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Protective effects of Asparagus Adscendens Roxb against statin induced Rhabdomyolysis and Nephrotoxicity in rats

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Abstract

The primary objective of this work was to assess the potential protective properties of a hydroalcoholic extract derived from *A. adscendens* against rhabdomyolysis produced by the administration of rosuvastatin and gemfibrozil in a rat model. HMG-Coenzyme A reductase inhibitors are extensively used drugs for managing hyperlipidemias; nonetheless, their prolonged administration has been linked to the occurrence of rhabdomyolysis. The rats were subjected to oral administration of rosuvastatin at a dosage of 80 mg/kg and gemfibrozil at a dosage of 600 mg/kg twice daily for a period of 30 days. This treatment showed a notable elevation in several serum parameters, including creatine phosphokinase, urea, and blood urea nitrogen. These findings suggest the occurrence of rhabdomyolysis and the manifestation of renal complications in the rats. The co-administration of rosuvastatin and gemfibrozil resulted in a significant elevation in lipid peroxidation levels and a reduction in the levels of antioxidant enzymes (such as reduced glutathione, superoxide dismutase, and catalase) in rats. In order to mitigate the occurrence of rhabdomyolysis resulting from the combination of rosuvastatin and gemfibrozil, a hydroalcoholic extract was administered orally at doses of 200mg/kg and 400mg/kg.

The rats exhibited an elevation in antioxidant enzyme levels and a reduction in lipid peroxidation levels, together with improvements in serum markers of rhabdomyolysis, after the administration of a hydroalcoholic extract of *A. adscendens*. The histopathological examination of the kidneys from rats treated with rosuvastatin and gemfibrozil alone revealed significant renal injury, but the groups treated with the extract exhibited reduced damage to the nephrotic cells. The observed enhancement in biochemical parameters and reduction in oxidative stress levels suggest that the hydroalcoholic extract of *A. adscendens* possesses antioxidant properties, thereby influencing the biochemical alterations associated with rhabdomyolysis. Consequently, this extract exhibits potential protective effects against rhabdomyolysis.

Keywords: Rhabdomyolysis, Rosuvastatin, Asperagus adscendens, Gemfibrozil, Oxidative stress.

Introduction

Cardiovascular illnesses represent a significant health concern and are the primary cause of mortality. Hypercholesterolemia is well recognized as the primary etiological factor contributing to the development and progression of cardiovascular illnesses. There is a definitive association between hypercholesterolemia and atherosclerosis. HMG-CoA reductase inhibitors, often referred to as antihyperlipidemic medicines, have become the predominant therapeutic class for managing hypercholesterolemia. Nevertheless, the administration of statin medication may lead to significant side effects, such as the increase in enzyme levels in the liver and the occurrence of skeletal muscle abnormalities, namely myositis and myopathy.[1]

Rhabdomyolysis is a medical condition characterized by extensive muscular necrosis and myoglobinuria, accompanied by renal failure and/or electrolyte problems. It is clinically diagnosed by a creatine phosphokinase (CPK) result over 5000 U/L. Rhabdomyolysis is a condition characterized by the breakdown of striated muscles, leading to skeletal muscle damage that disrupts the integrity of the sarcolemma. This causes the escape of cellular components from the muscle cells into extracellular fluids and circulation.[2] The co-administration of fibric acid derivatives with HMG-CoA reductase inhibitors has been seen to result in drug-induced myopathy.

Rhabdomyolysis results in alterations to the viscosity of the sarcolemma, mostly owing to the activation of phospholipase, as well as the occurrence of numerous vasoactive molecules and proteases.[3] The phenomenon induces heightened permeability of the sarcolemma, facilitating the release of intracellular contents and an elevation in intracellular sodium ions. Consequently, there is an elevation in the intracellular calcium concentration, thus augmenting the functionality of intracellular proteolytic enzymes and facilitating the degradation of intracellular structures. Furthermore, the presence of calcium ions in the body contributes to generating reactive oxygen species, also known as free radicals, which may harm muscle tissue.[4] The presence of neutrophils in the injured muscle exacerbates the damage via the release of proteases and more free radicals. The observed outcome is characterized by an inflammatory response that is self-perpetuating and involves the breakdown of muscle tissue rather than a process of pure necrosis. Rhabdomyolysis is further characterized by severe hypokalemia, elevated blood myoglobin levels, and compromised glycogen synthesis, contributing to diminished energy generation within muscle tissues.

