Original Article

Effects of *Corchorus olitorius* Extract on Certain Antioxidants and Biochemical Indices in Sodium Arsenite Exposed Rats

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ABSTRACT

Objective: *Corchorus olitorius* is a household leafy vegetable with several medicinal uses. The anti-oxidative and certain biochemical indices of the methanol leaves extract in an *in-vivo* and *in-vitro* experiments were studied.
Method: Phenols, Flavonoids were determined by Folin Ciocalteu and Aluminium chloride assays while Hydroxyl, 1-1-diphenyl-2-picrylhydraxyl (DPPH) radicals and Malondialdehyde levels measured using deoxyribose assay, DPPH and the reaction of Malondialdehyde with Thiobarbituric acid. Cholesterol, reduced glutathione (GSH) and enzyme assays were carried out using standardized methods.
Results: Extract showed antioxidant activities as it scavenged hydroxyl, DPPH radicals and inhibited lipid peroxidation respectively *in-vitro*. Animals fed with extract for 21 days at 50, 100, 150 and 200 mg/kg body weight significantly ($p<0.05$) decrease MDA in a concentration- dependent manner by 2.02, 1.34, 0.76 and 0.02 mg/ml respectively. However, significant increases ($p<0.05$) in the tissue level of GSH was observed (52.80, 68.7, 81.80 and 88.80 mg/ml). Furthermore, Extract at 100 mg/kg modulate sodium arsenite induced hepatotoxicity in rats, as combined treatment significantly ($p<0.05$) decreases cholesterol and marker enzymes activities (Alanine aminotransferase, Aspartate aminotransferase and Gamma glutamyl transferase) compared with intoxicated animals. Extract contained various amount of phenol and flavonoids from (2.250-3.500 mg/g) and (1.650-1.880 mg/g) respectively.
Conclusions: Extract shows anti oxidative properties reduces MDA and cholesterol level, enhance GSH increase and exhibit hepatoprotective ability, suggestive of their bioactive polyphenol components which may be explored as therapeutic agent for management, prevention and treatment of age – related diseases.

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INTRODUCTION

Despite decades of research, the roles of free radicals-induced organ injury is still a matter of debate and currently a serious issue in diagnosis of many diseases. It is well known that free radicals cause cell damage through mechanism of covalent binding and lipid peroxidation leading to injury of the cell membranes[1]. Common free radicals includes, superoxide anion (O_2^-) Nitric oxide (NO), Peroxyl (RO_2^-), Hydroxyl (OH), Lipid Peroxyl (LOO^-), Alkyl (R^-) and alkoxy (RO^-) radicals which are involved in various catalysis of enzymes, transition metals or biological systems[2]. However non-free radicals species such as Hydrogen Peroxide (H_2O_2), Singlet Oxygen (O_2^{-1}), Ozone (O_3), Lipid Peroxide (LOOH) are other forms of activated oxygen generated from aerobic organisms capable of reacting with most biological molecules including proteins, lipids, lipoprotein and DNA[3]. The reactive ability of free radicals have been considered responsible for a series of undesired processes such as aging, material degradation, food deterioration, oxidative stress and many pathophysiological disorders such as arthritis, diabetes, inflammation, neuro-degeneration and cancer[2-4].

Interestingly, antioxidants have been found to terminate or retard oxidative process by scavenging free radicals and as protective agents in humans reduces cellular oxidative damage and thus retard the progress of many chronic diseases as well as lipid peroxidation[5]. In addition, some antioxidants exert their effect by the levels of endogenous antioxidant defenses up regulating the expression of the genes encoding superoxide dismutase (SOD), catalase, or glutathione peroxidase[6].

However nature remains the primary source for cures to mans ailments and diseases as antioxidants of natural source have been identified in many medicinal plants which has raised various interest in this field[7]. Numerous indigenous vegetables in Africa were consumed innocently without information on their dietary and health benefits[8], while many medicinal plants are currently gaining approval in the traditional treatment of diseases such as malaria[9], about 800 plants are currently documented with beneficial effects for treatment of diabetes[10]. Interestingly, most suspected bioactive components of these plants are believed to have important roles with natural antioxidant properties which are presumed to be safe since they occur in plants hence the need for laboratory based experiments and investigations to validate these claims which forms the rationale for this study.

