

## Efficacy of Saponins From *Helianthus annuus* Roots on Antihyperglycemic, Antiperoxidative and Antihyperlipidemic Effects in Alloxan-Induced Diabetic Rats

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### ABSTRACT

The present study evaluates the antihyperglycemic, antiperoxidative and antihyperlipidemic activities of saponins from *Helianthus annuus* roots in alloxan induced diabetic rats. Thirty rat's weights between 100-150 g was used for the study and divided into six groups of five rats each. Group A was non-diabetic rats; the remaining five groups was induced intraperitoneal with 150 mg/kg of alloxan monohydrate. Group B was diabetic control, while group C, D, E and F was treated with saponin (100, 200, 300 and 500 mg/kg) for 21 days. Administration of saponins significantly reduced the elevated levels of glucose, decreased total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL) and increased high density lipoprotein (HDL) in the serum towards normalcy compared to the diabetic control ( $p < 0.05$ ). In addition, saponins exhibited strong inhibition of lipid peroxidation and increased the levels of antioxidant enzymes (superoxide dismutase and catalase) in the liver, kidney and pancreas compared to the diabetic control ( $p < 0.05$ ). Results suggest that saponins from *Helianthus annuus* root can enhance the antihyperglycemic, antihyperlipidemic and antioxidant properties in alloxan-induced diabetic rats, and may have the potential to be used in the prevention or in the management of diabetes.

**Keywords:** *Helianthus annuus* saponin roots, antihyperglycemic, antiperoxidative, antihyperlipidemic, antioxidant.

### INTRODUCTION

Diabetes mellitus (DM) is a key global health problem recognized as one of the leading causes of death worldwide, where the high prevalence of the disease could be attributed to improved nutritional status<sup>1,2</sup>. DM is defined as a metabolic condition characterized by an elevation of the blood glucose concentration due to lack of insulin leading to hyperglycemia. It is associated with abnormal metabolism of macromolecules like carbohydrates, fat and protein<sup>3-5</sup>. Hyperglycaemia in diabetic patients is linked with variations in glucose and lipid metabolism and alterations in liver enzyme levels<sup>6</sup>. Substantial evidence in the literature indicates that hyperglycemia can cause oxidative stress by various mechanisms. Though, excessive levels of glucose reaching the mitochondria lead to an overdrive of the electron transport chain, resulting in overproduction of superoxide anions which consequently result in damage of a variety of tissues<sup>7-9</sup>. Furthermore, hyperglycemia can stimulate oxidative stress by the autooxidation of glucose in the presence of transition metals as well as the generation of reactive oxygen species (ROS) during the process of glycation<sup>10</sup>. Hyperlipidemia has been incriminated as a contributory factor of atherosclerosis in

patients with diabetes<sup>11,12</sup>. Excessive intake of fatty acids leads to an accumulation of triglyceride in many tissues, particularly in the fat tissue, in which lipolysis is increased. In the liver which is the main organ of glucose metabolism, high free fatty acid concentration contributes to the resistant action of insulin by enhancing glucose output from the liver<sup>13</sup>.

*Helianthus annuus* L. is a folk remedy for bronchiectasis, bronchitis, carbuncles, catarrh, cold, colic, cough, diarrhoea, dysentery, dysuria, epistaxis, eyes, fever, flu, fractures, inflammations, laryngitis, lungs, malaria, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, urogenital ailments, whitlow, and wounds<sup>14</sup>. Anti-hyperglycaemic effects of these plants are due to their capability to improve the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes<sup>15</sup>. *Helianthus annuus* leaves are extensively used for whooping cough, asthma, anti-malaria, insect bites, snake bites, fevers and lung problems. Elsewhere, the leaves are used for treatment of diabetes<sup>16</sup>. Despite the usage of *Helianthus annuus* root in the management of diabetes mellitus, there is no scientific report on the

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antidiabetic activity of saponins from *Helianthus annuus* roots in alloxan-induced diabetic rats.

