Methanolic leaf extract of *Ficus Exasperata* attenuates Arsenate–mediated hepatic and renal oxidative stress in rats

***Oyewole O.I., Oladele J.O., Oladele O.T.**

Abstract

Objective: In furtherance of the scientific search for suitable antidotes for pro-oxidative toxicants which man is exposed to on daily basis, the study investigated the modulatory potential of *Ficus exasperata* leaf extract on arsenate-mediated hepatic and renal toxicity using rats as a model.

Methodology: Twenty-eight rats were sorted into four groups containing seven rats each. Group A (control) received distilled water while 10 mg/kg bw of sodium arsenate was administered intraperitoneally to groups B, C and D to induce hepatic and renal damage. Group C and D were treated with oral administration of 100 mg/kg bw and 200 mg/kg bw of methanolic leaf extract of *Ficus exasperata* respectively for 14 days.

Results: Arsenate significantly ($P<0.05$) induced hepatic and renal damage characterized by elevated levels of serum urea, creatinine, uric acid, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Arsenate also caused decreased serum concentrations of albumin, globulin and total protein as well as significant depletion in antioxidant status (glutathione–S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) in the liver and kidney of the rats. Administration of leaf extract of *Ficus exasperata* significantly ($P<0.05$) attenuated all these toxic effects by boosting antioxidants status and normalizing serum hepatic and renal markers.

Conclusion: These results are indicative of the modulatory potential of *Ficus exasperata* leaf on liver and kidney dysfunction arising from oxidative damage.

Keywords: Nephrotoxicity, Hepatotoxicity, Oxidative stress, Arsenate, *Ficus exasperata*

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L'extrait de feuille méthanolique de Ficus Exasperata atténue le stress oxydatif hépatique et rénal médié par l'arsénedi chez les rats

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Resume

Objectif: En vue de la recherche scientifique d'antidotes appropriés pour les substances toxiques pro-oxydantes dont l'homme est exposé quotidiennement, l'étude a étudié le potentiel modulateur de l'extrait de feuille de Ficus exasperata sur la toxicité hépatique et rénale médiée par un arséniate utilisant des rats comme modèle.

Méthodologie: vingt-huit rats ont été triés en quatre groupes contenant sept rats chacun. Le groupe A (témoin) a reçu de l'eau distillée tandis que 10 mg / kg pc d'arséniate de sodium a été administré par voie intrapéritonéale aux groupes B, C et D pour induire des dommages hépatiques et rénaux. Les groupes C et D ont été traités par administration orale de 100 mg / kg de poids corporel et 200 mg / kg pc d'extrait de feuille méthanolique de Ficus exasperata respectivement pendant 14 jours.

Résultats: l'arséniate significativement ($P<0,05$) induit des dommages hépatiques et rénaux caractérisés par des taux élevés d'urée sérique, de créatinine, d'acide urique, d'alanine aminotransférase (ALT) et d'aspartate aminotransférase (AST). L'arséniate a également provoqué une diminution des concentrations sériques d'albumine, de globuline et de protéines totales, ainsi que d'une diminution significative de l'état antioxydant (glutathion-S-transférase (GST), la superoxyde dismutase (SOD) et la catalase (CAT) dans le foie et le rein des rats. L'administration de l'extrait de feuilles de Ficus exasperata significativement ($P<0,05$) a atténué tous ces effets toxiques en stimulant l'état des antioxydants et en normalisant les marqueurs hépatiques et rénaux sériques.

Conclusion: Ces résultats révèlent le potentiel modulatif de la feuille de Ficus exasperata sur le dysfonctionnement du foie et du rein résultant de dommages oxydatifs.

Mots-clés: Néphrotoxicité, Hépatotoxicité, Stress oxydatif, Arsénate, Ficus exasperata

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INTRODUCTION

The global upsurge in the prevalence of many diseases including organ failure and cancer in recent years has been associated with increase in toxic chemical exposures (1). Evidences from experimental research indicates that exposure of human and animals to xenobiotics and other chemical substances resulted in serious adverse effects including liver and kidney failure as well as cancer (2). Sodium arsenate ($Na_3A_SO_4$) is a sodium salt of arsenic acid. Arsenates are naturally occurring environmental toxicants and like other arsenic compounds are capable of causing mutagenic, teratogenic and carcinogenic defects in humans and other animals. Arsenates are used as component of herbicide, fungicide and rodenticide and can result in contamination of air, soil, food and water (3). Arsenate can also be released into the environment through burning of coal in industries. The pentavalent metalloid can get to man and other animal species through dermal, respiratory and oral routes. Arsenate binds thiol groups in tissue proteins and impairs their functions. It also affects mitochondrial enzymes and interrupts metabolic energy production (4).

