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Effects of Fruits of *Momordica cymbalaria* against Sodium Fluoride Induced Nephrotoxicity in Male Wistar Rats

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Abstract:

Aim: The aim of the study is to evaluate the protective effect of Hydroalcoholic extract of fruits of *Momordica cymbalaria* against the sodium fluoride induced nephrotoxicity in male wistar rats.

Methods: HAEMC was subjected to preliminary phytochemical screening by standard qualitative tests.The extent of protective activity of HAEMCin recovery of kidney damage caused by sodium fluoride was determined by measuring the levels of serum albumin, total protein, urea, uric acid, creatinine, lipid peroxidation, reduced glutathione and catalase along with the change in total body weight and kidney weight. Animals were treated with sodium fluoride (100ppm) for 30 days and treated with HAEMC of doses (200mg and 400mg).

Results: NaF treated animals showed significant elevation of serum biomarkers like creatinine, urea and uric acid and significant decline in serum albumin and total protein. Considerably there was an increase in lipid peroxidation and decrease in reduced glutathione and catalase activity which indicates the damage of kidney compared to normal control. Treatment groups were found with significant recovery of sodium fluoride induced alterations in the antioxidant parameters compared to NaF treated animals.

Key words: Catalase, Lipid peroxidation,*Momordica cymbalaria*, Sodium fluoride, Reduced glutathione.

INTRODUCTION

Fluorine is a chemically electronegative element which is essential for both animals and humans[1]. Fluoride, depending on dose it is both beneficial and detrimental. Long term ingestion of fluoride leads to fluoride toxicity and also causes many adverse effects. The optimal value of fluoride in drinking water is 1.5 mg /L. Fluoride, when it is present in low concentration in drinking water it has beneficial effects on teeth (used in prevention of dental caries i.e., from 0.5 to 2.0 mg /day) bones (used in the prevention of osteoporosis i.e., from 20 to 50 mg/ day). Fluoride in excessive exposure in drinking water or in combination of other sources results in adverse effects which include teeth decay, osteoporosis and harm to kidneys, bones, reproductive organs, and muscle [2]. Among the other soft tissue kidney is more prone to fluoride toxicity [3].

The kidneys are one of the highly exposed sensitive organs for fluoride toxicity as the kidneys are able to excrete approximately 50% of an ingested dose of fluoride. The high concentration of fluoride causes adverse changes in the functioning of the kidneys [4]. The renal failure denotes failure of excretory functioning of the kidneys. The renal failure can be categorized into two type's namely chronic renal failure and acute renal failure.Chronic renal failure refers to an irreversible deterioration in the renal function leading to loss of excretory metabolic and endocrine functions and acute renal failure refers to the sudden and reversible loss of renal function [5].

The kidneys are the site for potential fluoride toxicity as fluoride produces high levels of oxygen and nitrogen species leading to increased oxidative stress which causes renal toxicity [6]. Because kidneys are the major routes of fluoride excretion therefore kidneys are important targets for fluoride toxicity. High fluoride levels cause disturbances in the kidney functioning and lead to renal histopathological changes [7]. Therefore several experimental studies were available on fluoride toxicity in kidney. Moreover the present study was aimed to demonstrate the effects of NaF on rat kidney through sequential biochemical changes in serum levels of different biochemical parameters and its possible effectiveness of nephroprotective activity by HAEMC.

MATERIALS AND METHODS

Plant material and preparation of extract

The fruits of *Momordica cymbalaria* were collected from cadapa, Andhra Pradesh. The fruit material were taxonomically identified and authenticated by Dr.A.Manohar Rao from Professor Jayashankar Telangana state agricultural university, Hyderabad, India. The plant material is shade dried with occasional shifting and powdered with a mechanical grinder and stored in an airtight container. The coarse powder of shade dried fruits of the medicinal plant *Momordica cymbalaria* was subjected to successive maceration extraction process with ethanol and water (70:30) for 24 hrs [8]. The obtained extracts were dried under reduced pressure by using rota flash evaporator.

Preliminary phytochemical screening

The hydroalcoholic extract of fruits of *Momordica cymbalaria* (HAEMC) were screened for the presence of phytochemical constituents by using standard qualitative chemical tests [9].