Corchorus olitorius (malvaceae), is a plant native to both tropical and subtropical regions throughout the world with mallow-leaves commonly consumed as a leafy vegetable[11,12]. The leaves are used in ethnomedical practices to treat ache and pain, dysentery, malaria, enteritis, fever, gonorrhea, pectoral pains and tumors[13]. The present study was aimed at evaluating certain antioxidative and biochemical indices of the extract of *Corchorus olitorius* leaves via *in vivo* and *in vitro* experiments.

MATERIALS AND METHODS

Reagents

Some of the reagents in this study includes; methanol, folin ciocalteu, gallic acid, quercetin, sodium carbonate,

thiobarbituric acid, trichloroacetic acid, 2-deoxyribose and 1, 1-dipheny-2-picrylhydrazyl radical (DPPH), all of which were products of Sigma chemical company USA.

Experimental animals

Twelve weeks old male wistar rats weighing between 120-140g obtained from the animal house of Biochemistry Department were maintained on commercial feed for about two weeks before the commencement of the experiment. The animals were randomly divided into four groups with six (6) animals per group in a battery cage and another set of four groups with six animals each for the first and second in vivo study respectively. They were fed *ad libitum* and handled based on the recommendations and guide lines of our institutional animal care and ethical committee for laboratory animals.

Plants material and extract preparation

The plants were collected from the teaching and research farm and authenticated by the botany unit. Fresh healthy leaves of the plant were dried and powdered while 10 grams of this was extracted in 100ml of methanol in the cold for 72 hours.

Administration of test substances to animals

48 animals were divided randomly into two major groups with the first group made up of 24 rats divided into four groups with 6 animals each and were treated with various doses (50, 100, 150 and 200mg/kg body weight) respectively for 21 days with the extracts while the other groups were made of 24 rats divided into four groups namely group A (control), B (sodium arsenite intoxicated rats), C (sodium arsenite and extract) and D (extract only) respectively. Arsenite intoxication was carried out at 2.5 mg/kg body weight while extracts were administered at 100mg/kg body weight for this group.

Collection of blood sample and preparation of liver homogenate

Rats were sacrificed by cervical dislocation. Blood was collected directly from the heart into plain and well-labelled sample bottles and centrifuged at 4000rpm for 5 minutes to obtain serum for analysis of biochemical parameters. The hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA, pH 7.4) and centrifuged at 12, 000 rpm for 60 minutes. The supernatant was used to assay for GSH and MDA concentrations.

Phytochemical study

Phenols and Flavonoids were measured spectrophotometrically using Folin Ciocalteu and Aluminium chloride assays by[14,15], using gallic acid and quercetin as standards.

Antioxidants activity

Radicals scavenging effects were determined using the methods of[16,17] for the hydroxyl and DPPH radicals respectively. MDA levels and GSH determination were carried out by the methods of[18,19] while Inhibition of lipid peroxidation was determined by the method of[20] with ascorbic acid as standard.

Biochemical indices

Protein and cholesterol concen-trations were determined by the procedure of[21,22]. Enzyme assays, ALT, AST and γGT were determined using the method of[23-25] based on the standardized methods by the International Federation of Clinical Chemistry.

Statistical analysis

Statistical analysis were based on the Duncan`s experimental analysis with mean standard deviation of sample analysed using the student T test and p< 0.05 i.e 95% level of significance[26].

RESULTS AND DISCUSSION

Considerable evidence indicates that increased oxidative damage is associated with, and may contribute to the development of all major neuro-degenerative diseases[27].

It is also well known that free radicals cause cell damage through mechanism of covalent binding and lipid peroxidation with subsequent tissue injury[1].

