

Hepatoprotective Activities of Ethanolic Roots Extract of *Ageratum Conyzoides* on Alloxan-Induced Hepatic Damage in Diabetic Wistar Rats

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J Morphol Sci 2019;36:39–45.

Abstract

Introduction The aim of the present study was to evaluate the hepatoprotective activities of the ethanolic roots extract of *Ageratum conyzoides* (AC) in alloxan-induced hepatic damage in diabetic rats.

Materials and Methods Diabetes was induced in Wistar rats by the administration of alloxan (150 mg/kg, intraperitoneal). The ethanolic roots extract of AC, at doses of 250 and 500 mg/kg of body weight, was administered to diabetes-induced rats at a single dose per day for a period of 28 days.

Results The effect of the ethanolic roots extract of AC on blood glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and hepatic oxidative stress markers was measured in the diabetic rats. The ethanolic roots extract of AC exhibited significant reduction of blood glucose ($p < 0.05$) at the dose of 500 mg/kg when compared with the standard drug glibenclamide (600 µg/kg). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels increased significantly ($p < 0.05$) in the diabetic group without treatment when compared with the control group. In addition, the levels of oxidative stress markers, such as superoxide dismutase (SOD), catalase (CT), glutathione peroxidase (GPx), and glutathione (GSH), were significantly decreased in the diabetic rats compared with the normal rats, while the lipid peroxidation significantly increased in the diabetic group without treatment compared with the control (normal) group. The results demonstrated that the morphological, functional and oxidative stress changes in the liver caused by the ingestion of alloxan were attenuated in diabetic rats treated with the ethanolic roots extract of AC.

Conclusion We concluded that the ethanolic roots extract of AC possesses significant antidiabetic, antioxidant and hepatoprotective effects on alloxan-induced diabetic rats.

Keywords

- ▶ hepatoprotective activity
- ▶ antioxidants
- ▶ alloxan
- ▶ rats
- ▶ *ageratum conyzoides*

Introduction

Diabetes mellitus (DM) is a chronic carbohydrate, lipid and protein metabolic disorder that contributes to several kinds of complications, including diabetic hepatopathy. Diabetes

mellitus has now become an epidemic, with a worldwide incidence of 5% in the general population.¹

Some reports have shown that antioxidant treatment reduces diabetic complications and protects the cellular components from oxidative damage.² It has been reported

received

April 24, 2017

accepted

August 3, 2018

published online

November 14, 2018

DOI <https://doi.org/>

10.1055/s-0038-1669423.

ISSN 2177-0298.

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that diabetic complications are associated with the pathogenesis of many serious systemic diseases, such as the overproduction of reactive oxygen species (ROS), and the accumulation of lipid peroxidation byproducts.³

Dietary fibers supplementation with powerful antioxidants, such as terpenoids, flavonoids, vitamins and medicinal plants, has been used to prevent the occurrence of DM and its complications.⁴

Plants have been the major source of drugs for the management of DM in Nigerian herbal medicine and in other ancient systems in the world, and for a long time DM has been managed with the use of orally administered herbal medicines or their extracts,⁵ because plant products are frequently considered to be less toxic and to cause less side effects than synthetic ones.⁶

Ageratum conyzoides (AC) belongs to the Asteraceae family, which includes other medicinal plants like *Vernonia amygdalina* and *Tridax procumbens*, which have a proven antidiabetic potency.^{7,8} *A. conyzoides* is an annual herbaceous plant which has a wide tropical distribution and is widely used in Southern Nigeria; it is a tree of up to 30 m high with a wide crown, fissured bark and fragrant white flowers.⁹ The plant is commonly found in West Africa and in abundance particularly in the Southern part of Nigeria. It is found in the savannah regions and in the swampy areas of Nigeria. Its stems are covered with fine white hairs. It is commonly called goat weed in English, Imi esu and rerinkomi in Yoruba, igwulube in Igbo, alkama tuturuwa in Hausa, otiti in Efik, and nnyano in Ibibio. The root of AC is used in India for the treatment of fever and gastrointestinal diseases such as diarrhea, dysentery and intestinal colic with flatulence.¹⁰