## MATERIALS AND METHODS

### Chemicals

Alloxan was purchased from Sigma (Sigma-Aldrich, Germany), while thiobarbituric acid was purchased from Fluka (Buchs, Switzerland). Randox kits were purchased from Randox Laboratories Limited, UK. The other reagents used for the experiment were of analytical grade.

### Plant Material

Roots of *Helianthus annuus* were collected at a local farm in the suburbs of Ado Ekiti, Nigeria. The plant was identified and authenticated by a senior taxonomist in the Department of Plant Science and a voucher specimen number (UHAE/2014/086) was deposited accordingly at the herbarium of Ekiti State University, Ado-Ekiti, Nigeria.

### Extraction of saponins from *H. annuus* root

A hundred gram (100 g) ground sample was extracted with 500 ml of petroleum ether (40–60 °C) in a Soxhlet extractor for 12 h. The air-dried, defatted sample was extracted with methanol (500 ml) for 12 h. The methanol extract was partitioned with n-butanol and water (1:1, v/v). After a thorough shaking, the mixture was allowed to stand overnight and the n-butanol layer was separated in the next day. The aqueous layer was washed five times with aliquots of n-butanol until it became colorless. The pooled butanol layer was evaporated under reduced pressure to give a residue which was dissolved in 100 ml methanol and precipitated by adding a large amount of diethyl ether to obtain a solid crystalline dark brown compound<sup>17</sup>.

### Animals

Thirty albino rats with an average weight of  $125 \pm 39$  g were obtained from Animal Unit Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. They were divided into six groups of five animals each and allowed to acclimatize to experimental condition for two weeks. They were housed in clean cages and maintained under standard laboratory conditions (temperature  $25 \pm 2$  °C with dark/light cycle 12/12 h). They were fed ad libitum on rat pellets (Top Feeds, Nigeria) and water. The principles of Laboratory Animal Care (Public Health Services, 1986) was followed throughout the duration of the experiment.

### Experimental protocol

Diabetes was induced through a single intraperitoneal injection of a freshly prepared alloxan solution in normal saline at a dose of 150 mg/kg body weight. Seventy-two hours later, the rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with blood glucose levels of 200–300 mg/dl) was chosen for the experiments. The rats (n = 30) were divided equally into 6 groups. Group I served as normal control, and were given 2 ml saline by gavage, group II served as diabetic control, groups III–VI were diabetic rats treated with saponin at doses of 100, 200, 300 and 500 mg/kg body weight respectively for 21 days. After 21 days of treatment, the rats were weighed and sacrificed by decapitation and the

blood collected into clean dry beakers for serum preparation and the liver, kidney and pancreas was used for the determination of malonaldehyde (MDA), catalase (CAT) and superoxide dismutase (SOD). Glucose levels were estimated using a glucometer. The tissues (liver, kidney and pancreas) were removed into 0.25 M ice cold sucrose solution in a ratio of 1:5 w/v.

### Biochemical parameters

Using the supernatant of the centrifuged homogenate of the liver and pancreas tissues, the SOD, GSH and CAT levels was determined according to the method described by Sun and Zigman<sup>18</sup> and Aebi<sup>19</sup> respectively; whereas, the level of lipid peroxidation was determined as described by Okhawa et al.<sup>20</sup>. The serum levels of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) was assayed by Randox commercial kit (United Kingdom).

### Statistical analysis

The data are expressed as mean  $\pm$  S.E.M. (standard error of mean). The differences among groups was analyzed using one-way analysis of variance (ANOVA). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 20.0 (SPSS Inc., Chicago, USA), was used for this analysis. For fasting blood glucose, repeated measures ANOVA followed by Duncan Multiple Range Test was used. Statistical different is expressed at  $p < 0.05$ .

