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Ameliorative action of aqueous extract of *Acalypha indica* against puffer fish *Lagocephalus lunaris* induced toxicity

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Abstract

Acalypha indica has been used in poisoning cases and are known to possess significant antioxidant activities. The objective of this study is to evaluate the protective effects of an aqueous extract (5 g/l) of *A. indica* (leaves and stems), on biochemical disorder, oxidative stress and histopathological changes induced by muscle extract of puffer fish *Lagocephalus lunaris* in swiss albino mice. Mice injected with *L. lunaris* muscle extract (1 ml/100g body weight) for 10 days showed (1) a reduced appetite and diarrhea resulting in a lower growth rate than controls, (2) an increase in serum Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase and Total & Direct bilirubin levels suggesting liver functional disorders, (3) an increase of serum creatinine and uric acid concentrations highlighting renal insufficiency and (4) increase in the level of serum triglycerides, total cholesterol, glucose and low density lipoproteins indicate the interference in the metabolism or biosynthesis of lipids (5) an oxidative stress as evidenced by the raise of TBARS and the inhibition of antioxidant enzymes (Superoxide dismutase, Catalase and Glutathione peroxidase) activities in liver, kidney and heart tissues (6) histopathological changes in liver, kidney and heart tissues. Absorption of *A. indica* aqueous extract as a drink, for 10 days demonstrated ameliorative action and prevented the disorders induced by *L. lunaris* muscle extract.

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Lagocephalus lunaris, toxicity assessment, biochemical disorders, oxidative stress and histopathology.

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INTRODUCTION

Puffer fish is the second most poisonous vertebrates in the world while on the other hand it is one of the most expensive Japanese delicacies prepared by trained chefs. Tetrodotoxin (TTX) is a heterocyclic guanidium alkaloid ($C_{11}H_{17}N_3O_8$) found in puffer fish,

the main causal agent for human intoxication after consuming the puffer fish. Puffer fish poisoning cases have been recorded right from the 17th century [1] and are still being recorded though the number of cases has reduced. It is particularly common in Japan but a number of cases have been reported in many other Asian countries such as China, Taiwan, Hong Kong, Philippines, Thailand, Bangladesh, India as well as other continents [2-3]. No antidote for puffer toxin has been discovered yet. Intoxicated subjects are treated symptomatically together with cardiovascular and respiratory supports, fluids and diuretics are given to accelerate the toxin excretion [4].Many years of studies have demonstrated that toxicity of puffer fish shows remarkable individual and regional variations [5]. In most marine puffers high concentration of TTX are found in liver and ovaries/eggs but significant amounts are also detected in digestive tissues, skin and muscles [6-8]. *Lagocephalus lunaris* (Lunartail Puffer) is a commonly available puffer fish in the southeast Indian coast and is eaten by the locals. Both intact as well as in disguise the meat of *L. lunaris* which tastes like chicken meat but relatively cheaper is sold in fresh market by unscrupulous vendors for making easy money. Although TTX was not detected and quantified by mouse bioassay in the tissue extracts of *L. lunaris* but symptoms of intoxication were observed (under publication) which contradicted to the other findings[6,9-10], so this work was taken up. It was shown previously that puffer fish *Lagocephalus lagocephalus* flesh or liver extracts could produce oxidative damages in liver, kidney and erythrocyte tissues[11-12].In this study it was found that when *L. lunaris* muscle extract (1 ml/100 g of body weight) was given to swiss albino mice by intraperitoneal injection, the mice exhibited biochemical disorders and oxidative stress. The increased intake of dietary antioxidants may help to shift the balance towards an adequate antioxidant status [13].