In Brazil, the roots and leaves are of value in the treatment of malaria, ovarian inflammation, amenorrhea, dysmenorrhea, rheumatism and dysentery.^{11,12} In Cameroon and in the Congo, it is used traditionally to treat fever, rheumatism, headache, and colic.^{13,14} The leaves are also used in dressing wounds and burns, and they have been shown to present antibacterial activities.^{15,16} In Nigeria, the leaves and roots are useful in the treatment of boils, leprosy, skin diseases, eye diseases, and inflammations.¹⁷ It has been shown to possess antidiabetic,¹⁸ fertility,¹⁹ antispasmodic and muscle relaxation properties.²⁰ The leaves have been found to be a potential source of anti-diabetic agents.²¹

The aim of the present study was to investigate the potentials of the ethanolic root extract of AC as an antioxidant and hepatoprotective agent in rats with alloxan-induced DM.

Materials and Method

Collection of the Plant Material

The AC roots were collected from a cultivated farmland at Ijebu Ilugun, northeast of Ijebu, Ogun State, Nigeria, in the month of June 2016. The plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), where a voucher specimen has been deposited in the herbarium (FHI 107873).

Preparation of the Plant Extract

The roots of the plant were shade-dried at room temperature for 7 days and then powdered using mortar and a pestle. The root powder (2,500 g) was soaked in a solution of 96% ethanol containing 4% water in 3 cycles using a Soxhlet extractor. The crude extract was filtered with Whatman filter paper 4 (Whatman, Maidstone, Kent, UK), and the filtrate was concentrated and dried in a rotary vacuum evaporator under reduced pressure in vacuo at 30° C to obtain 287.2 g of dry residue (11.5% volume) to yield a viscous brownish-colored extract that was stored in an airtight bottle kept in a refrigerator at 4° C until used.

Preliminary Phytochemical Analysis

The preliminary phytochemical screening of the powdered roots of AC for carbohydrates, glycosides, flavonoids, terpenoids, tannins, saponins, steroids and alkaloids was performed according to standard laboratory procedures.^{22,23}

Laboratory Animals

Twenty-five healthy Wistar rats weighing between 180 and 200 g were obtained from the Laboratory Animal Center of the College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The rats were housed in clean metallic cages and kept in a well-ventilated room at 24 ± 2°C at the Animal House of the Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ikenne, Ogun, Nigeria, with a 12 h light/dark cycle throughout the experimental period and were allowed to acclimatize to the laboratory condition for 1 week before being used. They were fed with standard animal pellet (Pfizer Feeds Plc., Ikoyi, Lagos, Nigeria) and had free access to water *ad libitum*.

Acute Toxicity Study

The toxicity study was performed using a group of 5 rats weighing an average of 200 g, after depriving them of food overnight with access to water only. The rats fasted for 14 hours before being administered a single dose of 5,000 mg/kg of the ethanolic roots extract of AC by gavages. They were closely monitored in the first 4 hours and then hourly for the next 12 hours, followed by hourly intervals for the next 48 hours after the drug administration to observe any deaths or changes in general behavior and in other physiological activities. The animals did not show any mortality at the dose administered, since 1/10th of the regular dose (500 mg/kg of body weight [bwt]) was chosen as the highest extract dose. The dose used is consistent with previous finding on the plant.²⁴

Alloxan-induced Diabetic Experiment

Alloxan was purchased from the representative of the Sigma Company (Zayo-Sigma Chemicals Ltd, Jos, Nigeria) and was prepared in freshly normal saline solution. Diabetes was induced by intraperitoneal (IP) injection of alloxan monohydrate (150 mg/kg bwt) in a volume of 3 mL.²⁵ After 72 hours, blood was drawn for blood glucose estimation monitored with an Infopia Finetest glucometer (Infopia Co Ltd., Anyang, Gyeonggi, Korea). The animals with blood

glucose level ≥ 250 mg/dl were considered diabetic and included in the experiment.²⁶

The diabetic animals were randomly distributed into four groups of five animals each while the last group, the positive control, had five normal rats.