Elevated amounts of oxygen-free radicals have been seen in individuals diagnosed with rhabdomyolysis prior to the manifestation of symptoms. Reactive oxygen species, often referred to as free radicals, play a significant role in the pathophysiology of several illnesses. Antioxidants play a significant role in cellular defense mechanisms against free radicals. Toxicity that is facilitated or influenced by a specific agent or process.[5]

The antioxidant capacity of *Asperagus adessendens* Roxb. has been extensively studied and shown to possess significant antioxidant properties. Multiple research studies have confirmed its efficacy as a powerful antioxidant, revealing the existence of many beneficial compounds such as Vitamin E, β-carotene, Shatavarin IV, among others. These vitamins are considered to be very significant in their ability to scavenge free radicals. The compound becomes concentrated inside cellular membranes and blood lipoproteins, so providing protection against oxidation. These substances are sometimes referred to as competitive antioxidants due to

their ability to intercept lipid peroxyl radicals, therefore effectively terminating chain reactions of lipid peroxidation.[6,7] On the basis of the above facts, the present study has aimed to investigate the protective effect of hydroalcoholic extracts of *Asperagus adessendens* Roxb in rosuvastatin and gemfibrozil-induced rhabdomyolysis and nephrotoxicity in rats.

Materials and methods

Drugs and chemicals: For the study, chemicals from HIMEDIA in Mumbai were used. They were of the best quality and scientific grade. Rosuvastatin came from Carysyon Pharmaceuticals and gemfibrozil was a gift from Sun Pharmaceuticals, Baroda. To figure out the blood values, Span Diagnostic Kits were used.

Plant material: The roots of *Asparagus adscendens* were obtained from the Patanjali Herbal Garden located in Haridwar. Subsequently, they were subjected to a week-long process of shade drying, after which they were finely powdered using a blender. Approximately 500 grams of dried, finely ground *Asparagus adscendens* powder were immersed in an extractor and subjected to maceration for a duration of 30 hours, using petroleum ether as the solvent. The compound was subjected to successful reflex with chloroform, followed by separate extraction using alcohol and water by a continuous hot percolation process employing a Soxhlet apparatus for a duration of 40 hours. The hydro-alcoholic extract underwent filtration and subsequent concentration at decreased pressure using a rotary flask evaporator. Following the concentration process, a hydroalcoholic extract of *Asparagus adscendens* was obtained, resulting in a brownish residue. This residue was then kept in an air-tight container.

Animals: Wistar albino rats, regardless of sex, weighing between 150-250 g, were obtained from the Food and Drug Laboratory Baroda and then kept inside the animal facilities of the respective department. The animals were kept in conventional polypropylene cages and subjected to regulated environmental conditions, including a room temperature ranging from 22 to 25 degrees Celsius and a humidity level of 55 ± 5%. Additionally, the animals were exposed to a light and dark cycle of 12 hours each. The animals were supplied with a commercially available rat normal pellet diet and had unrestricted access to water. The animal experimental experiments conducted in this study adhered to the norms set out by the Committee for Control and Supervision of Experiments on Animals (CCSEA), a governmental body in India. Furthermore, prior clearance was obtained from the institutional animal ethics committee to ensure compliance with ethical standards.

Treatment protocol

Animals were divided into six groups of six animals in each. They received the following treatment for 30 days.

Group I: Normal animals received distilled water and feed.

Group II: Gemfibrozil (600 mg/kg, PO twice) and rosuvastatin (80 mg/kg) were given to the toxic control group.[8]

Group III: In comparison to normal rats, animals were given HAEAA (200 mg/kg p.o.) alone.

Group IV: In comparison to normal rats, animals were given HAEAA (400 mg/kg p.o.) alone.

Group V: Along with HAEAA (200 mg/kg p.o.), the treatment group also got gemfibrozil (600 mg/kg, p.o.) and rosuvastatin (80 mg/kg, p.o.).