## RESULTS

### Effect of saponin on blood glucose

Table 1 showed that the effect of the saponin extract of *Helianthus annuus* roots on serum glucose in normal and diabetic rats. The results showed that serum glucose of diabetic rats increased when compared with normal rats. The administration of the saponin extract at doses of 100 ( $98.60 \pm 2.69$  mg/dl), 200 ( $102.60 \pm 6.11$  mg/dl), 300 ( $103.60 \pm 3.67$  mg/dl) and 500 ( $98.00 \pm 2.92$  mg/dl) mg/kg body weight tended to bring serum glucose ( $p < 0.05$ ) significantly toward normal values, while normal rats did not exhibit any significant alterations in serum glucose levels for the duration of the experiment.

### Effect of saponin on lipid peroxidation

Table 2 show the significant increase in ( $p < 0.05$ ) lipid peroxidation (LPO) products measured as thiobarbituric acid reactive substances in liver, kidney and pancreas of diabetes untreated rats compared to the normal control rats. However, treatment with saponin extract from *Helianthus annuus* roots significantly lowers the MDA concentration in the liver, kidney and pancreas with decreasing dosage compared to the diabetic control ( $p < 0.05$ ). Noteworthy is the fact that the alterations in MDA levels was restored to normal levels in the pancreas and lower than that of the control level in the liver and kidney.

### Effect of saponin on the activity of antioxidant enzymes

Table 3 show that the activity of SOD was significantly decreased in alloxan-treated rats in liver, kidney and pancreas compared to the control ( $p < 0.05$ ). Similarly, to that of SOD, the activity of CAT (Table 4) was significantly reduced in alloxan-treated rats in liver,

Table 1: Oral administration of saponin from *Helianthus annuus* Roots on blood glucose (mg/dl).

Treatment	Fasting Blood Glucose Level			
	0 Day	3 <sup>rd</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
Control Group	88.60±1.03 <sup>a</sup>	87.40±0.60 <sup>a</sup>	86.80±0.80 <sup>a</sup>	89.80±0.86 <sup>a</sup>
Untreated Group	93.00±1.70 <sup>a</sup>	345.80±4.65 <sup>bc</sup>	412.80±1.83 <sup>c</sup>	458.80±6.67 <sup>b</sup>
Group A (100 mg/kg)	88.60±3.66 <sup>a</sup>	415.20±7.22 <sup>c</sup>	162.80±8.16 <sup>b</sup>	98.60±2.69 <sup>a</sup>
Group B (200 mg/kg)	88.60±2.69 <sup>a</sup>	326.40±5.32 <sup>bc</sup>	139.00±5.57 <sup>b</sup>	102.60±6.11 <sup>a</sup>
Group C (300 mg/kg)	88.20±2.44 <sup>a</sup>	290.60±9.66 <sup>b</sup>	168.40±7.28 <sup>b</sup>	103.60±3.67 <sup>a</sup>
Group D (500 mg/kg)	90.60±2.99 <sup>a</sup>	376.00±7.42 <sup>bc</sup>	178.40±8.32 <sup>b</sup>	98.00±2.92 <sup>a</sup>

\*Values are expressed as mean of five determinations ± SEM.

\*Row values with different superscripts are significantly (p<0.05) different.

Table 2: Effect of saponin from *Helianthus annuus* Roots on MDA of diabetic rats.

Tissues	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
Liver	6.15±0.24 <sup>a</sup>	14.88±1.16 <sup>c</sup>	2.68±0.42 <sup>b</sup>	2.87±0.50 <sup>b</sup>	2.90±0.38 <sup>b</sup>	3.89±1.09 <sup>d</sup>
Kidney	6.67±0.43 <sup>a</sup>	12.81±1.33 <sup>b</sup>	4.40±0.76 <sup>a</sup>	4.52±1.01 <sup>a</sup>	4.60±0.74 <sup>a</sup>	4.74±1.19 <sup>a</sup>
Pancreas	3.45±1.15 <sup>a</sup>	7.47±0.85 <sup>c</sup>	1.45±1.00 <sup>b</sup>	1.51±0.32 <sup>b</sup>	1.77±0.30 <sup>b</sup>	2.10±0.12 <sup>b</sup>

\*Values are expressed as mean of five determinations ± SEM.