Interestingly, the interaction of oxygen radicals and other oxidants with proteins, lipids, biological membranes and even DNA have been linked with oxidative stress, lipid peroxidation, DNA. Single-stranded–breaks, changes in structures or loss of bases and DNA-DNA or DNA-Protein cross-linkages[28].

However foods, herbs, beverages and other plant products are found to be rich in polyphenolic compounds with, free radical scavenging and protective anti oxidant defenses in the body[29], due to their natural source.

Hence in the present study, rats orally exposed to the methanol extract of *Corchorus olitorius* for 21days at various doses (50, 100, 150 & 200mg/kg body weight) showed significant (P<0.05) decrease in the level of liver MDA and a significant increase (P<0.05) in the concentration of reduced glutathione (GSH) in a dose-dependent manner (Table 1).

Observation from table 1, is an indication of the ability of the extract to inhibit lipid peroxidation, maintain membrane integrity and boost the body's antioxidant status by increasing GSH levels.

Also, studies have shown that Sodium arsenite is capable of generating free radicals intracellularly[30,31] which in turn induces oxidative damage to tissues, thus in this study, sodium arsenate was used to induced hepatoxicity in the experimental animals. Sodium arsenite intoxication caused increase in serum protein, cholesterol, malondialdehyde level and activities of alanine aminotransferases, aspartate aminotransferases and gamma glutamyltransferase (Fig. 1, 2, 3, 4, 5 and 6). Elevated levels of these serum enzymes have been studied and could be indicative of an early liver damage as well as an assessment of its functions[32,33]. However results obtained showed that rats treated with sodium arsenite and the extract ameliorated these effects as these parameters were restored nearly to their control levels. This then suggest the possible modulatory role of the extract for a possible hepatoprotective ability[34]. The decreased cholesterol level may be an indication of the possibility of anti-hyper lipidemic property of the extract which may be employed in treating cholesterol disorders[35]. Results from the arsenic intoxication can be inferred that *Corchorus olitorius* methanol leaf extract possesses free radical scavenging activity which could have a beneficial effect.

Further assessment of the extract shows that phenols and flavonoids were expressed between (2.250-3.500 mg/g) and (1.650-1.880 mg/g) Garlic acid and quercetin equivalent respectively. (Table 2).

The extract also scavenged hydroxyl and DPPH radical maximally by, 60.01% and 93.48% while Fe^{2+}-induced lipid peroxidation was inhibited maximally by 60.04% respectively (Table 3).

The overall results of this study from the antioxidants and biochemical indices investigated clearly suggest that extracts of *Corchorus olitorius* were hepatoprotective had antioxidative and anti-lipiperoxidative effects which are linked to the main repository of appreciable amount of

phenolics, flavonoids and other antioxidative compounds found in this plants.

CONCLUSION

Conclusively, data and information obtained from this study are indication of *Corchorus olitorius* potential to enhance nutrition and health as a rich source of dietary antioxidants for biological systems susceptible to free radicals-mediated reactions leading to oxidative stress in diseases.

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Authors disclosure statement

"No conflict of interests exist".

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Table 1. Malondialdehyde (MDA) and reduced glutathione (GSH) concentrations in the liver of various treated groups with extract for 21days

Treatment group	Malondialdehyde (MDA) concentration (mg/ml protein)	Reduced glutathione (GSH) concentration (mg/ml)
A (control)	2.89 ± 0.109	47.00 ± 1.655
B (50mg/kg body weight)	2.02 ± 0.023	52.80 ± 6.870
C (100mg/kg body weight)	1.34 ± 0.250	68.75 ± 7.190
D (150mg/kg body weight)	0.76 ± 0.280	81.80 ± 6.990
E (200mg/kg body weight)	0.02 ± 0.001	88.80 ± 4.945

Values are given as mean and standard deviation of six determinations.