Liver, being the major site of metabolism of many chemicals and foreign compound has been documented to have high tendency of accumulating arsenate following prolonged chronic exposure (5). Hepatic changes recorded in arsenate toxicity include primary hepatic neoplasia, fatty degeneration, cirrhosis, portal hypertension, mitochondrial damage, acute yellow atrophy and impaired porphyrin metabolism (6). The kidney is the main target of many toxic agents due to its biochemical and physiological functions which make it susceptible to many xenobiotics and chemicals. The kidney is the major site of conversion of arsenates and their route of excretion (7). Arsenate exposure induced renal dysfunctions which includes cortical necrosis, renal failure, cancer, proteinuria and hematuria (8).

Plants have been reported as the major source of phytochemicals and biologically active natural agents (9). The prophylactic uses of phytochemicals in the treatment of chronic diseases such as organ failure, cancer and cardiovascular diseases have continuously been explored. The therapeutic potential of these plants have been attributed to their anti-inflammatory, antioxidant, and anticarcinogenic potentials (10).

Ficus exasperata (F. exasperata) popularly known as 'sandpaper tree' (Yoruba-"ipin") in Nigeria is a terrestrial Afro-tropical shrub with ovate leaves that grows up to about 20 m tall and prefers evergreen and secondary forest habitats (11). *F. exasperata* is among the most widely used medicinal plants having many therapeutic potential. The leaf of *F. exasperata* is chiefly employed in the treatment of many ailments including kidney disorders, venereal diseases, coughs, hemorrhoids, epilepsy, high blood pressure, rheumatism and arthritis (12, 13).The main objectives of the present study is to investigate the protective role of leaf extract of *F. exasperata* on arsenate-induced hepatic and renal oxidative toxicity in rats.

MATERIALS AND METHODS

Chemicals/Reagents

Sodium arsenate ($Na_3A_SO_4$) is a product of Sigma-Aldrich Co. St Louis, Missouri, USA. Assay kits (urea, creatinine, uric acid, albumin, globulin, total protein, ALT and AST are products of Randox Laboratories Ltd. UK). Antioxidant kits (SOD, GST and CAT) were obtained from Nanjing Jincheng Biological Engineering Institute, China).All other chemicals are of analytical grade and were obtained from Analar BDH Limited, Poole, England.

Collection of plant material and preparation of extract

Fresh leaves of *F. exasperata* were collected at Oke Baale, Area, Osogbo, South Western part of Nigeria. The plant was identified at the Botany Unit, Department of Biological Sciences, Osun State University, Osogbo. Dried samples of the plant (Voucher No: (OSU/001/1618) were deposited in the University herbarium for future reference. The samples were air dried for 2 months after which it was pulverized into powdery form using industrial grinder. Extraction of the phytochemicals was done by dissolving 700 g of the powder in 4.2 litres 98 % absolute methanol for 14 days after which the extract was filtered using a white moslin cloth. Crude extract was obtained by filtration followed by evaporation of the solvent in a rotatory evaporator at 65°C. The paste was weighed and used to prepare stock solution and different doses of the extract.

Experimental animals

Twenty-eight (28) Wistar strain albino rats (average weight 160 g) were used for this study. They were obtained and raised at the Central Animal House, Osun State University, Osogbo in accordance with the guidelines for the

care and use of laboratory animals. Rats were kept under laboratory conditions (25±2 °C and relative humidity of 50±15%) in cages cleaned of metabolic waste twice daily and were allowed to acclimatize for two weeks before the experiment. They were exposed to 12 hrs daylight and darkness, fed with rat pellet and water *ad libitum*. The rats were randomly divided into four groups of seven rats each: Group A (control) received distilled water while 10 mg/kg bw of sodium arsenate was administered intraperitoneally to groups B, C and D to induce renal and hepatic damage. Group C and D were treated with oral administration of 100 mg/kg bw and 200 mg/kg bw of methanolic leaf extract of *F. exasperata* respectively for 14 days while rats in group B were left untreated

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Preparation of serum

The rats were sacrificed 24 hrs after the last treatment by cervical dislocation. The jugular vein was cut and blood sample collected into clean, dry centrifuge tube. The blood was left for 10 min at room temperature to clot after which it was centrifuged at 4000 rpm in an MSC (Essex, UK) bench centrifuge. The clear supernatant (serum) was aspirated using a Pasteur pipette into clean, dry sample bottles and then stored at -4 °C for biochemical analyses.