Animals

Healthy adult male wistar rats weighing 200-250gms were used for the experiment. The animals were housed under conditions of controlled temperature and 12 hour day-night cycle and fed with standard rodent pellet diet and provided water *ad libitum*. The animals were acclimatized for one week and then used for the experimental study. Prior to the experiments the approval was obtained from Institutional Animal Ethical Committee (IAEC) with protocol No: IAEC/1657/CMRCP/T2/PhD-16/46.

Treatment procedure

The experimental animals were divided into five groups of six animals each.

Group 1: In thisgroup the ratsreceived only vehicle (5ml/kg) for 30 days which serves as normal control group.

Group 2: Rats were treated with sodium fluoride of 100ppm in drinking water for 30 days which serves as toxic control group.

Group 3: Rats were treated with plant extract (200mg/kg/day) for 30 days which is administered orally which serves as plant control group.

Group 4: Animals of this group were received plant extract at low dose (200 mg/kg/day) along with sodium fluoride of 100ppm in drinking water for 30 days per oral which serves as treatment control group-1.

Group 5: In this group animals were received plant extract (400mg/kg/day) along with sodium fluoride of 100ppm in drinking water for 30 days per oral which serves as treatment control group-2 [10].

On 31st day the blood samples were withdrawn from the retro orbital puncture, after the rats were anesthetized with diethyl ether. By centrifuging the blood at 1200 rpm for 10 min serum samples were prepared and stored at -20 degrees until the serum parameters were studied and at the end of the experimental period, the rats were sacrificed and the kidney was rapidly removed and preserved in formalin for histopathological studies.

Organo-somatic index:

The weights of the animals were noticed prior to the experiment and at the end of the treatment and the change in the body were calculated.Weights of the kidneys was noticed immediately after the dissection.

Estimation of biochemical parameters:

Different biochemical parameters were assayed such as serum total protein, serum albumin, serum urea, serum uric acid and serum creatinine.

Histopathological studies:

The isolated kidneys were preserved in 10% formalin and embedded in paraffin wax and longitudinally sliced by using a microtome. They were stained using hematoxylin and eosin (H&E) stain and observed under a trinocular microscope[11].

***Invivo* Antioxidant studies:**

Estimation of lipid peroxidation from PMS:

Lipid peroxide from post mitochondrial supernant (PMS) was evaluated by reaction with thiobarbituric acid. In this method 0.5 ml of PMS was allowed to react with 0.5 ml of Tris Hcl buffer and incubate at 37^0 C for 2 hours and then add 1 ml of freshly prepared ice-cold tri-chloro acetic acid (TCA) followed by centrifugation for 10 min at 1000 rpm and obtained supernant of volume 1 ml was added to 1 ml of thiobarbituric acid. Boil for 10 min in a water bath and brought to room temperature, after cooling add 1 ml of distilled water. Then measure the absorbance at the wave length of 532nm against the blank which is prepared without adding post mitochondrial supernant (PMS)**.**

Estimation of Reduced glutathione (GSH) from PMS:

Reduced glutathione from post mitochondrial supernant (PMS) was determined by the reaction with hydrogen peroxide. The process involves 0.7 ml of PMS was allowed to react with 0.75 ml of 4% sulfosalycilic acid and centrifuged at 1200 rpm for 5 minutes at 4^0 C. and obtained supernant of volume 0.5 ml was added to 4.5 ml of 0.1mM DTNB. Then measure the absorbance at the wave length of 412nm against the blank which is prepared without adding post mitochondrial supernant (PMS).

Estimation of catalase activity from PMS:

The catalase activity was evaluated by determining the amount of H2O2 hydrolysis by the enzyme. Briefly, 0.05 ml of PMS was allowed to react with 1.95 ml of PO_4 buffer and followed by adding 1.0 ml of H_2O_2. Then measure the absorbance at the wave length of 240nm for 2 minutes at an interval of 60 seconds against the blank which is prepared by adding only 1.95 ml of PO_4 buffer.