Table 2. Total phenolic and flavonoids contents of the methanol extracts of Corchorus Olitorius leaves

Concentration (mg/ml)	Total phenolic content (mg/g) Gallic acid Equivalent	Total flavonoids content (mg/g) Quercetin Equivalent
100	2.250 ± 0.004	1.650 ± 0.008
200	2.250 ±0.001	1.800 ± 0.039
300	3.000 ±0.004	1.810 ± 0.014
400	3.500 ±0.008	1.880 ± 0.011

Mean value ± standard deviation of three replicates.

Table 3. Percentage inhibition of lipid peroxidation, hydroxyl radical and DPPH scavenging effects at various concentrations by the extract

% inhibition of lipid peroxidation	% hydroxyl radical scavenging effects	% DPPH radical scavenging effects
41.10 (250.00mg/ml)	15.98 (25.00mg/ml)	51.820 (100.00 mg/ml)
47.65 (300.00 mg/ml)	20.03 (50.00 mg/ml)	80.340 (200.00 mg/ml)
50.31 (350.00 mg/ml)	24.67 (75.00 mg/ml)	90.580 (300.00 mg/ml)
54.40 (400.00 mg/ml)	32.23 (100.00 mg/ml)	92.610 (400.00 mg/ml)
58.08 (450.00 mg/ml)	44.63 (125.00 mg/ml)	92.900 (500.00 mg/ml)
60.74 (500.00 mg/ml)	60.01 (150.00 mg/ml)	93.480 (600.00 mg/ml)

Mean values ± standard deviation of three replicate.

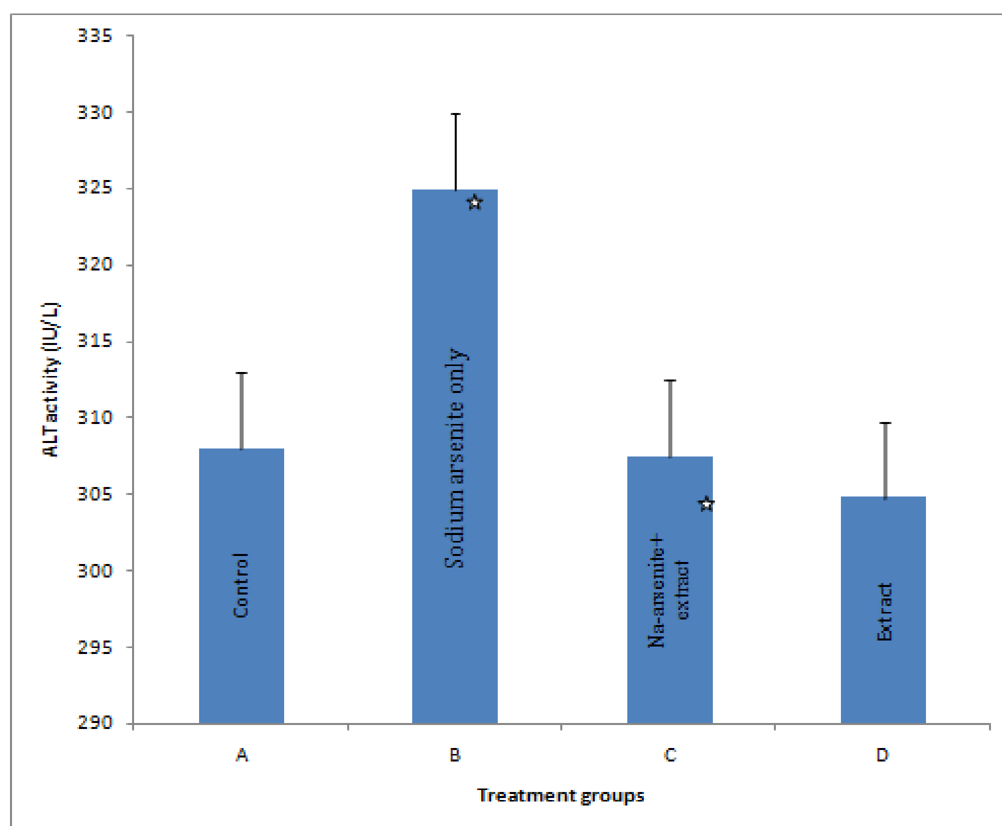

Figure 1. ALT activity in the serum of the various treatment groups

Values are given as mean and standard deviation of six determinations.
☆Values differ significantly from control ($P \leq 0.05$).

