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Effect of *Abelmoschus esculentus* (Okra) fruit extract on glucose level in alloxan-induced diabetic mice in Libya

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***Abstract*—This study was conducted to investigate the effect of okra fruit extract on diabetes mellitus induced by alloxan compound in male mice, where four solvents of ethyl alcohol, methyl alcohol, ethyl acetate and water were used. The results showed that the ethyl alcohol solvent was the best for the detection of alkaloids, flavonoids, tannins and phenols compared to other solvents. The phytochemical detection of the okra fruit extract confirmed that the extract is rich in alkaloids, flavonoids, tannins and phenols, and the results were clear in the ethyl extract, and the results were positive. In this study, 48 male rats were used, divided into the healthy group, which included 24 mice that were not injected with alloxan, and the infected group, which included 24 mice, that were injected with alloxan. Mice were dosed with okra fruit extract daily at doses (100,200,300 mg/kg) for a month, with blood sugar measured on day (0, 7, 14, 21, 30). Where the results showed that the induction of diabetes by alloxan caused an increase in the level of glucose in the blood serum of mice. The study indicated that the treatment of diabetic mice with ethyl extract of okra fruits at doses (100, 200, 300 mg / kg). As well as there was improvement in diabetic pancreatic tissue sections treated with ethyl extract of okra fruits, where the dose (300 mg/kg) was the most effective compared to other doses. The dose was taken once daily for a month which produced a significant decrease in the blood glucose level (P≥0.0005). The study also showed the dependence of the anti-hyperglycemic activity of okra fruit extract on dose and time, where the greatest effect on hyperglycemia was obtained at a dose of (300 mg/kg) in diabetic mice induced by alloxan compared to other doses.**

Keywords—Abelmoschus esculentus, Okra fruit, glucose level, alloxan-induced diabetes, mice.

Introduction:

Diabetes represents a group of autoimmune diseases, metabolic disorders (of carbohydrates, fats, and proteins), and genetic disorders that all share one of the main characteristics of the disease, which is hyperglycemia, which results when the pancreas is unable to produce the required amount of insulin for the body. In humans (dysfunction in insulin secretion), or when the human body is unable to effectively manage the insulin produced, or both (Egan and Dinneen, 2018) (Karamanou, 2016). Diabetes is divided into several types, namely type 1 diabetes, type 2 diabetes, other specific types of diabetes, and gestational diabetes. (Baynest, 2015). Humans have known medicinal plants since ancient times and used them to treat various diseases, and their medicinal value lies in the presence of some active substances (Galletto, 2004). It has also been observed in recent years that there is a continuous increase in the manufacture of many natural products for medicines and therapeutic preparations from different groups of plants. For this reason, treatment with these plants is now based on strong scientific foundations. Okra is an important medicinal plant. It has been widely used in many medicines. From traditional systems of medicine (Kumar et al., (2013). Okra is one of the important summer vegetable crops in the world. It belongs to the Malvaceae family. It is one of the most important and widely used types of vegetables, widely known and cultivated all over the world. It is the only vegetable crop of importance in this family. It is very popular in India, and ranks first in its consumption, although its original homeland is Ethiopia, Sudan, and the countries of northeastern Africa (Kumar et al., 2013). Okra is consumed either cooked, canned, frozen, or dried, and its pods are used in some countries as a substitute for coffee. It is also rich in phenolic compounds which have biological properties (Matloub et al., 1989). Moreover, Okra fruits have many medical and therapeutic benefits, including adjusting sugar levels, lowering the level of cholesterol in the blood, and lowering the level of toxic substances in the kidneys. They are also useful in treating digestive disorders because they contain a large percentage of fibers that facilitate bowel movement and reduce the absorption of carbohydrates in the small intestine (Kufe et al., 2015). Many previous studies have proven that okra fruits contain many active substances with various medicinal effects. Many phytochemical studies exhibited that polysaccharides, polyphenols, flavonoids, tannins, sterols and triterpenes are the major components of A. esculentus with various biological activities (Arapitsas et al, 2008). It has been reported that the okra powder plays antidiabetic

and antihyperlipidemic roles in diabetic rats. Dietary fibers and polyphenols which are abundantly found in A. esculentus, may contribute to the hypoglycemic and hypolipidemic effects of A. esculentus has suggested previously (Gunness, et al 2010)

This study was conducted to evaluate the effects of A. esculentus (okra) on glucose level in alloxan induced diabetic mice model.