The treatments for each group were as follows:

Group I: Normal rats (positive control).

Group II: Alloxan diabetic untreated (control negative).

Group III: Alloxan diabetic treated with glibenclamide at a dose of 600 $\mu\text{g}/\text{kg}$ bwt²⁶

Group IV: Alloxan diabetic treated with AC at a dose of 250 mg/kg bwt.

Group V: Alloxan diabetic treated with AC at a dose of 500 mg/kg bwt.

Weight Variation: The animals were initially weighed and then weighed every 7 days from the beginning of the treatment until the 28th day.

Animal Sacrifice

After 28 days of treatments (24 hours after the last dose), the animals were anaesthetized with diethyl ether and the blood was collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 4,000 rpm for 5 minutes to obtain the sera. The abdominal cavity of each rat was opened through a midline abdominal incision to expose the liver. The liver was excised and then weighed with an electronic analytical and precision balance. The liver of each animal was fixed in Bouin fluid for histological procedures.

Histological Analysis

The liver tissues were carefully dissected, and the organs were cut on slabs ~ 0.5 cm thick and fixed in Bouin fluid (10%) for 1 day, after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 minutes each in an oven at 57°C. Serial sections 5 μm thick were obtained from a solid block of tissue and were stained with hematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following the clearance in xylene, the tissues were oven-dried. Photomicrographs were taken.

Serum Alanine Aminotransferase and Aspartate Aminotransferase Parameters

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured spectrophotometrically as described by the method of Reitman et al (1957),²⁷ as modified by Rajan et al (2012).⁵

Hepatic Antioxidant Enzymes Assay

Assay of catalase (CT) activity: The catalase activity was evaluated according to the method described by Chance et al (1955).²⁸ The catalase activity was assayed spectrophotometrically in the supernatants by measuring the decrease in absorbance of H_2O_2 , and it was expressed as μmg^{-1} protein.

Superoxide Dismutase Activity Assay

The superoxide dismutase (SOD) activity was evaluated according to the method described by Marklund et al (1974).²⁹ It was expressed as μmg^{-1} protein.

Glutathione Peroxidase Activity Assay

The glutathione peroxidase (GPx) activity was determined by the method described by Pagila et al (1967).³⁰ The absorbance of the product was read at 430 nm and it was expressed as nmol^{-1} protein.

Liver Reduced Glutathione Concentration Assay

The reduced glutathione (GSH) was measured according to the method described by Rukkumani et al (2004).³¹ The absorbance was read at 412 nm, it was expressed as nmol^{-1} protein.

Lipid Peroxidation (Malondialdehyde) Assay

The lipid peroxidation in the liver tissue was measured colorimetrically by the thiobarbituric acid reactive substance (TBARS) method described by Park et al (2002).³² The concentration was estimated using the molar absorptivity of malondialdehyde, which is $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and it was expressed as nmol mg^{-1} protein.

Statistical Analysis

The Student t-test was used, and differences were considered significant at $p < 0.05$. All data were expressed as mean \pm standard error of the mean.

Results

The phytochemical analysis revealed the presence of carbohydrates, glycosides, flavonoids, terpenoids, steroids, saponins, tannins, and alkaloids (**Table 1**).

Effect on the Body Weight of Male Rats

The control group (I) gained weight over the 4 weeks of the experimental period, with the mean body weight increasing by 22.2 g after 4 weeks (**Table 2**). In contrast, the untreated

Table 1 Phytochemical composition of the extract of the roots of *Ageratum conyzoides*

Phytochemical	Availability
Carbohydrates	+
Glycosides	+
Cardiac glycosides	+
Saponins	+
Steroids	+
Flavonoids	+
Terpenoids	+
Anthracene derivatives	-
Alkaloids	+
Tannins	+

Abbreviations: +, present; -, absent.

