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Effect of aqueous extract of *Helianthus annus* on some biochemical parameters in alloxan-induced diabetic rats

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ABSTRACT

The hypoglycemic and other effect of the aqueous extract of Helianthus annus seed were studied in normal and alloxan-induced diabetic male rats. The phytochemical screening of the extract was also carried out. The aqueous extract was administered intraperitoneally at a dose level of 400mg/kg for 21 days to 2 groups of 4 rats each. The following biochemical parameters were determined in serum using standard laboratory procedures; Glucose, Urea, Creatinine, Total Protein, Aspartate aminotransferase, alanine aminotransferase, alkaline phosphate, Cl^- , K^+, and Ca^{2+}. The phytochemical screening shows the presence of Alkaloids, Saponins, tannins, flavonoids, cardiac glycosides, terpenes and steroids, balsam, carbohydrates and phenols in the extracts. Treatment with the Helianthus annus extract significantly ($p<0.05$) reduced elevated blood level of Glucose, Urea, Creatinine, Aspartate aminotransferese, Alanine aminotransferase and Alkaline Phosphatase associated with Alloxan- induced diabetic rats when treated with 400mg/kg body weight. There was also a significant ($P<0.05$) increase in the level of Cl^- and decrease in the level of K^+, Ca^{2+} when treated with the extract as compared with diabetic control. From the study, the sunflower seed have proved to be effective hypoglycaemic agent and therefore may be used for the management of diabetic mellitus at the said dosage.

Keywords: *Helianthus annus*, Diabetic mellitus, Hypoglycaemic, Phytochemicals, Alloxan.

INTRODUCTION

Plants form the main ingredients of medicine in traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs. Roughly about 50,000 species of higher plants have been used medicinally. This represents by far the biggest use of the natural world in terms of number of species. The medical uses of plant grade into their uses for other purpose as for food, cleaning, personal care and perfumery. Plants are used in medicine to maintain and augment health-physically, mentally and spiritually as well as to treat specific conditions and ailments. According to Erah [1], West African hypoglycaemic plants are grouped

into their phyto-pharmaceutical active ingredients. The hypoglycaemic activity is associated with its insulin sparing action resulting in an increased insulin activity as a result of a stimulation of pancreatic synthesis and secretion of hormone [2].

The *Helianthus annus* is an annual plant native to the Americans, that possess a large inflorescence (flowering head). It belongs to the family *Asteracease.* What is usually called the flower (formally composite flower) of numerous florets (small flowers) crowned together. The outer florets are the sterile ray florets and can be yellow, maroon, orange or other colours. The florets inside the circular head called disc florets, which mature into seeds, the florets with the sunflower cluster are arranged into a spiral pattern. Typically, each floret is oriented towards the next by approximately the golden angle of about 137.5^{o} production a pattern of interconnecting spirals where the number of left spirals and the number of right spirals are successive Fibonacci numbers. There are 34 spirals in one direction and 55 in the other; on a very large sunflower, there could be 89 in one direction and 144 in the other. This pattern produces the most efficient packing of seeds within the flower head.

MATERIALS AND METHODS

Animals Used

Twenty adult male albino wister rats weighing between 180-320g were used in this study. The rats were allowed to acclimatize to the laboratory condition for about three (3) weeks before any experimental work was undertaken. The rats were fed with standard feed (VITAL GROWERS MESH) from Grand Cereal and Oil Mills Limited, Jos, Plateau state and water ad libitum. The nutrients composition of the feed was crude protein 14.5%, fat 7.0%, crude fibre 7.2%, calcium 0.8%, and available phosphorus 0.4%.

Plant Source

Sunflower seeds (*Helianthus annus)* were purchased from Mangu L.G.A of Plateau state.

Preparation of sunflower seed extract

The *Helianthus annus* was collected, dried and the pounded using pestle and mortar into powdery form. The powdery seeds was poured into a beaker, mixed properly with water and then placed on an electric hot-plate to boil. The mixture was stirred continuously until it started boiling. Immediately, it boiled, it was filtered and the debris was discarded while the filtrate was placed in another beaker. The filtrate was evaporated to dryness at 60^{o} C; the dried extract was stored in a clean air-tight container.

Induction of experimental diabetes

Diabetes was induced in groups I and III rats by intraperitoneally injection (I.P) of Alloxan at a dose of 150mg/kg body weight.

Administration of sunflower extract solution

The *Helianthus annus* extract solution was administered through the oral route at a dose of 400mg/kg body weight daily for 21days; two days after experimental diabetes was induced.