Group VI: The treatment group was given HAEAA (200 mg/kg p.o.), gemfibrozil (600 mg/kg, p.o.), and rosuvastatin (80 mg/kg, p.o.).

Following the designated treatment period, every blood sample underwent analysis to determine the presence of prooxidants, antioxidants, and several other biochemical

variables. This study aimed to assess the extent of oxidative stress and metabolic abnormalities associated with the process of rhabdomyolysis. Blood samples were collected from the retroorbital plexus of rats that had undergone treatment. Subsequently, the samples were subjected to centrifugation at a speed of 2500 revolutions per minute (RPM) in a refrigerated centrifuge in order to obtain serum. Serum was used as a means to investigate several biochemical facets of rhabdomyolysis. Span Diagnostic kits were utilized on a RA chemical analyzer to conduct tests for the presence of rhabdomyolysis.

At the conclusion of the experiment, a volume of 5 ml of whole blood was collected from the rats who had undergone treatment, using an EDTA bulb. A spectrophotometer was used to quantify the levels of thiobarbituric acid reactive substances (TBARS), which serve as indicators of lipid peroxidation (LPO), as well as the activity of endogenous antioxidant enzymes including reduced glutathione (GSH), superoxide dismutase (SOD), and catalase, in all of the blood samples.

Assessment of several biochemical markers in rats treated with gemfibrozil and rosuvastatin-induced rhabdomyolysis: The colorimetric method at 340 nm9 was used to measure creatine kinase with Span Diagnostic Kits. Span Diagnostic Kits were used to figure out AST and LDH using the UV kinetic method 10 at 340 nm. Based on the picrate method at 500 nm, creatinine[11] was calculated. The urease method was used to figure out the color of Urea[12] and blood urea nitrogen (BUN).

Rats treated with gemfibrozil and rosuvastatin for rhabdomyolysis were examined to determine the amount of thiobarbituric acid reactive compounds (TBARS): Whole blood was taken in an EDTA tube after the treatment time. The blood was then centrifuged at 3000 rpm in a centrifuged machine (Remi-motors Ltd., Mumbai) to get plasma. The serum (supernatant) was used to measure thiobarbituric acid reactive chemicals, and the blood cells that had settled were used to measure SOD and catalase. We used a Spectrophotometer (UV-250, Shimadzu, Japan) and the method of Slater and Sawyer (1971)[13] to figure out the amount of TBARS.

A volume of 2.0 ml of plasma was combined with 2.0 ml of newly made trichloroacetic acid (TCA) solution, which had a concentration of 10% w/v. The resulting combination was then left undisturbed in an ice bath for a duration of 15 minutes. Following a 15-minute duration, the precipitate was isolated using centrifugation. Subsequently, 2.0 ml of the resulting clear supernatant was combined with an equal volume of newly made 0.67% thiobarbituric acid (TBA).

The solution obtained was subjected to heating in a water bath at boiling temperature for a duration of 10 minutes. Subsequently, the sample was promptly subjected to a cooling process in an ice bath for a duration of 5 minutes. The measurement of the produced color was conducted at a wavelength of 532 nm, relative to the reagent blank. Various amounts of malondialdehyde were used and subjected to the same procedures as described before for the construction of the standard graph. The results are quantified in nanomoles (nM) of malondialdehyde (MDA) per milligram (mg) of protein.

Measuring reduced glutathione in rats treated with gemfibrozil and rosuvastatin-induced rhabdomyolysis: The method of Moron et al. (1979)[14] was used to measure reduced glutathione in whole blood. Whole blood and 20% trichloroacetic acid were mixed in the same amount. The separated part was spun in a centrifuge, and 2 ml of 0.6 mM 5,5-dithiobis (2-nitro benzoic acid) solution was added to 0.25 ml of the supernatant. Phosphate buffer (0.2 M, pH 8.0) was used to make the total amount 3 ml. At 412 nm, the color that

was formed was compared to a blank reagent. Standard glutathione in different amounts (10–50 qg) was used and handled in the same way as above for the standard curve. pg of GSH/mg protein was used to show how much reduced glutathione there was.