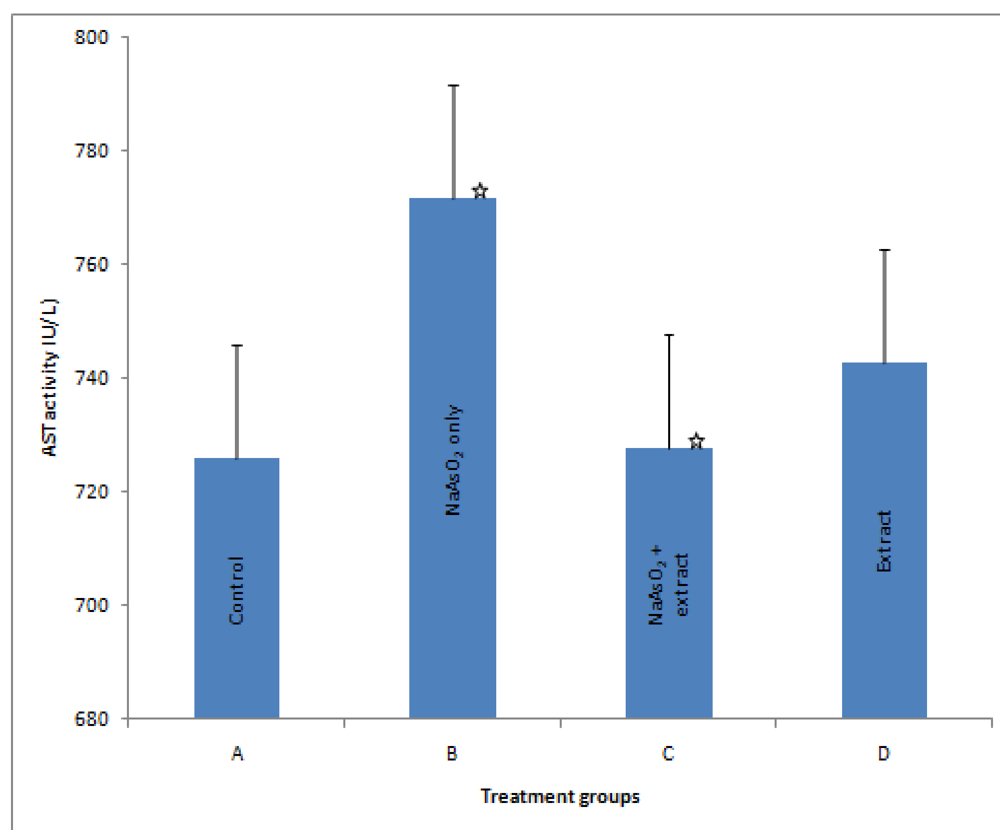

Figure 2. AST activity in the serum of the various treatment groups

Values are given as mean and standard deviation of six determinations.
☆Values differ significantly from control (P≤0.05).