Materials and Methods

Preparation of Extract

Extraction of Okra fruits

In this study, the fruits of the okra plant, the Whit Volvit variety, were collected from the local markets in the city of Zawia. They were washed well with water, dried, and placed in the oven for three continuous days at a temperature of 60 degrees Celsius, then grinded using an electric grinder to obtain a fine powder and preserved in nylon bags as a powder in the refrigerator at 4°C until used.

Preparation of okra fruit extract:

Weighed 20 grams of okra fruit powder and distributed it on four conical flasks. Put 5 grams in each flask and added to the first flask 50 ml of ethyl alcohol, to the second flask 50 ml of methyl alcohol, to the third flask 50 ml of ethyl acetate, and to the fourth flask 50 ml of distilled water, and they were mixed well. Then, put the flasks on a magnetic shaker and cover them with aluminum foil. They were left in a dark place for 24 hours at room temperature (25°C) and placed in a centrifuge at 1500 rpm for 5 minutes to get rid of suspended materials. Then it was filtered with Whatman filter paper No. (1) and the alcohol was evaporated from the extract by using a rotary evaporator at a temperature of 40°C until a thick liquid is reached. Then place the extract inside a tightly covered glass container, weighted, and stored it in the refrigerator at a temperature of 4°C until used (Roise and Viler,1987).

Preliminary Phytochemical Screening of Successive Extracts of okra fruit extract:

Test for flavonoids:

This detection was done by mixing 10 ml of ethyl alcohol (50%) with 10 ml of potassium hydroxide solution (50%), then an amount of this mixture was taken and an equal amount of plant extract was added to it. The appearance of yellow color indicates the presence of flavonoids (Patel *et al*,.2014).

Test for phenols:

Dilute 3 ml of the plant extract in 10 ml of distilled water, then add drops of ferric chloride reagent at a concentration of (1%), when a bluish-green color appears, indicating the presence of phenols (Patel *et al*,.2014).

Test for alkaloids:

Prepare 1% hydrochloric acid and add it to 2 ml of Okra fruit extract in a test tube. Heat the mixture for 20 minutes, shaking gently, then leave it to cool. Take 1 ml of the extract and add a few drops of Wagner's reagent to it. We notice the appearance of a creamy brown color indicating the presence of alkaloids (Patel *et al*,.2014).

Test for tannins:

Take 2 ml of the plant extract, put it in a test tube, heat it gently for two minutes, leave it to cool, then add three drops of ferric chloride (1%) to it. The appearance of an orange color indicates the presence of tannins. (Patel et al,.2014).

Detection of active compounds using thin layer chromatography:

To determine the chemical compounds that make up the alcoholic extract of the Okra plant, the thin layer TLC technique was conducted using different mobile phases, which are the mobile phase propane: ethyl alcohol: water in a ratio (1:2:1), methyl alcohol: ethyl acetate: water in a ratio (2:1:1), and Propane: methyl alcohol: water ratio (1:1:3)

By using thin sheets covered with silica gel, the sheet was loaded with small spots of alcoholic extract, using capillary tubes, ensuring that the spot did not exceed a diameter of 2 mm, and the distance between one spot and another was 2-3 cm. These spots dried completely, then this plate was placed in a special suitable glass container saturated with the separation fluid propane: ethyl alcohol: distilled water in a ratio of 1:2:1 and leaving the solvent to spread a distance of 15 cm from the original. Then the plate lifted from the glass container and marked with a pencil the limit reached by the solvent, then left the plate to dry at room temperature. The separated compounds were examined with the naked eye and then induced by spectral rays in an ultraviolet device (Saric et al, 2004 (Jemal et al., 2011) and then calculate the flow rate according to the following equation:

$$RF = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solute front TLC plate}}$$