Statistical analysis:

The results were expressed as the Mean ± SEM and statistical analysis were performed by using one way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison tests. Values of [a] $p<0.001$, [b] $p<0.01$ and [c] $p<0.005$ Vs toxic control; [d] $p<0.001$, [e] $p<0.01$ and [f] $p<0.005$Vs Normal control were considered as significant.

RESULTS

Phytochemical screening

The investigation have shown the presence of phytochemical constituents such as alkaloids, amino acids, carbohydrates, proteins, glycosides, flavanoids, steroids, vitamins, tannins and phenolic compounds in the hydroalcoholic extract of fruits of *Momordica cymbalaria*.

Kidney weight's

The value of change in kidneys weight in toxic control (1.19±0.05) showed significant decrease when compare to normal control (1.59±0.05). In treatment groups the values increased significantly (1.69±0.04[a,] 1.51±0.03[a] and 1.66±0.05[a]) when compared to toxic control (Table 1)(Figure 1).

Table 1-Effect of HAEMC treatment on NaF induced nephrotoxicity

Experimental group	Kidneys weight (g)
Normal control	1.59±0.05
Toxic control	1.19±0.05
HAEMC control	1.69±0.04[a]
HAEMC 200 mg dose	1.51±0.03[a]
HAEMC 400 mg dose	1.66±0.05[a]

Biochemical parameters

The value of serum levels of albumin (2.09±0.07) and total protein (6.40± 0.17) in NaF treated group were significantly decreased when compared to the value of serum levels of albumin (3.93±0.08) and total protein (7.36 ± 0.16) in the normal group. The value of serum levels of urea (36.26±1.256[d]), uric acid (1.29± 0.20) and creatinine (1.45± 0.07) in toxic control group significantly increased when compared to the value of serum levels of

urea (14.13± 1.175), uric acid (0.54± 0.09) and creatinine (0.66± 0.05) in normal control group. Treatment with HAEMC (dose 200mg and 400mg) showed significant recovery in serum levels of albumin (3.86±0.20[a], 3.22±0.10 [ae] and 3.82±0.14 [a]) total protein (8.20± 0.18[a], 6.66± 0.31 and 7.94± 0.15[a]) urea (15.12± 1.510[af], 27.87± 2.912[bd] and 21.94± 1.087[af]), uric acid (0.41± 0.05[a], 0.89± 0.09 and 0.58± 0.03[a]) and creatinine levels (0.41± 0.05[af], when compared to toxic control group. (Table 2)(Figure 2).

Histopathological studies

The results of histopathological studies were shown in Figure 1.The normal control group(A) showed a normal glomerulus and tubules while the kidney sections of disease control group(B) showed acute tubular necrosis, interstitial oedema, peri-tubular inflammation and interstitial haemorrhage. The kidney sections of standard control group(C), HAEMC 200mg/kg(D) and 400mg/kg(E) showed normal glomerulus, mild interstitial congestion, peri-tubular inflammation and interstitial haemorrhage. The histopathological changes in groups pre-treated with HAEMC showed marked protective effects when compared to groups treated with Gentamicin alone.

In vivo antioxidant studies

The value of lipid peroxidation (8.26±0.35) in NaF treated group was significantly elevated when compared to the lipid peroxidation (1.75±0.36) in the normal group. The value of reduced glutathione (0.39±0.098) and catalase (0.20±0.02) were significantly declined in toxic control when compared to the value of reduced glutathione (2.96±0.26) and catalase (1.13±0.04) in normal control group. Treatment with HAEMC (dose 200mg and 400mg) showed significant recovery in levels of lipid peroxidation (1.3±0.11, 5.29±0.47 and 2.51±0.43), reduced glutathione (9.24±0.78[ad], 3.48±0.49[c] and 9.48±1.19[ad]) and catalase (1.27±0.10[a], 0.24±0.02[d] and 1.09±0.09[a]) respectively when compared to toxic control(Table 3).