Superoxide dismutase (SOD) levels in rats treated with gemfibrozil and rosuvastatin-induced rhabdomyolysis were measured: Whole blood was taken in an EDTA tube after the treatment time. The blood was then spun at 3000 rpm in a machine made by Remi-motors Ltd., Mumbai. The blood cells that had been settled down were used to measure SOD and catalase. The method of Misra and Fridovich (1972) was used to figure out the amount of superoxide dismutase.[15] They mixed 0.5 ml of tissue purée with 0.5 ml of pure water. Then, they added 0.25 ml of ice-cold ethanol and 0.15 ml of ice-cold chloroform. A cyclo mixer was used to mix the ingredients well for 5 minutes, and the product was spun at 2500 rpm. 1.5 ml of carbonate buffer (0.05M, pH 10.2) and 0.5 ml of EDTA solution (0.49M) were added to 0.5 ml of supernatant. When 0.4 ml of epinephrine (3 mM) was added, the reaction began. The change in optical density per minute was recorded at 480 nm compared to a blank reagent. Units/mg of protein were used to show SOD function. The enzyme unit is that which changes the amount of light that passes through a substance every minute when epinephrine to adrenochrome transfer is blocked by 50%. 10-125 units of SOD were used to make the calibration curve.

Catalase (CAT) levels in rats treated with gemfibrozil and rosuvastatin-induced rhabdomyolysis: Hugo Aebi's method, described by Colowick et al. (1984)[16], was used to figure out the amount of catalase in blood cells that had settled down. As a start, 1 ml of hydrogen peroxide (30 mmol/1) was added to 2 ml of the weakened sample. To make the blank, 2 ml of the sample that had been diluted was mixed with 1 ml of phosphate buffer (50 mmol/1; pH 7.0). So that the original absorption is about 0.500, the reduction should be just right. At 240 nm, the drop in absorption was recorded. Catalase activity was shown as q moles of water used up per minute per mg of protein.

Histopathology of Kidney: The kidney obtained from the rats that underwent treatment was extracted and then preserved in a formalin solution. Subsequent procedures included dehydration and tissue cleaning. The 5mm thick sections were excised and then subjected to staining using Hematoxylin and Eosin (H/E) stains. The stained slices were observed quantitatively using magnifications of 40X and 100X.

Statistical analysis: The results were reported as the mean standard error of the mean (SEM). The unpaired t-test was used to analyze the data comparing two distinct groups. The data from different groups was subjected to statistical analysis using analysis of variance (ANOVA) followed by the Tukey test to determine the significance of the results.

Results

Impact of gemfibrozil with rosuvastatin on rhabdomyolysis serum parameters: The co-administration of rosuvastatin and gemfibrozil in rats resulted in a notable elevation in all the biochemical markers used for evaluating rhabdomyolysis, as compared to the control group of animals (as shown in Table 1). The findings indicate a notable occurrence of rhabdomyolysis after the administration of simvastatin in combination with gemfibrozil.

Assessment of HAEAA condition in rhabdomyolysis: The co-administration of rosuvastatin and gemfibrozil in rats resulted in a notable elevation in lipid peroxidation levels as compared to the control group, indicating the potential generation of free radicals in the context of

rhabdomyolysis (as shown in Table 1). The blood levels of glutathione (GSH), superoxide dismutase (SOD), and catalase exhibit a notable decrease after the administration of rosuvastatin in combination with gemfibrozil, as compared to their respective control groups. The occurrence of oxidative stress in rhabdomyolysis may be attributed to a notable rise in the production of free radicals and a substantial decline in the levels of antioxidant enzymes.

Changes in the biochemical parameter of rhabdomyolysis after an extract therapy: The findings indicate that the treatment of HAEAA to the normal rats did not result in any notable alteration in the biochemical parameter of rhabdomyolysis. However, when HAEAA was administered in conjunction with rosuvastatin + gemfibrozil, it led to a considerable improvement in the biochemical parameters of rhabdomyolysis, as shown in Table 2. The findings indicate that the administration of HAEAA led to enhancements in blood parameters and the restoration of high serum parameter levels in individuals with rhabdomyolysis.