Experimental Animals:

The animals for the study were obtained from the animal house of Zawia Medical Research Center. They were male Swiss albino mice (Mus musculus) (48), weighed between (30-35gm) and was kept in cages on optimum condition of temperature, humidity and light (Manohar et al., 2012). The mice were divided into 2 main groups; normal healthy group and diabetic group (24 mice/group), and then subdivided into 4 groups each group contain 6 mice (n= 6) as followed: -

1-Normal healthy groups (n=24):

Group1: normal control (n=6) (distilled water 3 ml/body weight by oral gavage needle).

Group 2: normal (n=6) tested compound (Okra fruit extract 100mg/kg by oral gavage needle)
Group 3: normal (n=6) tested compound (Okra fruit extract 200 mg/kg by oral gavage needle)
Group 4: normal (n=6) tested compound (Okra fruit extract 300 mg/kg by oral gavage needle).
2-Diabetic groups (n=24):
Group1: diabetic control (n=6) (distilled water 3 ml/body weight by oral gavage needle).
Group2: diabetic(n=6) tested compound (Okra fruit extract 100 mg/kg by oral gavage needle)
Group3: diabetic (n=6) tested compound (Okra fruit extract 200mg/kg by oral gavage needle)
Group4: diabetic (n=6) tested compound (Okra fruit extract 300mg/kg by oral gavage needle)

Drugs:

Alloxan monohydrate was received from Sigma Chemical Company (st. Louis. Mo.USA) and used in the induction of diabetes, to induce experimental diabetes.

Experimental procedures:

The mice were prevented from eating for 24 hours then, they were weighed and injected with alloxan dissolved in Phosphate Buffer Saline at a concentration of 150 mg/kg body weight into the peritoneal membrane of the abdomen (IP) (Berraaouanet al., 2015). After 48 hours, the mice were weighed and glucose was measured. In order to ensure the occurrence of diabetes, two consecutive doses of Alloxan were given two days after the first dose (100 mg/kg) and (150 mg/kg) two days after the second dose. Mice with a glucose level between (dl/(150-200mg) were considered diabetic and were chosen to complete the study (El-Desoukiet al,.2016). The animals were then dosed orally with okra fruit extract daily for 30 days, the glucose level readings were taken by using the Accu-check device on days (0/7/14/21/30) of the experiment. At the end of the study, the animals were dissected after being anesthetized with chloroform for the purpose of drawing blood from the heart by the needle directly. The blood was emptied into special tubes for collecting blood samples that did not contain an anticoagulant for the purpose of conducting blood tests.

Statistical analysis:

The data were analyzed using the statistical test One-way ANOVA to compare the means of the treated group and the control group for statistical difference using version 19 of the SPSS package, and values <0.05 were considered statistically significant.

Result:

Phytochemical Screening of Sequential Extracts of Okra fruits:

After preparing plant extracts using solvents (methyl, ethyl, ethyl acetate, and water) of Okra fruits by soaking and shaking for (24 hours), it is clear from Table (1,2) that the ethyl extract contains the all active compounds, phenols, flavonoids, alkaloids, and tannins compared to the rest of the extracts. Also, the aqueous extract is rich in all the previous compounds except flavonoids, while the ethyl acetate extract contains only flavonoids in a low percentage.

Table (1) shows the results of chemical detection of alkaloids, phenols, flavonoids, and tannins of okra fruit extract using solvents.

Extracts	Phenols	Flavenoids	Alkaloids	Tannines
+Methanol	-	+	-	+
+++Ethanol	++	+	++	+++
-Ethyl acetate	-	+	-	-
+++Water	++	-	++	+++

Table (2) shows the results of chemical detection of alkaloids, phenols, flavonoids, and tannins for the ethyl extract of okra fruits.