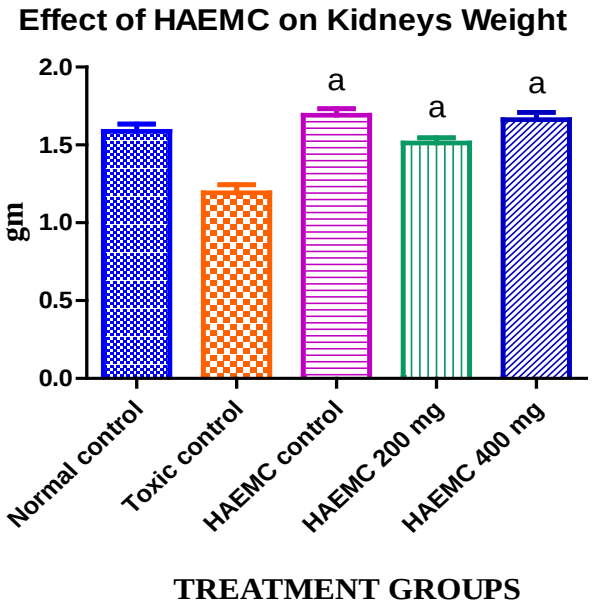

Figure 1.Effect of HAEMC on Kidney weights

Table 2- Effect of HAEMC treatment on NaF induced nephrotoxicity

Experimental group	Albumin (g/dl)	Total Protein (g/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Normal control	3.93±0.08	7.36 ± 0.16	14.13± 1.175	0.54± 0.09	0.66± 0.05
Toxic control	2.09±0.07	6.40± 0.17	36.26±1.256[d]	1.29± 0.20	1.45± 0.07
HAEMC control	3.86±0.20[a]	8.20± 0.18[a]	15.12± 1.510[af]	0.41± 0.05[a]	0.41± 0.05[af]
HAEMC 200 mg/kg	3.22±0.10 [ae]	6.66± 0.31	27.87± 2.912[bd]	0.89± 0.09	0.89± 0.04[af]
HAEMC 400 mg/kg	3.82±0.14 [a]	7.94± 0.15[a]	21.94± 1.087[af]	0.58± 0.03[a]	0.60± 0.07[a]

Table 3-Effect of HAEMC treatment on oxidative stress markers in NaF induced Nephrotoxicity.

Name of the Group	Lipid peroxidation (µMol/mg of tissue)	Reduced glutathione (µMol/mg of tissue)	Catalase (µMol/mg of tissue)
Normal Control	1.75±0.36	2.96±0.26	1.13±0.04
Toxic Control	8.26±0.35	0.39±0.098	0.20±0.02
HAEMC Control	1.3±0.11	9.24±0.78[ad]	1.27±0.10[a]
HAEMC 200 mg dose	5.29±0.47	3.48±0.49[c]	0.24±0.02[d]
HAEMC 400 mg dose	2.51±0.43	9.48±1.19[ad]	1.09±0.09[a]

