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Original Article

Comparative Study of Palm Leaves Extract and Glibenclamide in diabetic female rats induced by alloxan

Nabeel M. N. Al-Sharafi[*1] and Nadia K. J. Al-Dawah[1]

[1]Department of Physiology and Pharmacology/Faculty of Veterinary Medicine/ University of Kufa/Iraq.[*]Corresponding author: Nabeel M. N. Al-Sharafi

Email address: nabeelm.naji@uokufa.edu.iq

Abstract

This study was designed to compare the effect of palm leaves extract and glibenclamide in diabetic female rats induced by alloxan. Twenty four adult female rats were randomly divided into four equal groups (6 rats in each group), three groups were injected intraperitonealy (i.p.)with single dose alloxan(100 mg kg^{-1}B.W) and acted as G1, G2, and G3 and treated orally with (200mg/kg B.W.) palm leaves extract in G1 and (5mg/kg B.W) glibenclamide in G2 , while G3 and fourth group (C) consider as positive(+ve) and negative(-ve)control respectively. Fasting blood sample were collected at 15 and 30 day of experiment (after diabetes induction) for measuring of plasma glucose concentration, aspartate aminotransferase(AST) and alanine aminotransferase(ALT) activity.These parameters wereused as a guide for comparison between palm leaves extract and glibenclamide in ameliorating effects of diabetes. The results revealed that i.p. injection of alloxan caused hyperglycemia and significant increase in activity AST, ALT and serum glucose concentration in G3 treated group. The palm leaves extract exhibited significant anti-hyperglycemic activity in alloxan induced diabetic rats. A significant correction of the plasma glucose concentration and ALT and AST activity was observed in G1 in compare with the G1 treated group at 30 days and G2 glibenclamide treated group. In conclusion, these studies reveals that the palm leave extract was worked as anti-diabetic in the alloxan induce diabetic rats model for minimize the complication associated with the diabetic and related disorder.

Keywords: palm leaves, glibenclamide, alloxan, antidiabetic, rats

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Introduction:

Plants represent a good source of potentially useful dietary supplements for improving blood glucose control and preventing long- term complications in diabetics (Gallagher *et al*., 2003).The maincharacteristics of diabetes are hyperglycemia,polyuria, polydipsia and polyphagia, weight loss,muscle weakness and dyslipidemia(Robert *et al*.,2005). Chronic hyperglycemia is normally

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accompanied byincreased risk to hypertension, oxidative stress, decreased fibrinolytic activity, increased platelet aggregation, and severe atherosclerosis (Reusch,2003).The beneficial effect of synthetic drugs like glibenclamide provide good glycemic control but long term use have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems (Prasad *et al*., 2009).

Date palm (*Phoenix dactylifera L.*) is one of the oldest cultivated plants (Riad,2006) plants, *Phoenix dactylifera* was among the most frequently used plants to treat diabetes and hypertension (Tahraoui *et at*., 2007).Antioxidant activities were reported in many plants, among them is date palm fruit (*Phoenix dactylifera*) which possesses a potential antioxidant compounds that capable of scavenging free radicals (Biglari *et at.,* 2008).

This study was designed to evaluatethe potential beneficial effectsof date palm (*Phoenix dactylifera*) leaves extracts as antidiabetic in the alloxan induce diabetic rats model

Materials and Methods

Plant collection and preparation

Fresh leaves of palm were collected from Kufa Area in Najaf province in Iraq. The leaves were air dried on laboratory bench top, then pulverized into a coarsepowder and stored at 4^{o}Cuntil used.

Extraction

Extraction of palm leaves was preformed according to (Markham, 1982) in two steps as following:

Step one

200 g of palm leaves were crushed with 400ml of mixture methanol 95% and distilled water (9:1), mixed for 18h in magnetic stirrer at room temperature, and then filtered under vacuum using Whitman No. (1).