Compound	Reagents	Results	
Alkaloids	Wagner's reagent	Appearance of a creamy brown precipitate	
Phenols	Drops of (FeCl3)	Appearance of a bluish green color	
Flavonoids	Sodium hydroxide	Appearance of yellow color	
.Tannins	Drops of (Fe Cl_3)	Appearance of orange color	

Chromatographic Purification: TLC

To determine the degree of release of chemical compounds present in the extract of okra fruits, chromatographic separation was adopted by thin layer chromatography (TLC), and we adopted the results of the solvent system: methyl alcohol: ethyl acetate: water (2:1:1), and propane: methyl alcohol: water (2:1:1). 1:1:3), and propane: ethyl alcohol: water (1:2:1), where the solvent propane: ethyl alcohol: water (1:2:1) showed the best compared to other solvents, In this study relied on the properties in chromatography, which are primary data, and these properties are represented by the fluorescent color, and the disability constant Rf. Table (3.4) showed that each of the extracts (ethyl 4 spots, methyl 2 spots, and ethyl acetate 2 spots, and watery one spot), and a varying blocking agent, where the best of them was the ethyl extract, which gave four spots of different colors, varying (Rf means the presence of four compounds: compound Rf = 0.71, compound Rf = 0.77, and compound Rf = 0.82 , and compound Rf = 0.91. The methyl extract yielded two spots, meaning two compounds Rf = 0.71, and one

compound Rf = 0.77, with different colors, and the ethyl acetate extract yielded two spots, meaning two compounds Rf = 0.68, and one compound Rf = 0.77, with different colors. Finally, the aqueous extract, which gave one spot, means one compound, Rf=0.77.

Table (3) Thin Layer Chromatography (TLC) results for okra fruit extract:

Mobile phase	Rf			
	Ethyl acetate extract	Ethanol extract	Methanol extract	Water extract
(1:2:1) P:E:W	0.68, 0.77	0.71, 0.77, 0.82, 0.91	0.71, 0.77	0.77
(1:1:2) M:EA:W	0.075, 0.45, 0.54	0.029, 0.01, 0.42	0.028, 0.014, 0.050	-
(3:1:1) P:M:W	0.45, 0.85, 0.75	0.50, 0.67, 0.85	0.45, 0.72	-

Changes in blood serum glucose level:

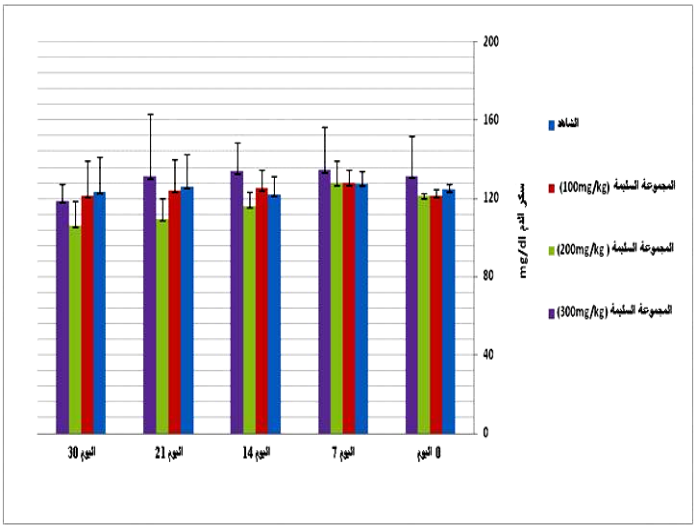
Effect of okra fruit extract on blood sugar level in healthy mice:

The results in Table (4) and Figure (4) indicate that the blood sugar levels on days 0, 7, 14, 21, and 30 in the mice of this group within normal levels (the healthy group), that reveals there are no significant differences in blood glucose levels. Using a dose of (100, 200, and 300 mg/kg) of the extract, the blood sugar level was within the normal range, as the average of the sugar readings was between (106.3 to 134.5). Therefore, there is no blood sugar lowering effect by using these doses of okra extract.