Experimental Design

The twenty Albino Wister rats were randomly divided into four groups (I-IV) with five rats in each group. They were fed with standard feed and water ad libitum. Diabetes was induced in groups I and III (as diabetic treated and diabetic control respectively). The rats were grouped and fed as follows:-

GROUP I- diabetic rats treated, GROUP II- normal treated rats, GROUP III- diabetic control and GROUP IV- normal control

Sample collection and preparation

At end of the 21days of extract administration, blood from the animals (both treated and control groups) was collected from the jugular vein into plain bottles. Each of the blood was in the plain bottle was allowed to clot at room temperature. The clotted blood sample was ringed and centrifuged for 10minutes at 5,000r.p.m. Pasteur pipette was used to separate the serum (supernatant) into clean bottles. The serum was used for chemistry assay.

Analytical Methods

Phytochemicals screening

The phytochemical screening of the *Helianthus annus* seed extract was carried out using standard qualitative procedures [3, 4].

Quantitative determination of serum Glucose level

The determination of serum glucose was carried out using kit product of fortress diagnostics [5].

Determination of Serum Enzymes

The determination of serum enzymes concentration was carried out using kit product of fortress diagnostic [6, 7].

Statistical Analysis

All grouped data were evaluated statistically. The hypothesis method include on one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. $P<0.05$ was considered significant while $P>0.05$ was considered insignificant. All results are expressed as the Mean ± standard deviation (S.D) for animals in each group.

RESULTS

Phytochemical Screening

Table 1 shows the results obtained when extracts was screened for phytochemicals such as Alkaloids, flavonoids, cardiac glycosides, saponins, tannins, carbohydrates, balsam, phenols and terpenes and steroids. All were detected except for saponins in ethanol extract.

Table I. Results of phytochemicals screening of *Hellanthus annus* extracts.

Phytochemical	Water extract	Ethanol extract
Alkaloids	+	+
Saponins	+	-
Tannins	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Terpenes & Steroids	+	+
Balsam	+	+
Carbohydrates	+	+
Phenols	+	+

+ = Present, - = Absent

Biochemical Parameters

Table 2 below shows serum glucose, total protein, urea and creatinine concentrations in the diabetic and normal groups of rats. The diabetic rats showed a significant increase when

compared with normal control rats (P<0.05) while on administration of *Hellanthus annus* aqueous extract, there was a significant decrease (P<0.05) when compared with the diabetic control rats except for total protein which increased.

Table II: Results of the effects of aqueous extract of *Hellanthus annus* on serum glucose, total protein, urea and creatinine concentrations in normal and diabetic rats

Parameters	Diabetic + extract	Normal + extract	Diabetic control	Normal control
Glucose (mmol/L)	6.90 ± 0.29^{a}	5.90 ± 0.16^{b}	13.10 ± 0.30^{c}	6.20 ± 0.10
Total protein (g/dL)	4.1 ± 0.09^{d}	3.70 ± 0.48^{d}	2.70 ± 0.10	3.80 ± 0.47^{d}
Urea (mg/dL)	9.0 ± 0.14^{a}	7.0 ± 0.85^{b}	10.3 ± 0.07^{c}	7.40 ± 0.11
Creatinine (mg/dL)	0.40 ± 0.16^{a}	0.20 ± 0.22^{b}	0.60 ± 0.04^{c}	0.30 ± 0.37

Values are mean ±SD for four determinations, n=4.
Data are Mean ± standard deviation for four determinations, n=4
a = statistically significant decrease compared to diabetic control (P<0.05)
b = statistically insignificant compared with the normal control (P>0.05)
c = statistically significant increase compared with the normal control (P<0.05)
d = statistically significant increase compared with diabetic control (P<0.05)

Enzyme Activity

Table 3 below shows the result of Aspartate transminase activity (AST), Alanine transaminase (ALT) and Alkaline phosphatise (ALP) activity in the serum of the normal and diabetic groups of rats. The diabetic control rats showed statistically significant increase when compared with the normal control rats (P<0.05) while on administration of *Helianthus annus* aqueous extract, the normal treated showed no statistical significance (P>0.05) when compared with the normal control rats, the diabetic treated showed statistical decrease (P<0.05) when compared with the diabetic control.

Table III. Results of the effect of *Helianthus annus* on serum enzymes activity in normal and diabetic rats

Groups	Enzyme activity		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Diabetic + extract	55.0 ± 0.13^{a}	33.0 ± 0.35^{a}	19.0 ± 0.23^{a}
Normal + extract	52.0 ± 0.28^{b}	31.0 ± 0.18^{b}	15.6 ± 0.05^{b}
Diabetic control	68.0 ± 0.24^{c}	52.0 ± 0.55^{c}	25.0 ± 0.43^{c}
Normal control	55.0 ± 0.43	33.0 ± 0.17	16.5 ± 0.12

Values are mean ±SD for four determinations, n=4.
a = statistically significant decrease compared to diabetic control (P<0.05)
c = statistically significant increase compared with the normal control (P<0.05)
b = statistically insignificant compared with normal control (P>0.05)