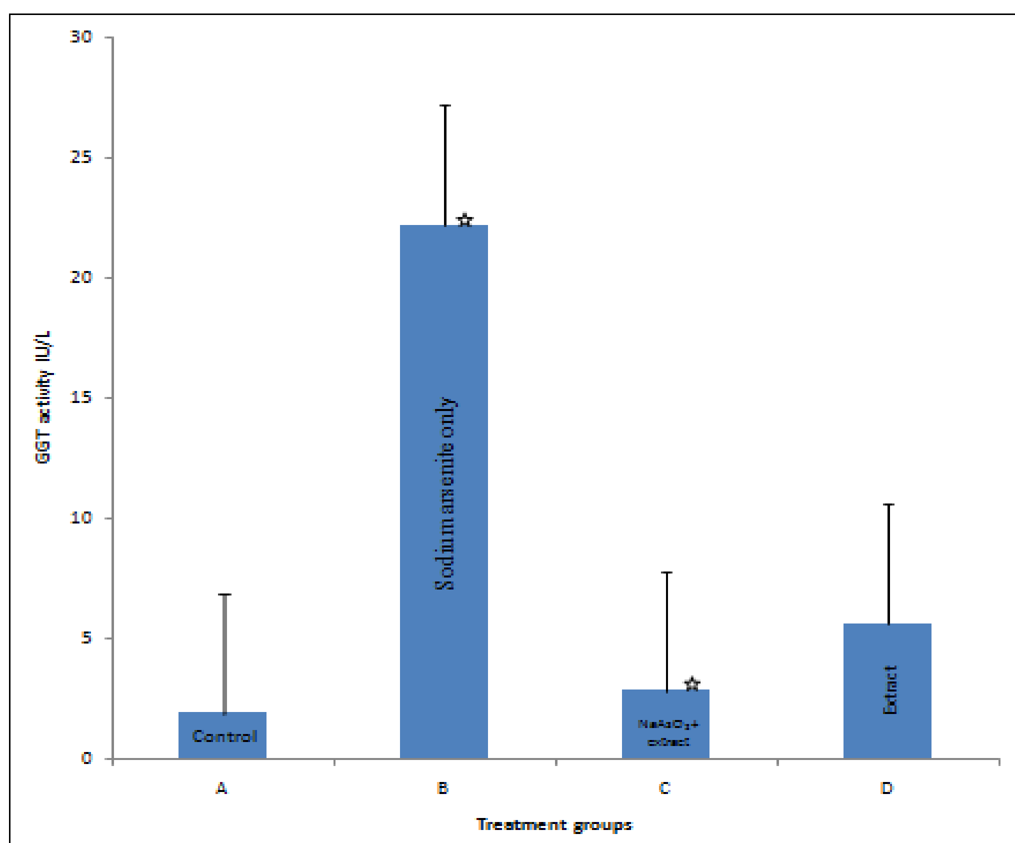

Figure 3. GGT activity in the liver of various treated groups

Values are given as mean and standard deviation of six determinations.
☆ Values differ significantly from control ($P \leq 0.05$).