Table (4) Effect of alcoholic extract of Okra fruits on glucose level (mg/kg) in the blood serum of healthy male albino mice.

Healthy groups	0Day	First week	Second week	Third week	Month
		7 days	14 days	21 days	30 days
control	±124.7 20.7	±127.5 21.9	±122.2 15.0	±126.2 31.7	8.3±123.7
Healthy group) mg/kg(100	±121.6 1.6	±128 11.2	±125.1.0 7	±124.1 10.7	±121.6 12.3
Healthy group) mg/kg(200	±121.3 2.8	±128 6.7	±116.3 9.3	±109.7.0 16	±106.3 17.5
Healthy group) mg/kg(300	±131.5 2.8	±134.5 6.3	±133.8 9.3	±131.2 16.3	±118.8 17.5

Figure (4) The effect of the alcoholic extract of Okra fruits on the glucose level (mg/kg) in the blood serum of healthy male albino mice. It shows that the okra extract and the dosing period have a non-significant effect, P≤0.05**.**



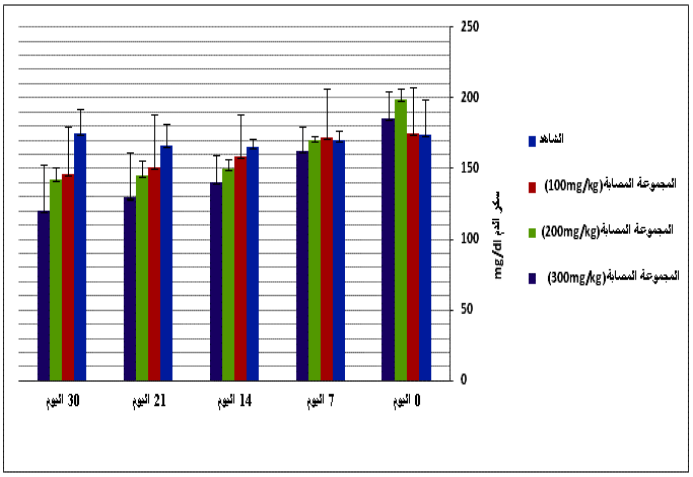
Effects of Okra fruit extract on fasting blood glucose level in diabetic mice:

The results of Table (5) and fig (5) indicate that the induction of experimental diabetes in experimental animals led to a significant increase (P≥0.05) in the level of blood serum glucose, compared to its level in the control group, which was not injected with alloxan. It is noted that treating diabetic animals with the extract Okra fruits, in doses of 100, 200 and 300 mg/kg, once a daily, for a month, caused a significant decrease (P≥0.0005) in the blood glucose level compared to the infected control group. The table also shows that the duration of dosing had a significant effect (P≥0.05) on the concentration of glucose in the blood serum of male albino mice, where the decrease was significant (P≥0.0005) a month after dosing with okra fruit extract, compared with a month after the induction of diabetes.

Table (5) Effect of alcoholic extract of okra fruits on glucose level (mg/kg) in the blood serum of diabetic male albino mice.

Diabetic group	Day 0	First week	Second week	Third week	Month
		7 days	14 days	21 days	30days
Control	± 174 19.1	± 170 .018	± 165.3 18.6	± 165.8 31.6	± 174.5 32.6
Diabetic group) mg/kg(100	± 175 7.5	± 172.1 2.6	± 158.8 6.2	± 150.8 10.4	146.1 8.1±
Diabetic group) mg/kg(200	198.8 ±.0 32	± 170.1 .0 34	150.3 29.9±	± 145.1 37.2	± 142.5 33.7
Diabetic group) mg/kg(300	± 185 25.0	± 162.1 7.2	± 140.8 5.7	± 130.1 15.8	± 120.2 17.5

Figure (4) The effect of the alcoholic extract of okra fruits on the glucose level (mg/kg) in the blood serum of male albino mice with induced diabetes. It shows that the okra extract and the dosing period have a significant effect *P≥0.05