Table 2 Body weight changes

Groups	Initial body wt (g)	Final body wt (g)	Difference in body wt (g)	Liver wt (g)
I	185.4 ± 0.7	197.6 ± 1.1	22.2	9.2 ± 1.0
II	192.8 ± 2.0	168.7 ± 2.2	-24.11	2.8 ± 0.8
III	195.6 ± 1.4	222.9 ± 1.7	27.3	8.5 ± 0.7
IV	189.2 ± 2.5	199.6 ± 1.5	10.4	7.4 ± 0.4
V	185.6 ± 1.8	198.3 ± 1.8	12.7	7.9 ± 1.0

Abbreviations: g, grams; wt, weight.

diabetic group (II) lost an average of 24.1 g after 4 weeks ($p < 0.05$). The treatments with glibenclamide and AC resulted in significant weight gain to levels approaching the control group (groups III, IV and V, versus group I). The mean liver weight in the diabetic untreated group decreased significantly compared with that of the control group, while the mean liver weight of the diabetic group treated with AC and glibenclamide decreased by improving the restoring activity of the extract of AC to the weight lost due to the administration of alloxan.

Effect on Blood Glucose Level

The blood glucose level in the diabetic group was significantly higher ($p < 0.05$) than that of the control group (→ Fig. 1). On the other hand, the administration of the ethanolic roots extract of AC for 28 days was found to lower significantly the blood glucose levels in a dose dependent manner in the treated diabetic groups ($p < 0.05$) when compared with those of the diabetic untreated (negative) group. The antihyperglycemic effect of the ethanolic extract of AC (250 /500 mg/kg) was found slightly effective than the reference standard

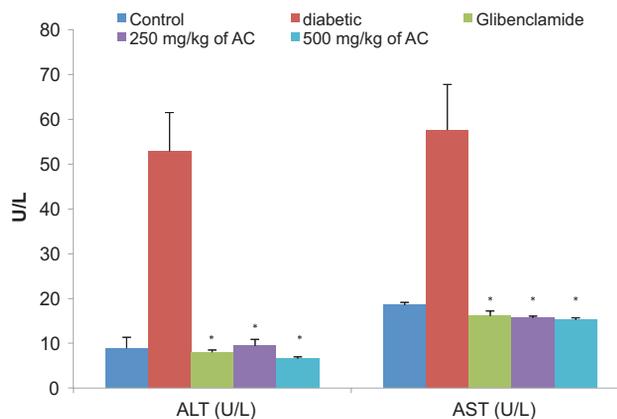


Fig. 2 Biochemical profiles of untreated diabetic rats; diabetic but treated with glibenclamide and extract respectively; and the normal rats untreated for 4 weeks. Mean ± SD, (n = 8) * $p < 0.05$ vs control group. Abbreviations: AC, *Ageratum conyzoides*; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

glibenclamide produced a significant reduction in blood glucose compare with diabetic control.

Effect on Some Biochemical Parameters

The study also indicates that ALT and AST increased significantly ($p < 0.05$) in the diabetic group compared with the control group, as shown in → Fig. 2. However, the administration of the ethanolic roots extract of AC for 28 days was capable of slightly lowering the ALT and AST levels in the diabetic groups.

Effect on Hepatic Antioxidant Enzymatic and Non-enzymatic Markers

The present study indicated that the diabetic group showed a statistically significant decrease ($p < 0.05$) in SOD, CT and GPx