Preparation of tissue homogenates

The kidney and liver were quickly excised from the rat and immediately placed on a blotting paper to remove blood stains. The tissues were then rinsed in 1.15% KCl to remove haemoglobin followed by homogenization in 4 volumes of ice-cold 0.01 M potassium phosphate buffer (pH 7.4) using Teflon homogenizer. The homogenates were centrifuged at 12,500 g for 20 min at 4 °C to obtain supernatants (post-mitochondrial fractions) which were stored till required for assay.

Determination of biochemical parameters

Serum creatinine, urea and uric acid were determined using colorimetric method described by Cheesbrough (14). Determination of protein concentration was done using bovine serum albumin as standard (15) while serum globulin was estimated using the method of Mokady et al. (16). Albumin concentration in the serum was measured using the bromocrecsol green method (17). Serum ALT and AST activities were determined based on the principle described by Reitman and Frankel (18). Catalase (CAT) activity was determined based on the method of Sinha (19). GST activity was measured using 1, 2-dichloro 4-nitrobenzene (CDNB) as substrate (20). SOD activity was determined by the method of Misra and Fridovich (21) based on the ability of the enzyme to inhibit auto-oxidation of epinephrine at pH 10.2 and 30 °C.

Statistical analysis

Data obtained were expressed as mean value ± standard error of mean (SEM). Comparison was done using one-way analysis of variance (ANOVA) between the control and treatment groups. P values <0.05 were considered statistically significant.

RESULTS

Figure 1 and 2 shows the effects of co-administration of *F. exasperata* leaf and sodium arsenate on serum liver markers (ALT and AST) and kidney markers (urea, creatinine and uric acid) respectively. Administration of arsenate caused significant ($P<0.05$) increase of all the measured parameters in the serum compared with group A (control). However, treatment with *F. exasperata* leaf extract significantly ($P<0.05$) reversed the elevated parameters close to that obtained in the control.

The results of serum concentrations of total protein, albumin and globulin in the rats are shown in Figure 3. Arsenate administration caused significant ($P<0.05$) decrease in serum levels of total protein, albumin and globulin while treatment with *F. exasperata* leaf significantly elevated these serum parameters close to that obtained in the control.

Figure 4 and 5 shows the effects of co-administration of *Ficus exasperata* leaf and sodium arsenate on activities of antioxidant enzymes (SOD, Catalase and GST) in the liver and kidney of rats respectively. Arsenate administration caused significant ($P<0.05$) depletion in antioxidant status in the liver and kidney of the rats. The antioxidants status was however increased in the tissues following treatment with *Ficus exasperata* leaf extract.

DISCUSSION

Results from this study indicate nephrotoxic effect of arsenate as rats exposed to 10 mg/kg bw sodium arsenate showed marked elevation of serum urea, creatinine and uric acid. Elevated levels of these serum metabolites are indicative of disruption of renal function (22). In addition, the observed significant elevation of serum AST and ALT by arsenate in rats is an indication of its hepatotoxic effects. This result

agrees with previous result that documented hepatotoxicity of arsenate through generation of reactive oxygen species (ROS) and oxidative modification of liver membrane (23). Elevation of these hepatic marker enzymes might be due to their leakage out of the liver into the blood system due to destruction of hepatic membranes. Arsenate administration also caused significant reduction in total protein, albumin and globulin which is indicative of hepatic dysfunction. Protein concentration is an essential tool in assessing the hepatotoxicity profile of xenobiotics. Low levels of these proteins including albumin and globulin have been associated with liver damage, malnutrition and dehydration (24). Globulin and albumin are globular proteins which are synthesized in the liver and transported by blood circulation thus found in the serum. Serum levels of these proteins are markers of liver's ability to synthesis them (25).

