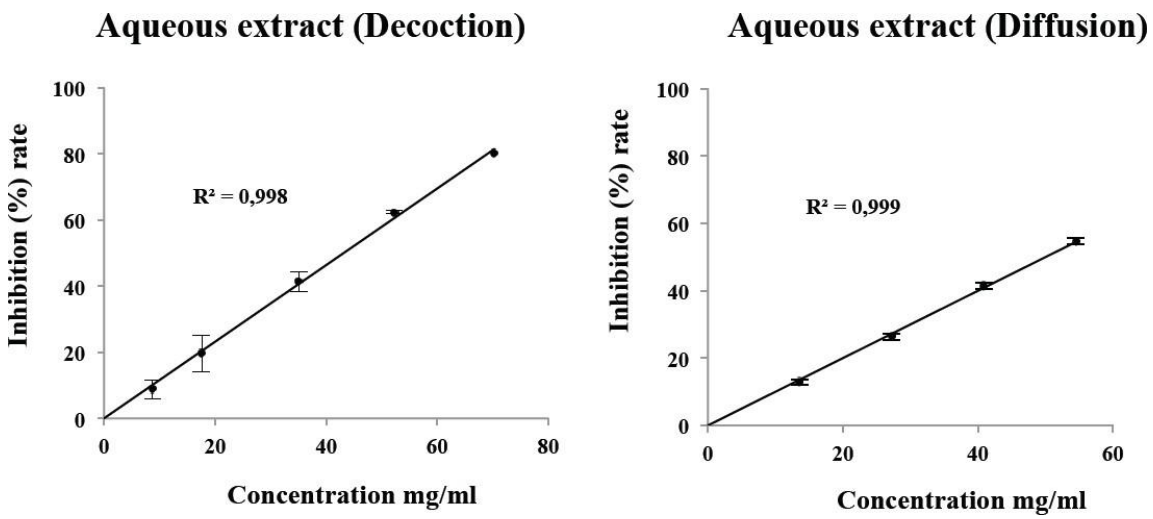

Fig. (1). The inhibition rate against α-amylase activity of the aqueous extracts of *Haplophyllum tuberculatum.*

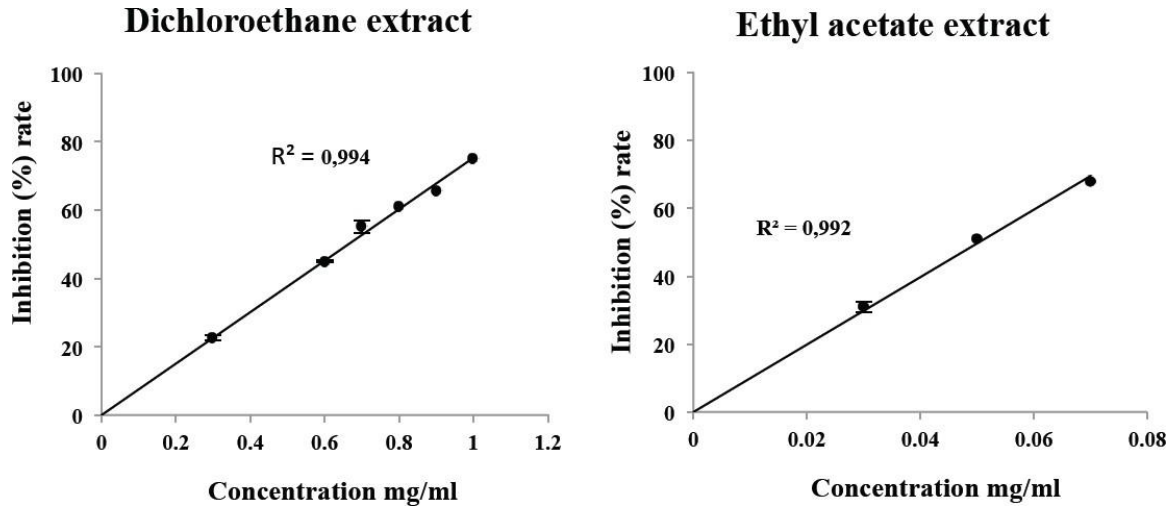

Fig. (2). The inhibition rate against α-amylase activity of the organic extracts of *Haplophyllum tuberculatum.*

3.2. α-amylase Inhibitory Activity

The present study evaluated the *Haplophyllum tuberculatum* extracts inhibitory capacities on the α-Amylase enzyme in order to control the postprandial hyperglycemia [24]. α-Amylase inhibitory was observed in all tested extracts, the inhibition rates of H*aplophyllum tuberculatum* extracts ranged from 40.33% to 88.65%. The obtained values are reported in Table **3**.

The percentage of aqueous extract varied from 44.31% to 61.87% as depicted in Fig. (**1**). The highest and the lowest percentages were observed in organic extracts Methanol and the Dichloromethane with 88.65% and 40.33% respectively as shown in Fig. (**2**). The percentage of essential oil is about 42.61% (Fig. **3**).

The Ethyl acetate extract shows a high α-amylase inhibitory activity with an IC_{50}=0.05 ± 0.00 mg/ml followed by Methanol extract with an IC_{50}=0.18 ± 0.01 mg/ml (Table **3**) in the first hand. On the other hand, the lowest α-amylase inhibitory activity was found in diffusion extract with an IC_{50}=50.03 ± 0.10 mg/ml. The IC50 value for the essential oil is 34.95 ± 0.11 mg/ml.