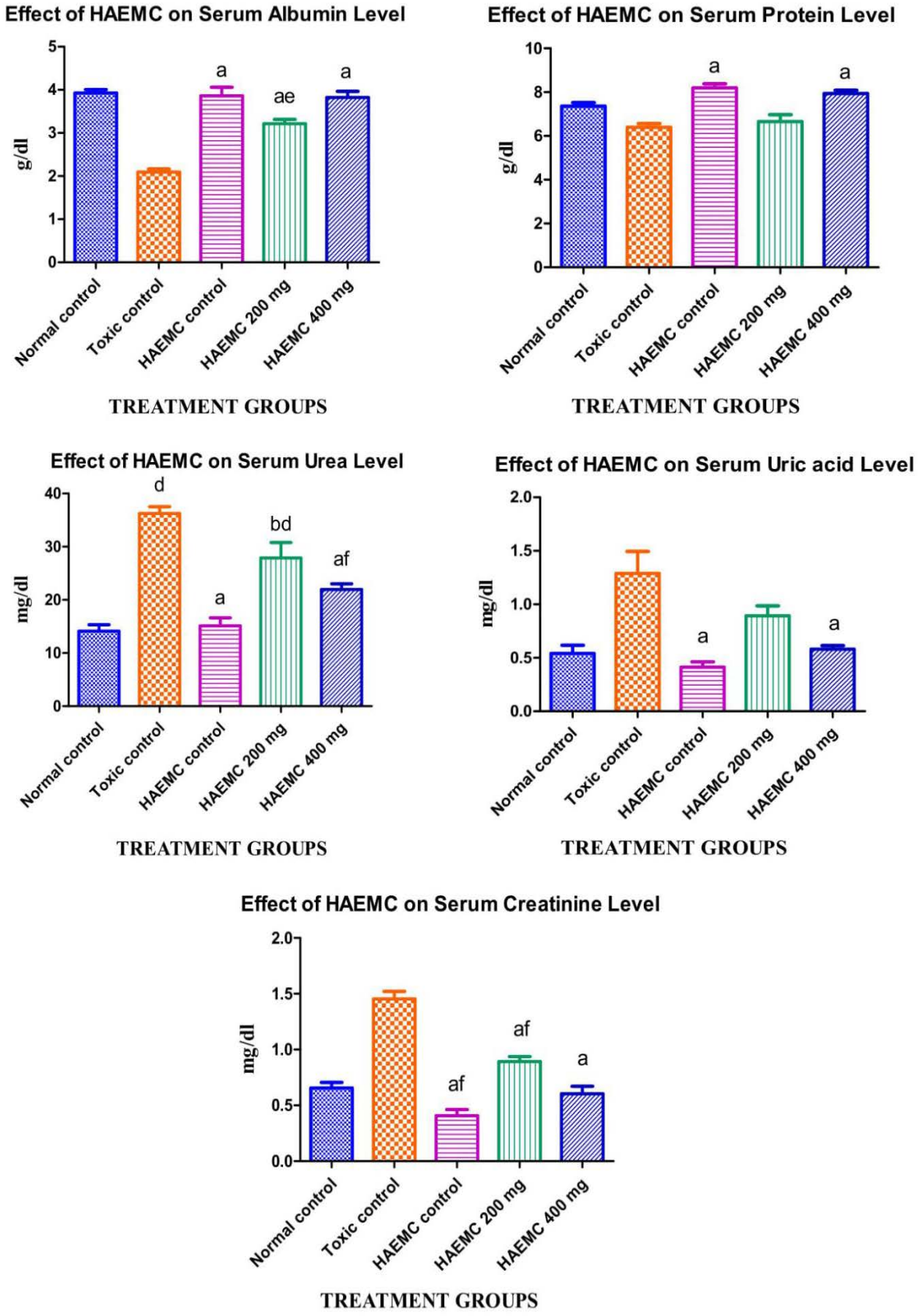

Figure 2. Effect of HAEMC on Biochemical parameters