There was significant depletion of SOD, CAT and GST activities in the liver and kidney of rats administered 10 mg/kg bw of sodium arsenate which might indicate overwhelming protecting activities of the enzymes. It has been reported that decreased activities of these antioxidant enzymes resulted in accumulation of O^{-2} and H_2O_2, which may promote additional radical generation such as highly reactive hydroxyl radical (26). Oxidative stress is a result of the imbalance between ROS and antioxidants in the body which can lead to oxidative damage of macromolecules and it has been implicated in the pathogenesis of many diseases (27, 28). Antioxidant protection and cellular defense of the body are provided by GST, SOD and CAT (29). SOD is the enzyme in antioxidant defense that scavenges superoxide radicals to form H_2O_2 thereby reducing the toxic effects of the radical (30). The high concentration of H_2O_2 is detoxified by CAT. GST is a group of multifunctional proteins that play a crucial role in the removal of potentially harmful hydrophobic compounds from blood and detoxification of electrophilic chemicals (31). The decrease in GST activity observed in rats administered arsenic may be the result of the decrease in the availability of substrate (GSH) and also alterations in its protein structure under oxidative conditions (32).

However, treatment with *F. exasperata* leaf extract ameliorated these kidney and liver anomalies by normalizing the serum levels of metabolites and enzymes in the rats. The plant extract also boosted the antioxidant capacity in the liver and kidney of rats which were already depleted by arsenate. These results indicate that *F. exasperata* leaf protect against renal and hepatic dysfunctions by protecting the structural integrity of the tissues membrane and enhance their healing and recovery from oxidative damage (33). The protective effect of *F. exasperata* leaf may be due to its antioxidant content and ability to protect the hepatic membrane thereby preventing enzymes leakage into the blood stream. These results suggest that *F. exasperata* offer protective role on the tissues by enhancing the activities of antioxidant enzymes and their synthesis; preventing production of reactive oxygen species and scavenging generated free radicals (34).

CONCLUSION

It is evident from this study that *F. exasperata* leaf posses hepatorenal protective activities via its ability to improve antioxidant status and normalizing renal and hepatic serum markers. Thus, *F. exasperata* leaf might be useful as therapeutic remedy for the treatment of kidney and liver related diseases arising from oxidative damage.

Conflict of interest: The authors declare no conflict of interest.

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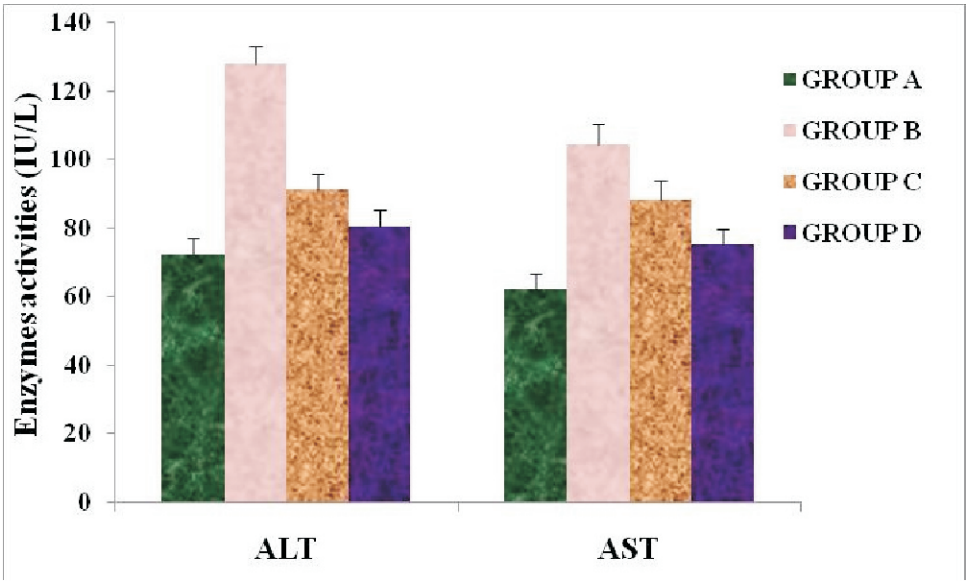

Figure 1: Serum ALT and AST activities in rats administered arsenate and *Ficus exasperata* leaf extract