\*Row values with different superscripts are significantly (p<0.05) different.

Table 3: Effect of saponin from *Helianthus annuus* Roots on SOD of diabetic rats.

Tissues	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
Liver	96.75±2.98 <sup>c</sup>	34.27±1.49 <sup>a</sup>	89.65±1.83 <sup>b</sup>	87.87±1.20 <sup>b</sup>	88.67±1.33 <sup>b</sup>	90.27±2.40 <sup>b</sup>
Kidney	96.07±1.99 <sup>c</sup>	35.09±1.37 <sup>a</sup>	86.07±3.62 <sup>b</sup>	87.47±0.80 <sup>b</sup>	90.12±2.19 <sup>bc</sup>	90.04±2.20 <sup>bc</sup>
Pancreas	95.94±1.73 <sup>b</sup>	33.33±1.33 <sup>a</sup>	88.29±4.28 <sup>b</sup>	87.73±4.17 <sup>b</sup>	87.56±2.24 <sup>b</sup>	87.23±3.94 <sup>b</sup>

\*Values are expressed as mean of five determinations ± SEM.

\*Row values with different superscripts are significantly (p<0.05) different.

Table 4: Effect of saponin from *Helianthus annuus* Roots on CAT of diabetic rats.

Tissues	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
Liver	331.39±2.00 <sup>b</sup>	118.91±1.62 <sup>a</sup>	310.67±1.85 <sup>c</sup>	289.43±3.07 <sup>d</sup>	226.01±2.21 <sup>e</sup>	193.47±1.61 <sup>f</sup>
Kidney	558.81±2.28 <sup>b</sup>	284.79±1.93 <sup>a</sup>	529.56±2.49 <sup>c</sup>	488.05±2.70 <sup>d</sup>	401.88±1.39 <sup>e</sup>	348.02±2.63 <sup>f</sup>
Pancreas	544.31±1.90 <sup>b</sup>	258.08±3.51 <sup>a</sup>	519.45±2.45 <sup>c</sup>	490.82±2.52 <sup>d</sup>	427.44±2.49 <sup>e</sup>	392.62±1.91 <sup>f</sup>

\*Values are expressed as mean of five determinations ± SEM.

\*Row values with different superscripts are significantly (p<0.05) different.

kidney and pancreas compared to the control (p < 0.05). Though, the levels of SOD and CAT was significantly restored after saponin treatment of *Helianthus annuus* roots suggesting that saponins have effective antioxidative properties and could scavenge excess free radicals (p < 0.05). However, Table 5 show the glutathione reduced (GSH) levels in the liver, kidney and pancreas of diabetes-induced rats, the activities of GSH decreased significantly relative to the control. *Helianthus annuus* treatment to the diabetes-treated groups caused a significant (p < 0.05) increase in the GSH level.

#### Effect of saponin on the lipid profile of diabetic rats

Table 6 show that there was a significant increase (p < 0.05) in TG, TC, LDL and VLDL and a significant reduction in HDL in the serum of the diabetic control

compared to the normal control. Administration of saponin extract of *Helianthus annuus* roots significantly brought back these parameters in dose dependent manner compared to the diabetic control (p < 0.05). However, the reversal of the lipid profile by saponin in diabetic treated rats was even lower than that of normal control, suggesting that saponin itself seems to have lipid lowering potentials.

#### DISCUSSION

*Diabetes mellitus* is a metabolic syndrome characterized by hyperglycemia and alterations in carbohydrate, fat, and protein metabolism, associated with deficiencies in insulin secretion or insulin action. In the present study, for the first time the normoglycemic and anti-diabetic

Table 5: Effect of saponin from *Helianthus annuus* Roots on GSH of diabetic rats.