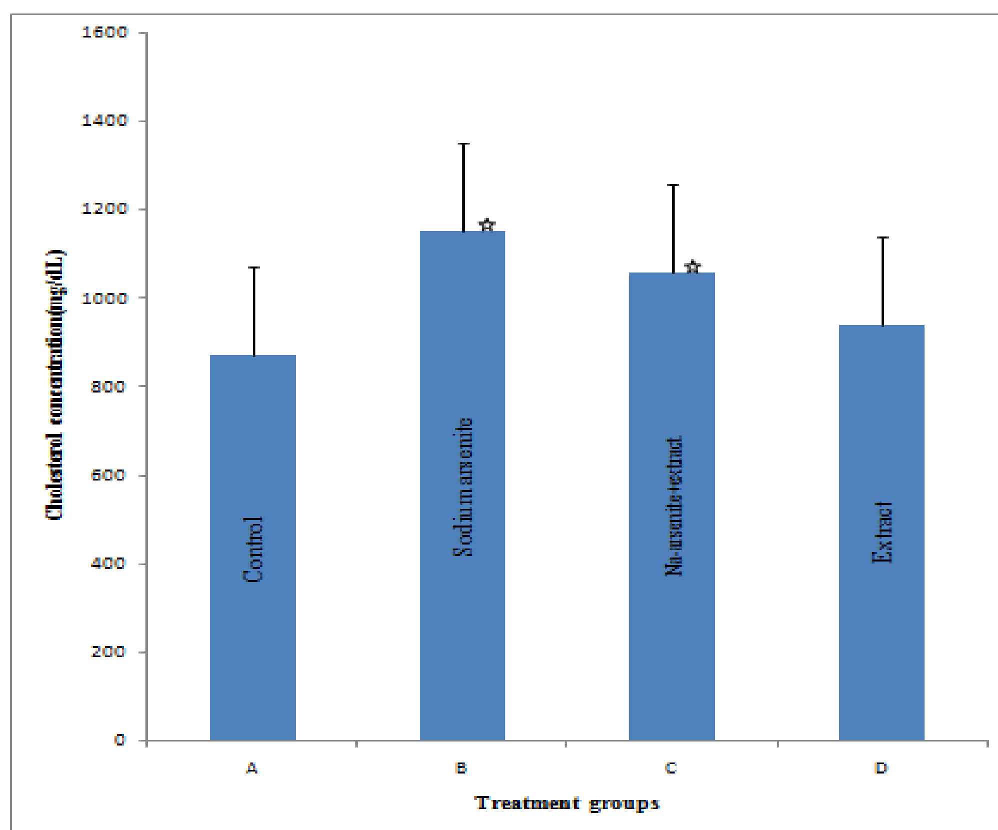

Figure 4. Cholesterol concentrations in the serum of various treatment groups

Values are given as mean and standard deviation of six determinations.
☆ Values differ significantly from control (P≤0.05).

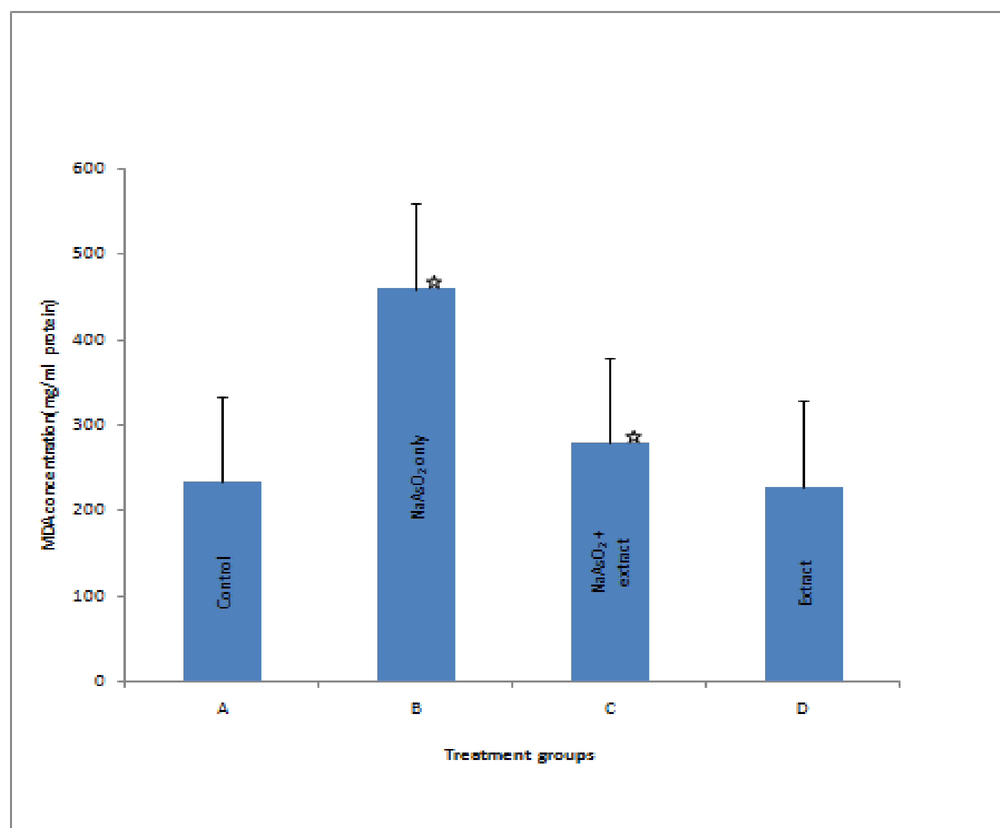

Figure 5. MDA concentrations in the liver of each treated groups

Values are given as mean and standard deviation of six determinations.
☆ Values differ significantly from control ($P \leq 0.05$).