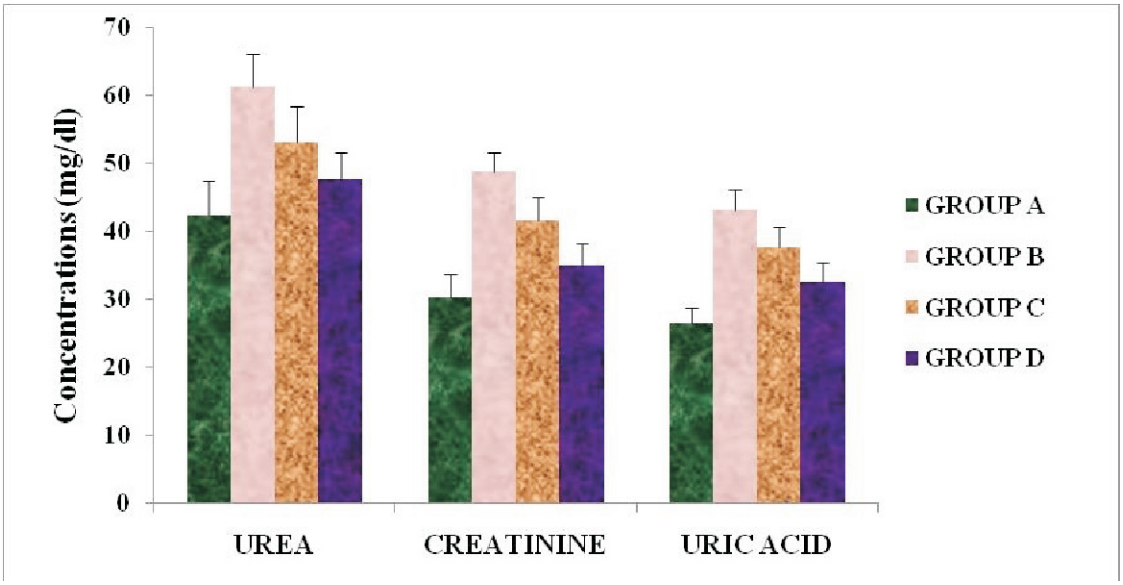

Figure 2: Concentrations of serum urea, creatinine and uric acid in rats administered arsenate and *Ficus exasperata* leaf extract

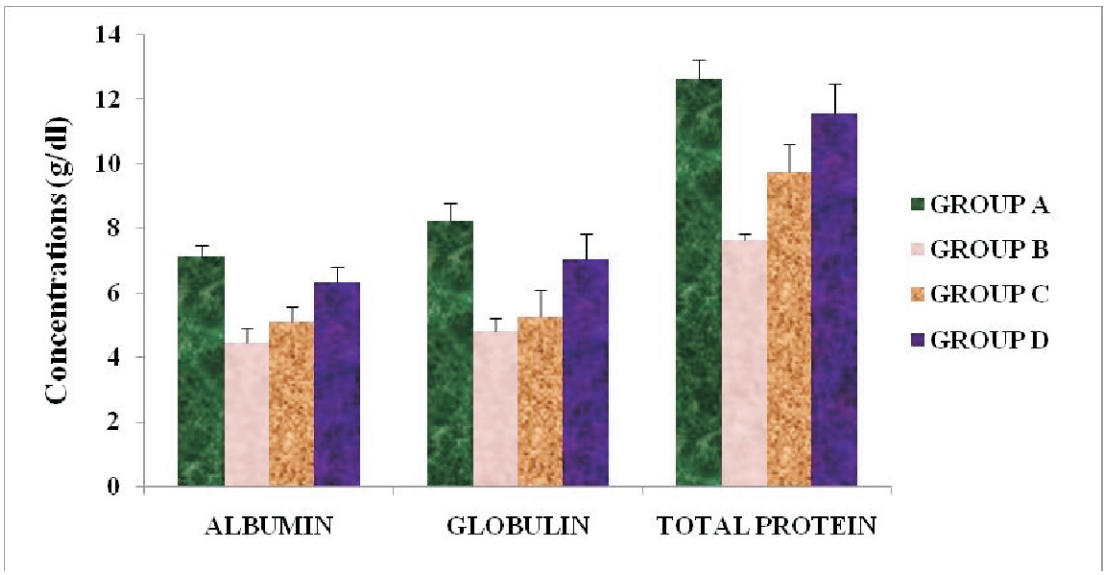

Figure 3: Concentrations of serum albumin, globulin and total protein in rats administered arsenate and *Ficus exasperata* leaf extract