The highest activity of the organic extracts compared to the aqueous extracts is due to the activity of polyphenol oxidase enzyme with the ability of the degradation of the polyphenols in aqueous extracts, while this process does not occur in the organic extracts [3]. Furthermore, water is a better environment for the occurrence of micro-organisms [25, 26], the latter harms the activity of aqueous extracts.

The ethyl acetate and methanol extracts of the *Haplophyllum tuberculatum* are the best inhibitors of α-amylase. This is may be due to the existence of the polar phenolic molecules with a close chemical structure responsible for the α-amylase inhibition.

The results showed that the ethanol extract contains more quantity of the total phenolic compound than Dichloromethane extract. However, the Dichloromethane extract has more α-amylase inhibitory activity than the first one. It may be thought that the inhibitory potency of the plant extracts is

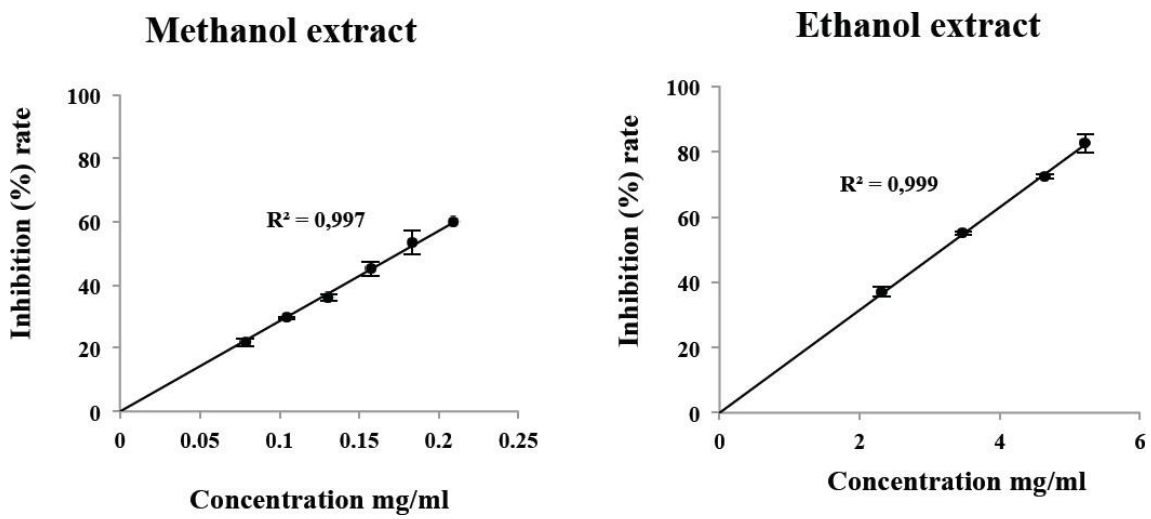

Fig. (3). The inhibition rate against α-amylase activity of essential oils of *Haplophyllum tuberculatum.*

Table 2. The inhibition rates of *Haplophyllum tuberculatum* extracts.

Extracts		α-amylase Inhibition Rates (%)
Aqueous	Decoction	44.31 ± 2.97
	Diffusion	61.87 ± 1.53
Organic	Dichloromethane	40.33 ± 0.00
	Ethyl acetate	56.68 ± 0.00
	Ethanol	88.65 ± 0.00
	Methanol	81.32 ± 0.00
Essential oil		42.61 ± 2.24

Table 3. IC_{50} values of *Haplophyllum tuberculatum* extracts.

Extracts		α-amylase IC_{50} (mg/ml)
Aqueous	Decoction	43.90 ± 0.00
	Diffusion	50.03 ± 0.10
Organic	Dichloromethane	0.65 ± 0.02
	Ethyl acetate	0.05 ± 0.00
	Ethanol	3.17 ± 0.03
	Methanol	0.18 ± 0.01
Essential oil		34.95 ± 0.11

not limited to the phenolic content but the presence of some active individual phenolic compounds [3]**.** Besides, the composition of the extract depends upon the type of extraction, time of extraction, temperature, nature of solvent, solvent concentration and the polarity of the solvents. The length of the extraction period must be also taken into consideration which depends on: the solvent used, pH of the solvent, temperature, particle size of the plant tissues and the solvent to sample ratio [25]. On the other hand, by comparing the values of the IC_{50} to the amount of total phenols and flavonoids, it is clear that the inhibitory activity is proportional to the concentration of phenolics compounds and the flavonoids with a correlation r= 0.998 and r= 0.947, respectively. In other words, the inhibitory activity against α-amylase enzyme is commonly related to the content of phenolic compounds and flavonoids.

CONCLUSION

Our study is the first investigation to monitor the effect of different extracts of *Haplophyllum tuberculatum* which was harvested at Laghouat city in the steppe region of Algeria on the inhibition of α- amylase, which are key enzymes in the digestive system relevant to type 2 diabetes. The results showed that Ethyl acetate and Ethanol extracts of *Haplophyllum tuberculatum* exhibited the best $\alpha - amylase$ inhibition, by using *Haplophyllum tuberculatum* extracts for the normalization of blood sugar disorders. We need complementary studies aimed at isolating and identifying the structures of the molecules responsible for the inhibition of α-amylase enzyme and determine the toxic effect of these extracts *in vivo* for safe use.

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