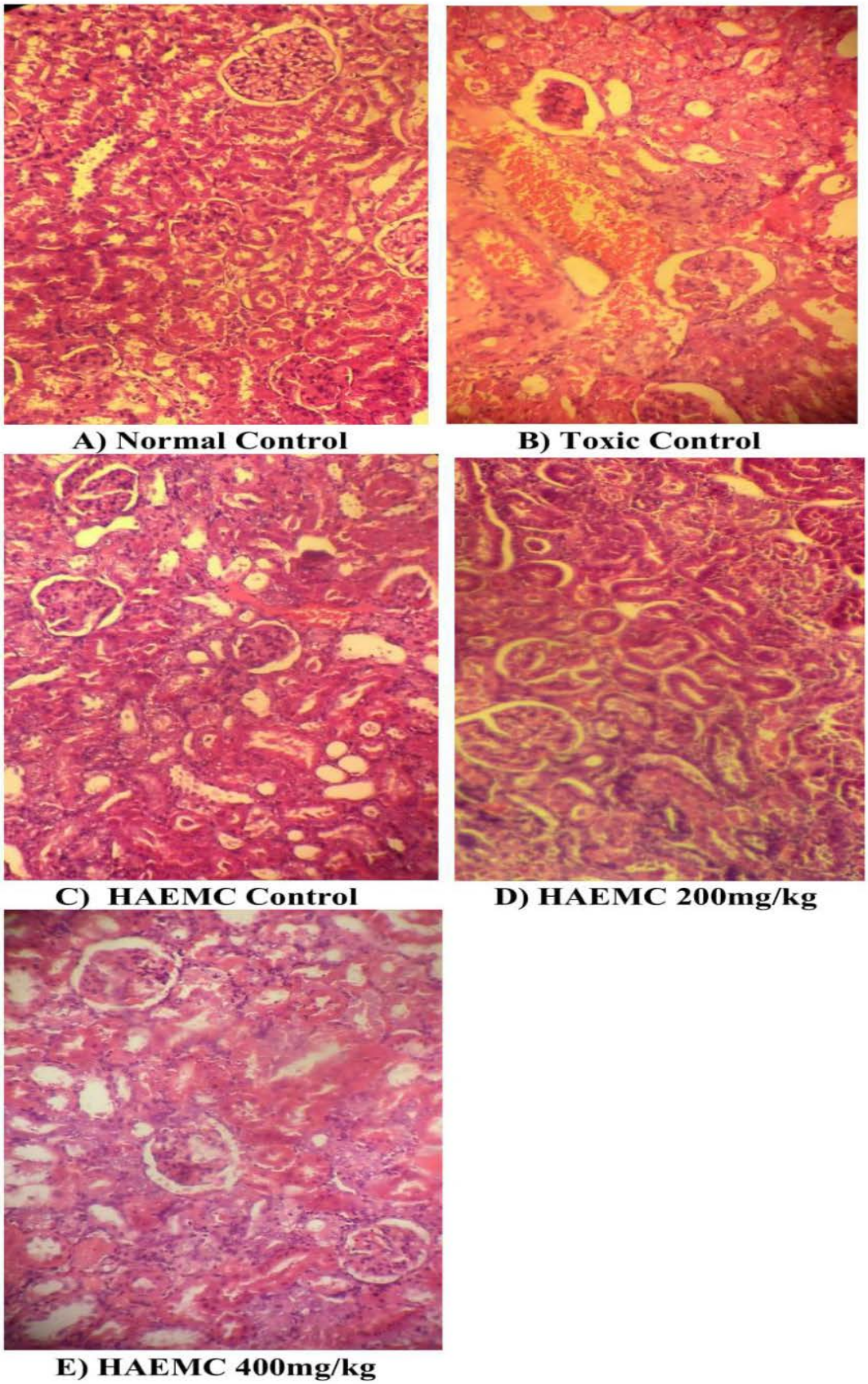

Figure 3. Light microscopic study (H&E) of kidney tissue in various experimental groups