TABLE IV: Results of the effect of *Hellanthus annus* on Potassium, Calcium and Chloride ions concentration in normal and diabetic rats

Groups	ELECTROLYTES		
	K^{+} (mM/L)	Cl^{-} (m/L)	Ca^{2+} (mg)
Diabetic + extract	7.0 ± 0.60^{c}	47.0 ± 0.16^{b}	8.9 ± 0.26^{c}
Normal + extract	7.0 ± 0.38^{d}	47.2 ± 0.03^{d}	9.3 ± 0.10^{d}
Diabetic control	5.7 ± 0.37^{a}	65.0 ± 0.037	7.7 ± 0.61^{a}
Normal control	7.2 ± 0.76	47.4 ± 0.10	8.1 ± 0.07

Values are mean ±SD for four determinations, n=4.
a = statistically significant decrease compared with Normal control (P<0.05)
b = statistically significant decrease compared with diabetic control (P<0.05)
c = statistically significant increase compared with diabetic control (P<0.05)
d = No statistically significant difference compared with normal control (P>0.05)

Electrolytes

Table IV below shows the level of potassium, chloride and calcium ions in the normal and diabetic groups of rats. The effect of treatment with *Helianthus annus* extract on potassium, and calcium ions showed significantly ($p<0.05$) increased compared to the diabetic control while chloride was significantly ($p<0.05$) reduced when compared to the diabetic control.

DISCUSSION

The results obtained from the preliminary phytochemicals screening of *Helianthus annus* extracts showed the presence of alkaloids, tannins, cardiac glycosides, carbohydrates, balsam, phenols, terpenes and steroids as shown in the table 1. Erah [1] reported that flavonoids are frequently found in plants with hypoglycaemic activity. The presences of cardiac glycosides are also known to reduce the effect of diabetic complications [8].

The table 2 shows the fasting blood serum glucose, total protein, and urea and creatinine levels in normal and diabetic groups of rats. There was significant increase in blood glucose, urea and creatinine levels in the alloxan-induced diabetic rats. The diabetic rats treated with *Helianthus annus* showed a marked decrease in their blood glucose, urea and creatinine levels ($P<0.05$). The normal rats treated with *Helianthus annus* extract showed no significant difference in blood glucose, urea and creatinine levels ($P>0.05$) when compared with the normal control. George (1998) reported that *Helianthus annus* lowers the level of glucose in the blood. Medicinal plants that exhibit anti-diabetic activity usually possess active substances which are able to mimic the action of insulin or which exert similar effect on the beta-cells of the pancreas, causing them to synthesize and secrete insulin [9].

The enzyme activity, Aspartate transaminase, Alanine transaminase and Alkaline phosphate levels in blood serum of normal and diabetic rats as shown in table 3, there was significant increase in the enzyme activity (AST, ALT and ALP) of the diabetic control ($P<0.05$) and on administration of *Helianthus annus* extract, there was significant decrease compared with the diabetic control ($P<0.05$). The increase in ALT activity in diabetes is always due to hepatocellular damage which is accompanied by AST activity [10]. The reversal of AST and ALT activity in *Helianthus annus* treated diabetic rats towards normalcy is evidence of prevention of cellular and tissue damage under diabetic condition [11].

In alloxan-induced diabetic rats, there was a significant decrease in the level of potassium, calcium ions when compared with normal control ($P<0.05$) on administration of *Helianthus annus* extract, there was no significant difference when compared with the normal control ($P<0.05$), but for Chloride ion level increased in diabetic rats when compared to normal control.
A fall in plasma potassium and ions arising due to a rise in blood glucose is as a result of the syndrome of hyperosmolar non-ketotic state [12]. The observed decrease in the serum levels K^+ ions of alloxan-induced diabetic rats in this study may be due to hyperosmolar non-ketosis. The significant increase of K^+ ions in serum of *Helianthus annus* extract treated diabetic rats as compared to the diabetic control seems to suggest that *Helianthus annus* enhances transfer of intracellular K^+ ions to the extracellular space there by reducing or preventing hyperosmolar non-ketotic state.

In this study it may be possible that the decrease in serum calcium ions of diabetic control rats may be due to decreased extra skeletal inability to absorb dietary calcium.

The observed significant elevation of serum Cl^- ion in this study is suggestive of renal tabular acidosis or metabolic acidosis. The lowering of serum Cl^- by *Helianthus annus* extract observed in this study would suggest that the extract enhances rehydration or prevent against metabolic acidosis.

In conclusion, the ability of *Helianthus annus* to significantly decrease the raised concentration of blood glucose, serum enzymes (AST, ALT and ALP) in diabetic rats proves that *Helianthus annus* possess anti-diabetic property. *Helianthus annus* does not possess toxicity effect as indicated by the lowered AST, ALT and ALP levels and may be beneficial in the management of diabetes mellitus.

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