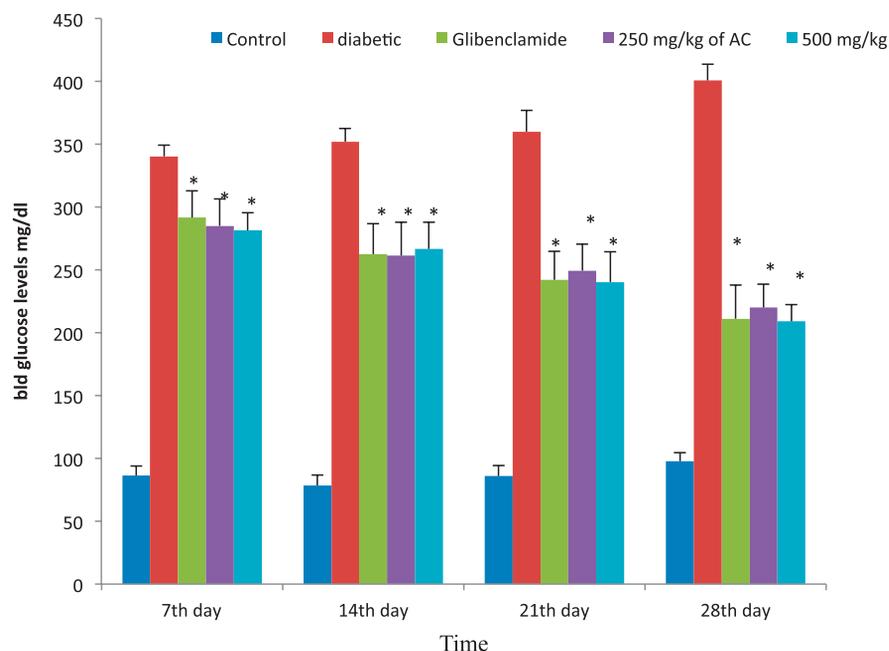


Fig. 1 Blood glucose level of Wistar rats treated with glibenclamide and ethanolic roots extract of *Ageratum conyzoides* for 4 weeks. Values represent Mean ± SEM (n = 8). $P < 0.05$ vs control group*: Statistically significant when compared with control group at $p < 0.05$.

activities compared with normal rats without treatment. Diabetic rats treated with AC significantly increased ($p < 0.05$) the liver SOD, CT and GPx activities compared with diabetic untreated animals, however. There was no significant ($p < 0.05$) change in the liver content of GSH and MDA on the diabetic group following the treatment with AC when compared with the normal rats without treatment. However, a significant reduction ($p < 0.05$) in the GSH content as well as a significant increase in the MDA content were observed in the diabetic untreated group when compared with the normal animals. The diabetic group treated with AC, however, presented a significantly elevated liver content of

GSH, but also a significantly reduced liver content of MDA compared with the diabetic untreated group.

Hepatocytoarchitectural Findings

The cytoarchitecture of the normal hepatic tissues (→ **Fig. 3a**) showed hepatocytes radially arranged from the lobular margins toward the center vein with each column interspaced by sinusoids.

The hepatic tissues of the diabetic group (→ **Fig. 3b**) showed a marked distortion of the liver cytoarchitecture resulting from the degeneration of the hepatic parenchyma.