Acalypha indica L. (Indian nattle) is a small annual shrub which generally occurs as a troublesome weed in gardens, roadsides and throughout the plains of India [14]. The plant is used as an antidote [15] for snakebite in Northern India [16]. *A. indica* has find application in different fields of pharmacology. The plant was traditionally used as an expectorant against asthma and pneumonia and also as an emetic, emenagogue and anthelmintic [17]. *A. indica* contains acalyphine which is used in the treatment of sore gums and to have a post-coital antifertility effect [18], anti-venom properties [19], wound healing effects [20], antioxidant activities [21], anti-inflammatory effects [22], acaricidal effects [23], diuretic effects [24] and antibacterial activities [25]. Therefore, this study was designed to investigate the protective effects of aqueous extract of *A. indica* against *L. lunaris* induced toxicity.

MATERIALS AND METHODS

Sample collection

Specimens of puffer fish *L. lunaris* were collected from local fishermen who usually catch the fish using trawl net from Chennai coast, Tamil Nadu in the southeastern part of India. It was collected during the month of February 2011 and transported to the laboratory in dry ice. Samples were frozen at -20 ºC until use. The specimens were identified by Prof. S.S. Khora, Division of Medical Biotechnology, Vellore Institute of Technology University (VITU), Vellore, India. Specimens of plant *A. indica* were collected from the campus of VITU, Vellore, India and were identified by the Taxonomist, in the Department of Plant Biotechnology, School of Biosciences and Technology, VITU, Vellore, India.

Fish extracts preparation

10 g of muscle was carefully dissected from *L. lunaris* and minced properly. To this minced tissue 2.5 volumes of 0.1% acetic acid was added and boiled in water bath for 10 min. Then it was cooled and centrifuged at 3000 rpm for 10 min and the

supernatant was collected. This process was repeated thrice, to make up 5 volumes of the sample taken [26]. The supernatant was finally stored at -30 ºC.

Plant extracts preparation

Aerial part (stem and leaves) of the plant *A. indica* was taken. Air-dried samples were ground, washed, put in boiling water (5 g/l, v/w) for 15– 20 min, and then filtered using Whatman filter paper [27]. The aqueous extract was given as beverage instead of normal water.

Animals

Adult male Swiss albino mice weighing about 25-35 g were obtained from VIT animal house (VIT University, Vellore, Tamil Nadu, India). They were housed at 22 ± 3 ºC with light/dark periods of 12 hour and a minimum relative humidity of 40%. Mice were fed with a commercial balanced diet (V.R.K. Nutritional Solutions) and drinking water was offered *ad libitum*. This study was carried out in strict accordance with the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Guidelines for laboratory animal facility. The protocol was approved by the Institutional Animal Ethical Committee (IAEC), VIT University (Permit Number: VIT/IAEC/IV/014/2011). All surgery was performed under anesthesia by intraperitoneal injection of chloral hydrate, and all efforts were made to minimize suffering.

Experimental design

After 1 week of acclimatization, mice were divided into two batches: 12 control mice drinking normal water and 12 treated mice drinking *A. indica* extract for 10 days [27]. Again, each group was divided into two subgroups and one of them was injected daily, for 10 days, with *L. lunaris* muscle extract (1 ml/100 g, v/w) [12]. Therefore, the mice were divided into 4 groups: Group A (Control drinking normal water), Group B (drinking normal water + i.p. injection of *L. lunaris* extract), Group C (drinking aqueous extract *of A. indica*), and Group D (drinking aqueous extract *of A. indica* + i.p. injection of *L. lunaris* extract). The choice of *L. lunaris* muscle extract was based on our previous studies (under publication). Body weight and food consumption were monitored during treatment.

Mice were sacrificed under anesthesia by intra peritoneal injection of chloral hydrate. The blood was collected without anticoagulant by heart puncture. The liver, kidneys and heart were excised and investigated for oxidative stress.

Serum parameters

The blood collected was centrifuged (4000 rpm/15 min, 4 ºC) and serum and blood cells were obtained. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Creatinine (CRE), Uric Acid (UA), Triglyceride (TG), Total Cholesterol (TC), HDL Cholesterol (HDL-C), LDL Cholesterol (LDL-C), Total Bilirubin (T Bil), Direct Bilirubin (D Bil), Albumin (ALB), Globulin (GLB), Total protein (TP) and Glucose (GLU) were determined in the serum using standard commercial kits (Span Diagnostics, India).