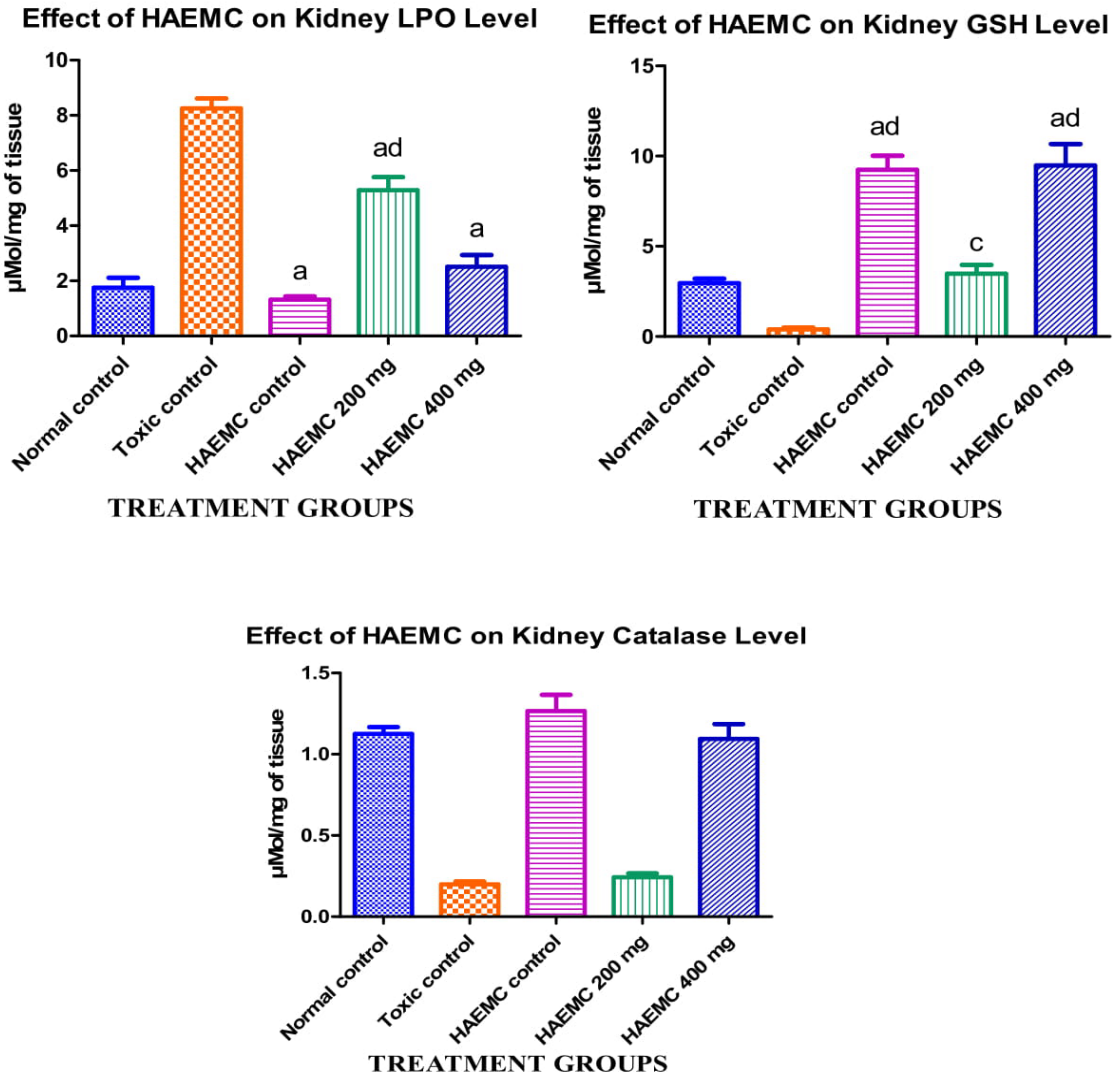

Figure 4. Effect of HAEMC on Antioxidant parameters

DISCUSSION:

In the present study, administration of NaF for 30 days caused a significant decrease in the serum albumin and total protein as well as significant increase in the serum urea, uric acid and creatinine levels indicating the induction of nephrotoxicity in rats when compared to the normal control. Therefore treatment with HAEMC for 30 days have shown significant changes in albumin, total protein, urea, uric acid and creatinine levels in treatment control groups when compare to toxic control group.

Considerably decrease in serum albumin and total protein as well as increase in serum urea, uric acid and creatinine levels suggests the impairment in glomerular function by the administration of NaF. These elevated serum levels indicates depressed clearance and lowered rate of filtration which results in reduced functioning of the kidney to eliminate the toxic metabolic substances. As kidney is the major organ for the removal of fluoride which shows harmful effects of fluoride retention and therefore results in disturbances in renal function [12].

Furthermore NaF treated group showed significant increase in lipid peroxidation due to increased ROS generation and free radic and the fluoride ions decreased the activity of reduced glutathione and catalase which indicated the induction of oxidative stress and serious damage in the biological membrane structure and cell activities [13]. The treatment with HAEMC with the doses of 200 and 400 mg/kg body weight per day for 30 days decreased the lipid peroxidation as well as increased the activities of reduced glutathione and catalase by producing the dose depending effects on the oxidative stress parameters of lipid peroxidation, reduced glutathione and catalase.

The histopathological results obtained correlated well with the biocgemical results and standard and EECC treated groups showed significant improvement when compared to disease control.

CONCLUSION:

To conclude, the present studies have shown that hydroalcoholic extractof fruits of *Momordica cymbalaria* prevents sodium fluoride nephrotoxicity in the rats through its antioxidant properties of the plant.

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