Alterations in antioxidant enzymes and lipid peroxidation levels subsequent to o-tocopherol administration in rhabdomyolysis: The administration of HAEAA in isolation did not result in any significant alteration in the levels of antioxidant enzymes in normal rats, as seen in Table 1. The administration of o-tocopherol resulted in a noteworthy elevation in the levels of antioxidant enzymes, namely reduced glutathione, superoxide dismutase, and catalase, in a group of subjects with rosuvastatin + gemfibrozil caused rhabdomyolysis, as compared to a separate group treated with rosuvastatin + gemfibrozil (Table 4). The findings indicate a significant increase in the levels of antioxidant enzymes subsequent to the administration of HAEAA.

The hydroalcoholic extract of A. ascendens, when administered at both dosages, did not result in a statistically significant alteration in lipid peroxidation levels in normal rats. However, it did lead to a substantial reduction in lipid peroxidation levels in rats with rosuvastatin + gemfibrozil induced rhabdomyolysis, as compared to the corresponding control group. The findings indicate a significant decline in lipid peroxidation and a decrease in the generation of free radicals after administration of o-tocopherol.

Effect of rosuvastatin + gemfibrozil on histopathology of kidney: The histopathological examination of the kidney (Figure 2) reveals the presence of minor renal injury in the group treated with a combination of rosuvastatin and gemfibrozil. It has been shown that severe instances of rhabdomyolysis may result in renal damage. However, it is worth noting that the modest renal damage seen in this research may be attributed to the 30-day period of therapy and the dosage of the statin administered. The kidney preparations in the treatment group were subjected to histological assessment, which showed a reduction in tubular necrosis generated by statins (Fig. 3,4).

Discussion

Statins and fibric acid derivatives have been widely recognized as the primary pharmacological agents for managing hypercholesterolemia. However, the administration of these medications has been linked to the occurrence of myopathy and rhabdomyolysis. In the current investigation, the treatment of a combination of rosuvastatin and gemfibrozil resulted in the occurrence of rhabdomyolysis in rats. This condition was characterized by elevated levels of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), creatinine (CRTN), urea, and blood urea nitrogen (BUN). Additionally, the

administration of [the specific intervention] resulted in an elevation in oxidative stress levels, a reduction in the concentration of antioxidant enzymes, and the manifestation of muscular weakness in the experimental rat population. These findings strongly imply the initiation of rhabdomyolysis. There is currently no established association between the signs and symptoms of rhabdomyolysis in humans and animal models. However, the measurement of certain biochemical indicators in the serum of these animal models does indicate the development of rhabdomyolysis. The administration of HMG-CoA reductase inhibitors resulted in a decrease in blood concentrations of Coenzyme Q A[17]. Elevated amounts of oxygen free radicals were seen in individuals diagnosed with rhabdomyolysis prior to the manifestation of symptoms. The liberation of myoglobin from muscular tissues has the potential to induce the production of free radicals, which may subsequently lead to renal damage. The hypothesized mechanism behind statin-induced rhabdomyolysis is a reduction in the production of ubiquinone and dolichol. Ubiquinone functions as an electron transporter in the process of oxidative phosphorylation, serves as a stabilizer for cellular membranes, and operates as an intracellular scavenger of free radicals in order to mitigate lipid peroxidation.

The hydroalcoholic extract derived from A. adscendens has antioxidant properties and plays a significant role in cellular defense against the harmful effects of free radicals in the human body. Antioxidant defense systems function to mitigate the harmful effects of reactive oxygen species via detoxification or scavenging processes.

The ingestion of HAEAA resulted in a normalization of serum parameters in cases with rhabdomyolysis. Additionally, the administration of HEAA resulted in a reduction in lipid peroxidation levels and an increase in the levels of antioxidant enzymes, namely glutathione (GSH), superoxide dismutase (SOD), and catalase. These findings suggest that HEAA has a protective impact in the context of rhabdomyolysis. Based on the aforementioned discovery, it can be inferred that the administration of *Asperagus ascendens* extracts, known for their strong antioxidant properties, effectively mitigates the heightened oxidative stress generated by statin usage in the body. Furthermore, these extracts demonstrate the ability to restore the raised serum parameters back to their normal levels in rats afflicted with statin-induced rhabdomyolysis.