Discussion

In diabetes management the primary aim is to reduce blood glucose levels to the normal range. Many hypoglycemic medications are available for this purpose but most of them have sever adverse effects. The results had been shown in tables (1, 2, 3) showed that the ethyl alcohol solvent is better at extracting alkaloids, flavonoids, tannins, and phenols, compared to other solvents. Through phytochemical screening of the Okra fruit extract, the detection gave a positive result in the ethyl extract, compared to the rest of the extracts. Flavonoids are characterized by being soluble in strong bases, as they are phenolic compounds. They are characterized by their weak acidic properties, and their polarity increases if they contain a larger number of free hydroxyl groups, or a sugar molecule, or more. This is what makes them soluble in polar solvents, ethyl, methyl, ethyl acetate and water. The presence of sugar in the compound molecule makes it more soluble in water (Temidayo, 2013)

The concentration of the active compounds in the extracts varies according to the solvent used, it depends on its polarity, and the degree of solubility of the active substances in it, as well as according to the plant organ studied, as well as on the chemical nature of the active substances presented in the extract. This was confirmed by previous studies carried out by (Sultana et al., 2009) and (2009). Siddhuraju and Becker (2003) found that the amount of phenolic compounds is affected by the method of extraction, as well as the type of plant organ, and most importantly the effect of the systems (solvents). Each system is characterized by characteristics that make it different from another system.

The results showed that inducing diabetes with alloxan caused an increase in the level of glucose in the blood serum of mice. This result is consistent with the findings of studies on rats conducted by (Chahlia, 2009 and Daisy et al., 2009). This increase in glucose concentration may be attributed to the ability of alloxan to attack the pancreatic beta cells, which specialize in secreting the hormone insulin, and destroy them by accumulating free radicals that are toxic on the beta cells, which leads to the cessation of the process of dissolving glucose by the cells, that causing stimulation the process of gluconeogenesis and glycogen breakdown (Benrebai et al., 2007).

The study also indicated that treating diabetic animals with ethyl extract of okra fruits, at doses (100, 200, 300 mg/kg) of body weight once a daily, for a month caused a significant decrease in blood glucose levels (0.0005P≥). The study also showed the dependence of the anti-hyperglycemic activity of okra fruit extract on dose and time, as the greatest anti-hyperglycemic effect was obtained at a dose (300 mg/kg) in mice with diabetes caused by alloxan compared to diabetic mice treated with dose (100 mg/kg), this result is consistent with the results of a study (Probhat et al., 2017).

It was observed that the blood sugar level returned to the normal level, after giving repeated doses of Okra fruit extract in the group suffering from diabetes, as the chemical examination of the Okra fruit extract revealed the presence of alkaloids, carbohydrates, terpenoids, tannins, flavonoids, phenolic compounds, and volatile oils. It is known that flavonoids, alkaloids and phenols are biologically active anti-diabetic compounds. The anti-diabetic effect of the ethyl extract of the fruit was due to many causes that led to low blood sugar. One could be due to the stimulating effect of ethyl extract of okra fruits on the remaining beta cells (Prohabt et al., 2017).

A study on the ethyl extract of okra fruits showed that the extract contains effective anti-hyperglycemic compounds without causing any blood sugar-lowering effect, unlike insulin and other synthetic drugs, that was evident in healthy and diabetic mice, and that was consistent with a study (Prohabt et al., 2017).

Conclusion:

It can be concluded from this study that Okra fruit extract has a hypoglycemic effect, as it helps lower blood sugar levels according to the results indicated in the previous tables, as the medicinal plant contains biologically active secondary metabolites that possess anti-hyperglycemic properties, such as alkaloids, phenols, flavonoids, terpenoids, steroids, and saponins, as this study showed that repeated oral administration of okra fruit extract in doses (200 mg/kg and 300 mg/kg) for 30 days, has beneficial effects on high blood sugar, after further investigation it can be said that Okra fruits can be exploited as an alternative herbal supplement for diabetes management.

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