Tissues	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
Liver	243.21±1.02 <sup>c</sup>	175.14±8.9 <sup>a</sup>	225.59±3.23 <sup>b</sup>	238.30±1.90 <sup>bc</sup>	238.58±1.59 <sup>bc</sup>	243.10±4.29 <sup>bc</sup>
Kidney	243.17±1.01 <sup>c</sup>	160.62±2.79 <sup>a</sup>	236.38±2.01 <sup>b</sup>	237.79±1.13 <sup>bc</sup>	237.18±1.45 <sup>b</sup>	238.98±2.47 <sup>b</sup>
Pancreas	241.51±0.96 <sup>c</sup>	157.31±1.64 <sup>a</sup>	235.46±1.81 <sup>b</sup>	236.69±1.50 <sup>b</sup>	237.03±1.78 <sup>bc</sup>	239.58±2.40 <sup>b</sup>

Values are expressed as mean of five determinations ± SEM.

\*Row values with different superscripts are significantly (p<0.05) different.

Table 6: Effect of saponin from *Helianthus annuus* roots on lipid profile of diabetic rats.

Parameters	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
TC (mg/dl)	360.2±1.77 <sup>e</sup>	393.8±1.46 <sup>f</sup>	236.0±4.45 <sup>a</sup>	285.8±5.54 <sup>b</sup>	321.8±4.35 <sup>c</sup>	342.0±4.09 <sup>d</sup>
TG (mg/dl)	62.0±1.30 <sup>c</sup>	125.6±3.54 <sup>e</sup>	34.0±3.85 <sup>a</sup>	51.60±3.87 <sup>b</sup>	70.80±4.13 <sup>c</sup>	94.80±3.72 <sup>d</sup>
HDL (mg/dl)	174.0±1.82 <sup>d</sup>	40.60±2.93 <sup>a</sup>	186.2±4.91 <sup>e</sup>	166.40±6.62 <sup>d</sup>	115.4±4.15 <sup>c</sup>	81.60±6.10 <sup>b</sup>
VLDL (mg/dl)	12.6±0.24 <sup>c</sup>	26.2±0.80 <sup>e</sup>	6.80±0.77 <sup>a</sup>	10.32±0.77 <sup>b</sup>	13.90±0.75 <sup>c</sup>	19.0±0.84 <sup>d</sup>
LDL (mg/dl)	173.60±1.12 <sup>c</sup>	327.0±2.68 <sup>e</sup>	43.0±4.74 <sup>a</sup>	109.28±1.22 <sup>b</sup>	192.40±7.70 <sup>c</sup>	241.40±3.17 <sup>d</sup>

\*Values are expressed as mean of five determinations ± SEM.

\*Key: TC – Total Cholesterol, TG – Triglycerides, HDL- High density lipoprotein, VLDL – Very Low density lipoprotein, LDL – Low density lipoprotein.

\*Row values with different superscripts are significantly (p<0.05) different.

effect of saponins, secondary metabolite from *H. annuus* roots which is acclaimed to have an anti-diabetic activity was investigated. Results demonstrated that administration of saponin from *H. annuus* roots showed marked antihyperglycemic, antiperoxidative and antihyperlipidemic effects in alloxan-induced diabetic rats. Alloxan a toxic glucose analog widely used to induce experimental diabetes in animals. It selectively destroys insulin-producing cells in the pancreas in animals, establish a redox cycle with the formation of superoxide radicals and undergoes dis-mutation to hydrogen peroxide with the formation of hydroxyl radicals<sup>21-23</sup>. However, insulin deficiency (Type-I diabetes) or decreased insulin action (type-II diabetes) results in a decrease in glucose utilization by insulin requiring tissues like liver and an elevation in glucose production through an increased rate of gluconeogenesis; both resulting in hyperglycemia. Thus, the antihyperglycemic effect of saponin from *H. annuus* root may be due to the restoration of insulin response or induction of enzymes inhibited by alloxan as shown in Table 1<sup>6</sup>.

Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation. The generation of free radicals may lead to lipid peroxidation and associated complications in diabetes mellitus. Administration of saponin extract from *H. annuus* root to diabetic rats (Table 2) significantly decreased the levels of lipid peroxidation (LPO) at all concentrations in group A, B, C and D. It is probable that ROS may induce LPO and modify SOD, CAT and treatment with the saponin extract may mitigate this action. It is suggested that the saponin extract from *Helianthus annuus* root may function as an anti-diabetic

agent and lower alloxan-induced lipid peroxidation damage and oxidative stress in diabetes. Increased in the activities of these de-novo antioxidant enzymes might be responsible for the scavenging effect, with subsequent protection of cells against lipid peroxidation. This present study also substantiated the earlier report of an increase in hepatic and renal MDA concentration in alloxan induced diabetic rats compared with the normal rats. The treatment with saponin extract from *Helianthus annuus* root lowers significantly the MDA concentration in the liver, kidney and pancreas with decreasing dosage (500 mg/kg, 300 mg/kg, 200 mg/kg, 100 mg/kg) compared with the diabetic control (p < 0.05) indicating that, saponin extract from *H. annuus* root could inhibit oxidative damage due to the anti-peroxidative effect of the ingredients present. Diabetes mellitus is linked with increased oxidative stress and decreased antioxidant status<sup>24</sup>. As presented in Table 3, the activity of SOD was significantly decreased in alloxan treated rats in the serum, liver and pancreas compared to the control (p < 0.05). The decreased levels of CAT and SOD observed in diabetic rat can be explained by the accumulation of superoxide anion and hydrogen peroxide respectively, which would have otherwise been effectively scavenged by these enzymes. According to the results obtained, the levels of SOD and CAT was significantly restored after saponin treatment, suggesting that saponins have effective antioxidative properties and could scavenge excess free radicals (p < 0.05, Tables 3 and 4). Consequently, saponin may have an efficient protective mechanism in response to ROS which may help to regenerate β-cells and protect pancreatic islets against cytotoxic effects of alloxan. However, decrease in GSH levels could probably be due to its increased use by the

hepatic cells as a result of decreased synthesis or increased degradation of GSH by oxidative stress<sup>25,26</sup>.

There was a significant ( $p < 0.05$ ) decrease in HDL and a significant increase in triglycerides (TG), total cholesterol (TC), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the serum of the diabetic control compared to the normal control. Furthermore, the diabetic treated group administered with saponin extract from *H. annuus* root had these parameters reversed even lower than that of normal control. The level of serum lipids is usually raised in diabetes due to the increase of blood glucose and such an elevation represents a risk factor for coronary heart disease<sup>27</sup>. The decrease in TG observed in this study, could be due to various factors which may include: i) decrease in fatty acid synthesis, ii) enhanced LDL receptors, iii) activation of Lecithin-cholesterol acyl transferase (LCAT) and lipases, and also iv) inhibition of acetyl-CoA carboxylase. The observed reduction in TC level in diabetic rats could be due to a decrease in cholesterol absorption from the intestine, through binding to bile acids, and an increase in fecal bile acid excretion<sup>28,29</sup>.

## CONCLUSION

Overall, the present study demonstrated for the first time the anti-diabetic effect of saponin from *H. annuus* root which was observed by attenuating the hyperglycemia-mediated oxidative stress, and hyperlipidemia, a contributory factor of atherosclerosis. However, saponin was effective enough to alleviate alloxan-induced diabetes by decreasing the level of lipid peroxidation and increasing the antioxidant defense system in the liver, kidney and pancreas. Results suggest that the beneficial effect of saponins is at least in part, mediated by its antioxidant, anti-dyslipidemic and antihyperglycemic activities and seems to justify the traditional use of *H. annuus* root.

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## COMPETING INTERESTS

The authors declare no conflict of interest.

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