Step two

The filtrate residues from step one was mixed again with 200ml of mixture methanol 95% and distill water (1:1) for 18h in magnetic stirrer at room temperature and the filtered was collected as described in step one . Then, the filtrate collected in step 1 and 2 was evaporated in the incubator (42°C) to reach one –third of original volumes.The concentrated extract was separated from low organic materials by addition of chloroform 20:100 (extract:chloroform) in separator funnel, then the mixture was left for one hour to separate in two layers: lower layer contain chloroform and upper layer contain (total polyphenol). The upper layer was separated with chloroform 10:100 (extract:chloroform), from the upper layer, total polyphenol was collected and dried in incubator at (40^{o}C), and then collected as powder.

Experimental animals

Adult female Sprague-Dawley rats weighing 150-200g were housed inclean cages and kept in well ventilated room witha 12 h light/ dark cycle at 22-25°C. The rats were maintained on a standard rat pellets. This study was approved by animal ethical committee / Kufa University.

Induction of diabetic rats

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Hyperglycaemia was induced in rats after fasting of the animals for 24 h by a single intraperitoneal (i.p.) injection of alloxan100mg/kg (B.W) (Stanley et al., 2001). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, to prevent hypoglycemia the rats were then kept on 5% glucose solutionfor the next 24 h.Three days after injection, threats with fasting blood glucose higher than 150 mg/dL , were considered as hyperglycemic/diabetic(Stanley et al., 2001). Twenty four adult female rats(18 diabetic rats and 6 control)were divided into four groups for 30 days as follow :(i) G1: Diabetic rats were gavages orally with 200mg/kg B.W. palm leaves extract. (ii) G2: Diabetic rats were gavages orally with glibenclamide 5mg/kg B.W/day (Sigma Chemical Co., St. Louis, USA) (5 mg/kg B.W). (iii) G3: Diabetic control rats. (iv) C: normal control rats. Fasting blood samples were drawn from heart puncture of rat at days 15 and 30 of experiment for measurement plasma glucose concentration ,aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity(using semi-automatic chemistry analyzer Belgium using kit Cyan com./Belgium).

Statistical analysis

The results are expressed as the mean values with their standard error. Two-way ANOVA followed by Duncan's variance was performed to compare between treatment groups. Significance was set at $p<0.05$.by used Statistical Package for Social Science (SPSS 20) Ready statistic program 20.

Result

Plasma glucose concentration (mg/dL)

Table (1) shows the mean value of plasma glucose concentration (mg/dL) of control and treated groups. Intraperitoneal injection of alloxan 100mg/kg B.W caused significant elevation ($P< 0.05$) in plasma glucose concentration in alloxan induced groups compared with the control groups. While oral gavages of palm leaves extract G1 or glibenclamide G2 caused significant decrease in the elevated plasma glucose concentration with mean value (148.46±4.82) compared with +ve control G3 with mean value (185.44±6.01) in day 30 of experiment indicating the hypoglycemic effect of palme leaves extract in alloxan-induced hyperglycemic rats. However this value is higher than the G2 group (130.06±3.25) value which was treated glibenclamide.

Table (1) Effect of palm leaves extract and glibenclamide on plasma glucose concentration (mg/dL) in alloxan induced diabetic rats.

Days / Groups	15	30
G1	158.90±4.15aAB	148.46±4.82aB
G2	144.13±3.87aB	130.06±3.25bC
G3	164.66±5.16aA	185.44±6.01bA
C	107.01±6.36aC	113.67±4.54aD

-C= control.

- G1=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + Palm extract (200mg/kg orally).
- G2=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + glibenclamide (5mg/kg orally).

ISSN 2307-8073

- G3=Animal intraperitonealy injected with alloxan (100mg/kg B.W.).
-Capital letter denote difference between groups, P< 0.05.
-small letter denote difference within groups, P< 0.05.
± SE

Plasmaaspartate aminotransferase (AST) and plasma alanine aminotransferase (ALT) activity (U/L)