The histopathological examination reveals the presence of minor renal injury in rats afflicted with rhabdomyolysis. It is plausible that the length of therapy might account for this particular finding.

The biochemical analyses provided evidence supporting the occurrence of rhabdomyolysis and renal failure as a result of the long-term administration of rosuvastatin in combination with gemfibrozil. The pro-oxidant-antioxidant balance also indicates the potential role of oxidative stress in the pathogenesis of rhabdomyolysis. The decrease in the body's antioxidant defense system caused by statins has been shown to contribute to the development of rhabdomyolysis. However, it has been observed that the hydroalcoholic extract of A. ascendens may restore the level of antioxidant defense and provide substantial protection against rhabdomyolysis generated by statins and gemfibrozil.

Table 1.

Group No.	Treatment Dose (mg/Kg)	MDAu/ mg Protein	GSH u/mg Protein	CATu/mg protein	SODu/mg Protein	GPu/mg Protein
I	Normal control	2.11±0.18	15.15±0.40	55.30±1.40	15.30±0.40	23.20±0.90
II	Toxic control rosuvatatin+ gemfibrozil	4.90±0.42*[a]	4.40±0.20*a	10.45±0.35*a	6.15±0.16*a	10.10±0.32*a
III	Alone HAEAA 200mg/kg	2.01±0.24*[b]	13.40±0.36*[b]	55.90±0.75*[b]	14.05±0.32*[b]	22.15±0.56*[b]
IV	Alone HAEAA 400 MG/KG	2.65±0.32*[b]	14.52±0.26*	55.45±0.45*[b]	15.90±0.25*[b]	22.40±0.62*[b]
V	Treatment control HAEAA 200mg/kg + rosuvatatin+ gemfibrozil	3.05±0.26*b	11.25±0.30*b	34.50±0.68*b	12.60±0.28*b	18.25±0.68*b
VI	Treatment control HAEAA 400mg/kg + rosuvatatin+ gemfibrozil	2.02±0.26*b	14.25±0.29*b	45.50±0.84*b	14.60±0.31*b	20.25±0.78*b

Table 2

Group No.	Treatment Dose (mg/Kg)	CPK (IU/L)	Serum creatinine	Blood urea nitrogen mg/dl	Alkaline phosphate mg/dl	Urea mg/dl
I	Normal control	203.5 ± 1.9	0.5±0.02	18.55±0.58	155.60±0.75	17.2±0.40
II	Toxic control rosuvatatin + gemfibrozil	1119± 2.1*a	5.310±0.05*[a]	104.30±5.5*a	345.20±1.58*a	78.6±1.65*a
III	Alone HAEAA 200mg/kg	206.1 ± 1.4*b	0.5±0.03*b	18.75±1.28*b	155.75±0.90*b	17.5±0.48*b
IV	Alone HAEAA 400 MG/KG	205.4 ± 1.6*b	0.5±0.03*b	18.10±2.05*b	155.60±1.05*b	17.4±0.56*b
V	Treatment control HAEAA 200mg/kg + rosuvatatin+ gemfibrozil	367 ± 2.7*b	0.8±0.03*b	44.25±1.60*b	175.45±0.96*b	17.2±0.50*b

VI	Treatment control HAEAA 400mg/kg + rosuvatatin+ gemfibrozil	325 ± 1.6*b	0.61±0.03*b	22.55±0.58*b	145.60±0.75*b	18.0 ±0.45*b