Oxidative stress analysis

Preparation of tissue

The liver, kidneys and heart were excised and rinsed with ice cold saline. The liver was homogenized (1:2, w/v) in 50 m mol/phosphate buffer (pH 7.4) and centrifuged at 5000 rpm for 30 min at 4 ºC [27] The supernatants obtained were stored at -30 ºC until use. The same procedure was repeated for kidney and heart.

Assay of antioxidant enzymes

Superoxide dismutase (SOD) activity was determined according to the method of Marklund and Marklund [28] which involves pyrogallol auto oxidation at pH 8.0. It is expressed in units/mg protein. One unit of

enzyme activity is defined as the amount of enzyme required to give 50% inhibition of pyrogallol auto-oxidation and the absorbance was read at 420 nm. Catalase (CAT) activity was determined by the method of Sinha [29]. In this method, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of H2O2, with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced was measured calorimetrically at 610 nm. The specific activity of catalase has been expressed as μmol of H_2O_2 consumed/min/mg protein. Glutathione peroxidase (GPx) activity was determined spectrophotometrically by using Ellmans reagent (DTNB) as a coloring reagent following the method described by Rotruck et al. [30] and the absorbance was read at 412 nm. The specific activity of GPx is expressed as μg of Glutathione (GSH) utilized/min/mg protein.

Assay of lipid peroxidation

Lipid peroxidation was determined by the procedure of Ohkawa et al. [31]. Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of oxidative stress expressed as n mol MDA/mg protein. The absorbance was measured at 530 nm.

Histopathology

Immediately after sacrifice, a of portion liver, kidney and heart were fixed in 10% formalin. The washed tissues were dehydrated in descending grades of isopropanol and finally cleared with xylene. These tissues were then embedded in molten paraffin wax. Sections were cut at 5 μm thickness and stained with haematoxylin.

Statistical analysis

Statistical analysis was performed using the Prism software package (GraphPad, InStat Version 3). Data were expressed as the mean and the standard deviation of the mean (SD). The analysis of variance (ANOVA) was used to compare the differences between the groups. Differences were considered significant at the 95% confidence level ($p < 0.05$).

RESULTS

Effects on mice body weight and food consumption

As shown in Fig. 1, during the experiment, the increase in body weight was similar in Group A (control) and Group C (drinking aqueous extract of *A. indica*) but lower in Group B (drinking normal water + i.p. injection of *L. lunaris* extract). In Group B the weight loss was accompanied by a significant decrease in diet consumption, as compared to the controls. The weight gain in Group D (drinking aqueous extract *of A. indica* + i.p. injection of *L. lunaris* extract) was lower when compared to control (Table 1).

Effects on Serum parameters

A marked decrease in ALT, AST, and ALP activities and HDL-C while a significant increase in CRE, UA, TC, TG, LDL-C, T Bil, D Bil, ALB, GLB, TP and GLU was found in the serum of mice treated with *L. lunaris* muscle extract compared to control. Post treatment with *A. indica* extract showed significant increase in ALT, AST, ALP activities and HDL-C while a significant decrease in CRE, UA, TC, TG, LDL-C, T Bil, D Bil, ALB, GLB, TP and GLU when compared to the toxic group. No significant change was observed in mice treated with only *A. indica* when compared to control (Table 2).

Effects on Antioxidant enzymes

A significant decrease of SOD, CAT and GPx activities was found in the liver, kidney and heart of mice treated with *L. lunaris* muscle extract compared to control whereas, post treatment with *A. indica* extract showed significant increase in SOD, CAT and GPx activities when compared to the toxic group. No significant change was observed in mice treated with only *A. indica* when compared to control (Fig. 2, Fig. 3, and Fig. 4).