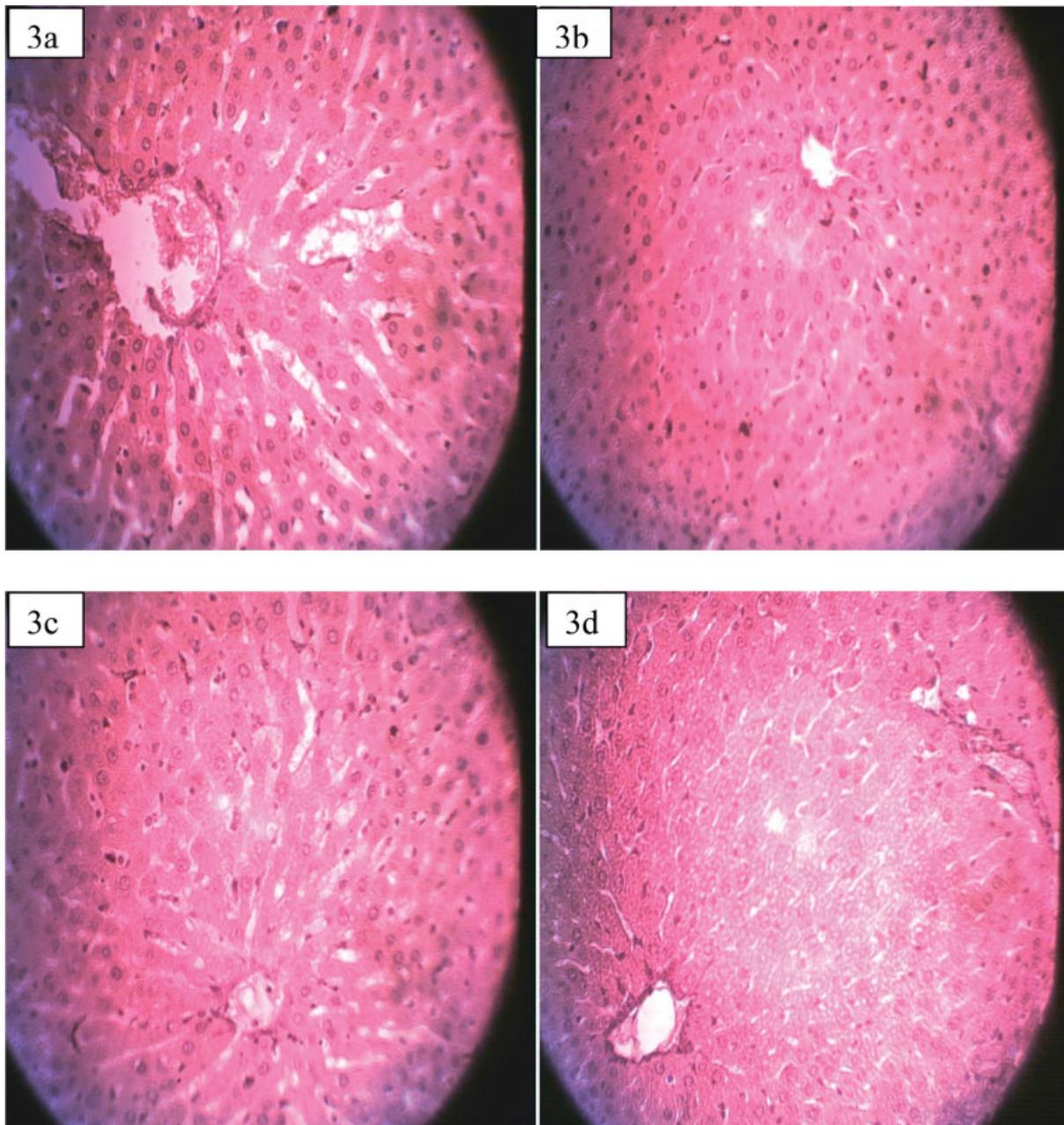


Fig. 3 (a) Cross section of (Group I) normal rat (positive control) stained with H and E $\times 400$. (b) Cross section of (Group II) diabetic rat (negative control) stained with H and E $\times 400$, Figure (c) Cross section of (Group III) diabetic rat treated with glibenclamide stained with H and E $\times 400$, (d): Cross section of (Group IV) diabetic rat treated with *Ageratum conyzoides* stained with H and E $\times 400$.

Table 3 Effect of oral administration of *Ageratum conyzoides* extract after 4 weeks on hepatic antioxidant enzymatic in alloxan-induced diabetic male rats

Parameters	Groups				
	I	II	III	IV	V
CT (μmg^{-1} protein)	17.2 \pm 1.6	5.2 \pm 2.4*	15.8 \pm 2.8**	13.9 \pm 2.6**	14.7 \pm 1.4**
SOD (μmg^{-1} protein)	44.3 \pm 4.2	12.4 \pm 1.7*	38.1 \pm 3.1**	29.3 \pm 2.8**	38.5 \pm 2.5**
GPx (nmolmg ⁻¹ protein)	0.85 \pm 0.2	0.25 \pm 0.1*	0.72 \pm 0.3**	0.57 \pm 0.2**	0.69 \pm 0.3**
GSH (nmolmg ⁻¹ protein)	2.7 \pm 0.2	0.57 \pm 0.1*	2.5 \pm 0.1**	1.4 \pm 1.1**	2.2 \pm 0.2**
MDA (nmolmg ⁻¹ protein)	0.7 \pm 0.1	4.5 \pm 0.4*	1.45 \pm 0.2**	1.5 \pm 0.2**	1.1 \pm 0.1**

Values are the mean values \pm standard error of the mean of 8 rats. *: Statistically significant when compared with control group (I) at $p < 0.05$. **: Statistically significant when compared with untreated diabetic group (II) at $p < 0.05$.

Abbreviations: CT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.