Fig. 1 Normal control

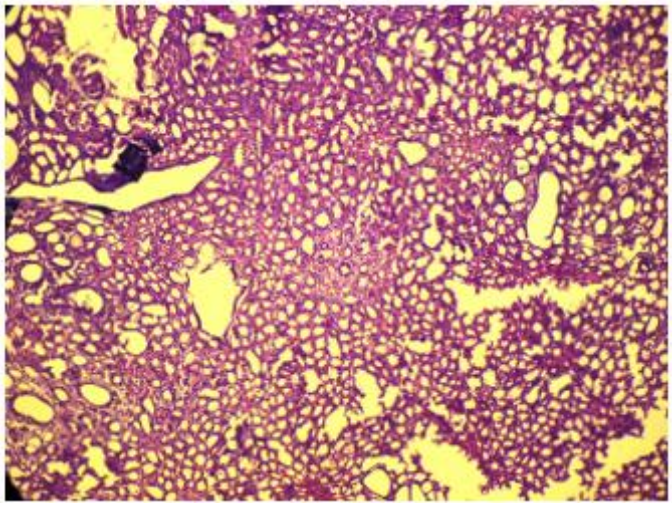


Fig. 2 Toxic control

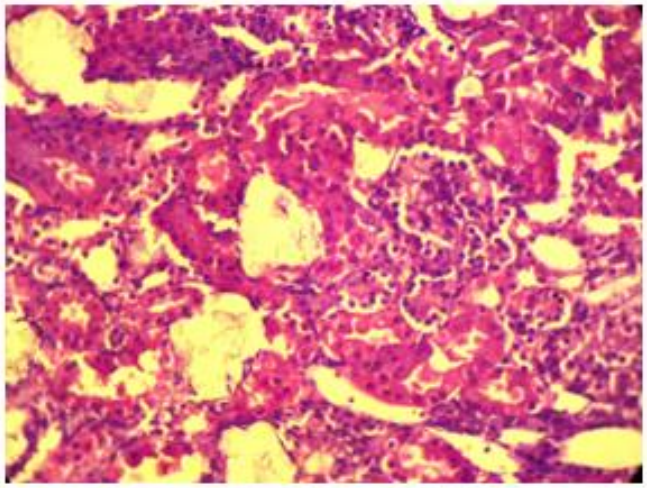


Fig. 3 Treated group HAEAA 200 mg/kg

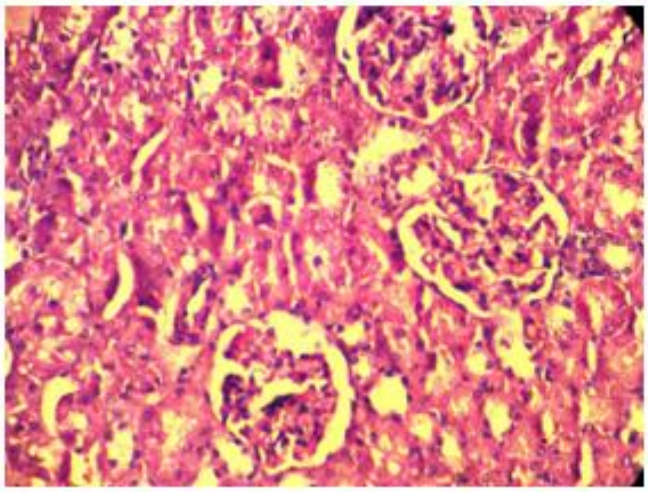
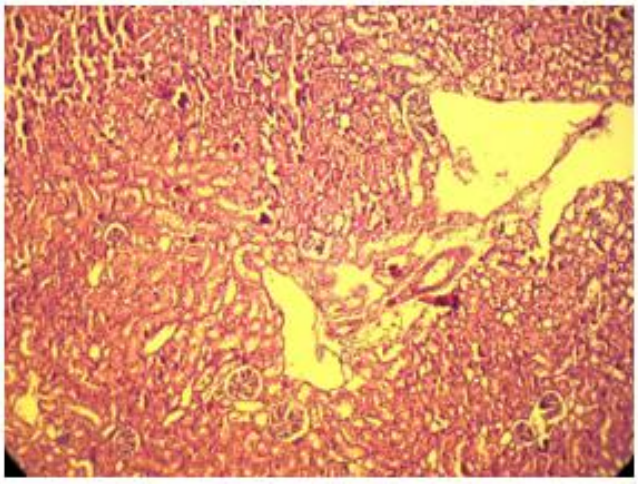


Fig 4 Treated group HAEAA 400 mg/kg



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