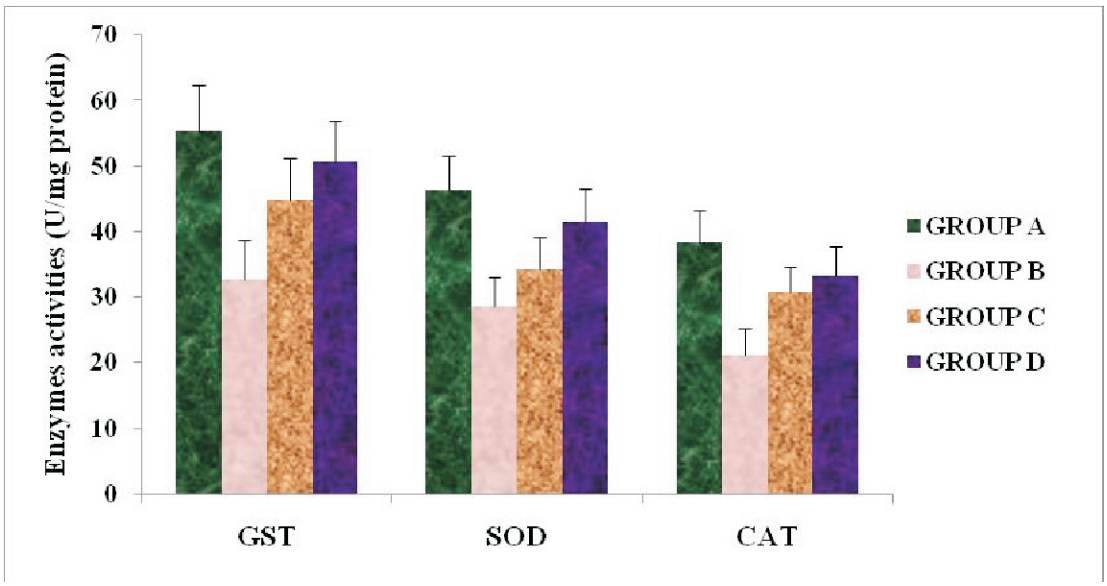

Figure 4: Activities of antioxidant enzymes in the liver of rats administered arsenate and *Ficus exasperata* leaf extract

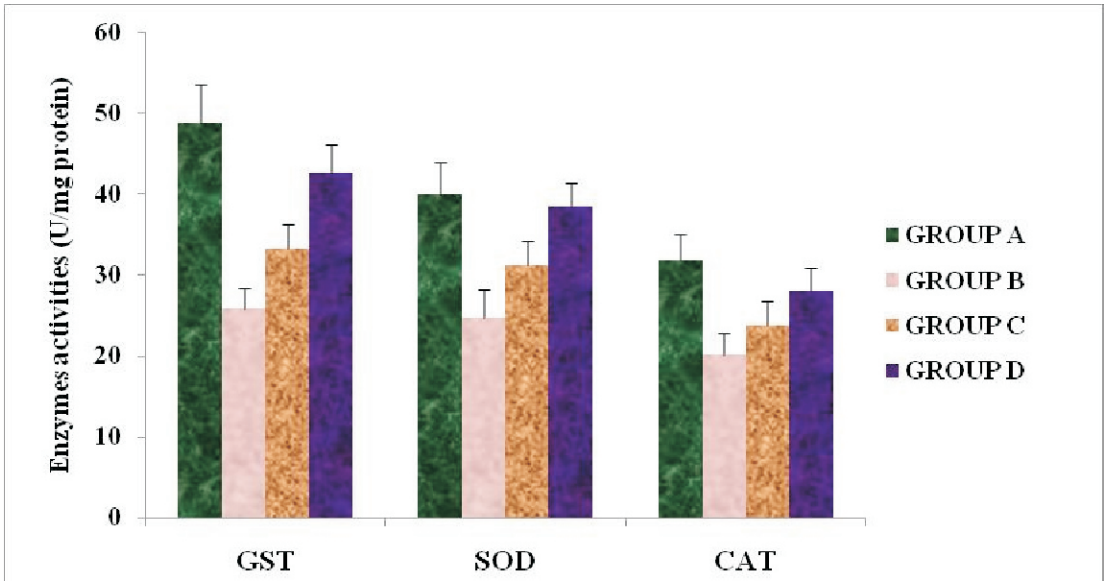

Figure 5: Activities of antioxidant enzymes in the kidney of rats administered arsenate and *Ficus exasperata* leaf extract