Tables (2) and (3) illustrate the mean value of plasma aspartate aminotransferase (AST) and plasma alanine aminotransferase (ALT) activity (U/L) of control and treated groups. The results show that intraperitoneal injection of alloxan caused significant (P< 0.05) increase in AST and ALT activity in all treated groups compared with control group at day 15 of experiment. Glibenclamide orally gavages in G2 group caused significant (P< 0.05) decrease in AST activity with mean value (16.26±0.93)compared with control +ve G3groups (21.58±1.41) at day 30 of experiment as well as, non-significant(P> 0.05) difference between G1 and G2 which received palm leaves extract and glibenclamide respectively when compared each other at the same period. Beside, orally gavages Palm leaves extract orally gavages group G1 and glibenclamide G2 revealed significant (P>0.05) decrease in the elevated ALT activity, (10.12±0.52) and (8.60±0.61) respectively in compare with group G3 (14.85±0.45) . In addition, plasma alanine aminotransferase ALT in G1and G2 appears near to the value of the control group C (9.54±1.02) at the end of experiment.

Table (2) Effect of palm leaves extract and glibenclamide on plasma aspartate aminotransferase (AST) activity (U/L) in alloxan induced diabetic rats.

Days / Groups	15	30
G1	30.95±2.42aA	18.87±0.83bAB
G2	26.13±1.08aA	16.26±0.93bBC
G3	27.17±2.26aA	21.58±1.41bA
C	14.37±1.28aB	13.54±0.56aC

-C= control.
- G1=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + Palm extract (200mg/kg orally).
- G2=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + glibenclamide (5mg/kg orally).
- G3=Animal intraperitonealy injected with alloxan (100mg/kg B.W.).
-Capital letter denote difference between groups, P< 0.05.
-small letter denote difference within groups, P< 0.05.
± SE

Table (3) Effect of palm leaves extract and glibenclamide on plasma alanine aminotransferase (ALT) activity (U/L) in alloxan induced diabetic rats.

Days / Groups	15	30
G1	19.59±2.04aA	10.12±0.52bB
G2	18.15±1.05aA	8.60±0.61bB
G3	20.57±0.86aA	14.85±0.45bA
C	9.09±0.57aB	9.54±1.02aB

-C= control.

ISSN 2307-8073

- G1=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + Palm extract (200mg/kg orally).
- G2=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + glibenclamide (5mg/kg orally).
- G3=Animal intraperitonealy injected with alloxan (100mg/kg B.W.).

-Capital letter denote difference between groups, $P< 0.05$.
-small letter denote difference within groups, $P< 0.05$.
± SE

Discussion

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial destruction of the β-cells of Islets of Langerhan's (Szkudelski, 2001). It causes to elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides (Dhanabal, 2007). The results of this study revealed that palm leaves extracts have good anti-diabetic activity and exhibited significant anti-hyperglycemic activity in alloxan-induced hyperglycemic rats; they can also improve the condition of Diabetic mellitus as indicated by parameters like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity. Similar studyhad been performed by Rosalina, *et al*., (2011). They showed that Oil palm leaves (OPL) ethanolic extract treatment dose-dependently reduced blood glucose and oxidation in the STZ rats, and restored antioxidants enzymes levels. The optimum dose was 100mg/kg, which effectively reduced liver and kidney damage to the level of normal rats.

The renewal of β cells in diabetes have been studied in several animal models. The total β cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet ß cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug (Gorray, 1986).

This study demonstrated that palm leaves extract at orally dose (200 mg/kg B.W.) is effective and shows similar curative effects as standard drug glibenclamide orally dose (5mg/kg B.W.), which was used as a comparative drug. The anti-diabetic curative effective of palm leaves extract could be due to the possibility that some β-cells are still surviving to act upon by palm leaves extract to exert its insulin releasing effect. It is well known that certain flavonoids exhibit hypoglycemic activity and are able to help regenerate the beta cells of the pancreas. The significant antihyperglycaemic effect of palm leaves is probably due to it flavonoids contents (Mard, *et al.* 2010).

In conclusion, the results of this study strongly suggest that palm leaves extract is potentially useful for the alleviation of diabetic andits secondary complications. Further work is needed to investigate the actual active components in the palm leaves. The present investigation reports the first anti-hyperglycemic activity of palm leaves which may be a new potential alternative in the treatment and management of diabetes.

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ISSN 2307-8073

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