The hepatic tissue of the glibenclamide treated group (**Fig. 3c**) showed mild pyknotic changes coupled with early periportal inflammation. The hepatic tissue of the AC treated groups (**Fig. 3d**) showed regeneration of the liver parenchyma and portal tract dilatation. (**Table 3**)

Discussion

Diabetes mellitus has been identified as one of the leading metabolic disorders worldwide. It is characterized by hyperglycemia associated with impairment in insulin secretion as well as with an alteration in the intermediary metabolism of carbohydrates, proteins and lipids. Reactive oxygen species play a major role in the etiology and in the pathogenesis of DM and of its complications. Lipid peroxidase-mediated tissue damage has been demonstrated in insulin-dependent and non-insulin-dependent DM.³³

It could be concluded from the result that the median acute toxicity (LD_{50}) value of the extract was 5,000 g/kg bwt. According to Locke (1983),³⁴ the extract can be classified as being non-toxic, since the LD_{50} by oral route was found to be much higher than the toxicity index of 2 g/kg of the World Health Organization (WHO).

The significant slight weight gain observed in the diabetic animals treated with the extract clearly suggested that the extracts might not have had the obesity forming tendency compared with glibenclamide treated improved weight gain which is one of the undesirable side effects encountered when treating diabetes with sulphonylureas. There were also no changes observed in the microscopic examinations of the organs of the diabetic animals treated with the extract or with glibenclamide.

The present study reveals that the administration of the extract of AC presented a significant antihyperglycemic activity by improving the blood glucose level, and that it was considerably effective compared with glibenclamide.

Glibenclamide, being a second-generation sulfonylurea class of the oral hypoglycemic agents, is known to mediate its hypoglycemic effect by stimulating insulin release from the pancreatic β cells, causing a reduction in the hepatic insulin clearance by stimulating the release of somatostatin and suppressing the secretion of glucagon.³⁵

Sulfonylureas have also been shown to induce hepatic gluconeogenesis.³⁶

Comparing the results of glibenclamide with those obtained for the extract of AC in the present study, it appears that the AC may have its antihyperglycemic effect through the induction of hyperinsulinemia by the utilization of peripheral glucose.

The present results show that the injection of alloxan induces hepatocellular damage, as evidenced by the high levels of ALT and AST in the untreated diabetic groups. These increases may be due to the leakage of these enzymes from the liver cytosol into the blood stream, which causes a change in the permeability of the membranes of the liver cells. On the other hand, the oral administration of the extract of AC lowered the ALT and AST levels slightly effective than the standard drug, glibenclamide.

Furthermore, AC has an ability to restore the protein breakdown and enhance the glycogenesis process in the liver of diabetic rats. The ALT and AST activities are known as cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage.^{37,38}

In the present study, **Fig. 2** shows the activities of AST and ALT in experimental rats compared with normal rats. The diabetic rats showed more activities of serum AST and ALT. Therefore, both enzyme activities were used as indicators of hepatic damage.

Therefore, it is possible to suggest that the AC extract is safe and might confer protection against ALX-induced hepatocellular damage as evidenced by the normal serum levels of ALT and AST in the diabetic group treated with AC. The hepatoprotective activity of AC might be due to the presence of antioxidant compounds such as flavonoids, terpenoids, liminoids and vitamins. It has been reported that AC contains terpenoids, which shows that the results found in the present study are in conformity with the previous report by Ekundayo et al (1988).¹⁸

In addition, hepatotoxicity and oxidative stress mediated by diabetic are exhibited by a significant increase in the activities of antioxidant enzymes, SOD, CT, GPx and the liver content of MDA, and a significant decrease of GSH. By contrast, the diabetic group treated with the ethanolic roots extract of AC remarkably modulated the oxidative stress caused by alloxan induction because it has been reported

that the oxidative stress associated with the increased generation of reactive oxygen species (ROS) and the defective antioxidant defense mechanism system in the body are the main contributors of DM and its complications.³⁹

It is possible to suggest that this extract might directly improve the structural and functional integrities of the liver.

Conclusion

The present study indicates that AC has the potential to manage DM and prevent DM-associated hepatic damage.

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