Effects on lipid peroxidation

Exposure to *L. lunaris* muscle extract alone significantly increased the TBARS levels in the liver of mice whereas *A. indica* alone did not change the degree of TBARS formation compared to controls. The combination of *L. lunaris* muscle extract and *A. indica* showed significant decrease in TBARS level when compared to the toxic group (Fig. 5).

Histopathological changes

Liver section from control group (Group A) showed normal lobular architecture and normal hepatic cells (Fig. 6a). The liver section from animals injected with *L. lunaris* muscle extract (Group B) showed liver damage in the form of congestion, degeneration and hepatocellular necrosis (Fig. 6b, 6c, 6d). The histological pattern of liver of the mice injected with *L. lunaris* muscle extract and then treated with *A. indica* aqueous extract (Group D) showed no necrosis however it showed congestion (Fig. 6e).

Kidney section from the control group (Group A) showed normal renal tubules and Bowman's capsule (Fig. 7a) while the group receiving *L. lunaris* muscle extract (Group B) showed tubular epithelial cell degeneration capsule showed mononuclear cell infiltration and congestion was also observed (Fig. 7b, 7c, 7d). The histological pattern of kidney of the mice injected with *L. lunaris* muscle extract and then treated with *A. indica* aqueous extract (Group D) showed mild tubular epithelial cell degeneration (Fig. 7e)

Heart section from the control group (Group A) showed normal features (Fig. 8a) while the group receiving *L. lunaris* muscle extract (Group B) showed mononuclear cell infiltration, myocardial degeneration and necrosis (Fig. 8b). The histological pattern of heart of the mice injected with *L. lunaris* muscle extract and then treated with *A. indica* aqueous extract (Group D) showed congestion (Fig. 8c).

DISCUSSION

This is the first study which identifies the protective role of *A. indica* aqueous extract against *L. lunaris* muscle extract induced toxicity in male swiss albino mice through comprehensive analysis of toxicity and oxidative stress biomarkers.

A. indica has higher scavenging action for DPPH radical thereby showing higher antioxidant activity which can be attributed to the presence of phenolic compounds, flavanoids, coumarins, quinines etc. as proved by the phytochemical analysis [32].

The present findings are consistent with Saoudi et al. [27] that puffer fish *Lagocephalus sp*. extract induces oxidative damage and biochemical disorders. The daily injection of *L. lunaris* muscle extract in mice for 10 days led to reduced appetite and diarrhea resulting in a lower growth rate than control while no change in growth rate was observed in mice treated with *A. indica* aqueous extract.

Usually the serum ALT and AST activities are elevated in viral and other forms of liver diseases associated with hepatic necrosis [33-35]. But in the present study there was a significant decrease in serum ALT and AST activities in mice injected with *L. lunaris* muscle extract which can be attributed to the damaged structural integrity of the liver observed in the form of congestion, cellular degeneration and hepatocellular necrosis in the histopathological liver section. Serum ALP measurement is of particular interest in the hepatobiliary diseases and in bone diseases. Decrease in activity of ALP is found in rare congenital defect, hypophosphatasemia and in pernicious anemia [34, 36-37]. In this case the decrease in serum ALP activity in the group injected with *L. lunaris* muscle extract may be due to the induced hepatic disorder. The tendency of these enzymes to return to normal levels in the *A. indica* extract administered group is a clear manifestation of hepatoprotective [38-39] effect of the *A. indica* extract. Therefore, treatment with *A. indica* aqueous extract seems to preserve the structural integrity of hepatocellular membranes.

Elevated bilirubin level was observed in the toxic group. Total bilirubin concentration is increased mildly in chronic haemolytic disease, moderately to severely in hepatocellular diseases and markedly in cholestatis [40-41]. The levels of total protein and albumin were reduced in the *L. lunaris* extract injected group. The reduction can be attributed to the initial damage produced and localized in the endoplasmic reticulam which could result in the loss of P-450 leading to fatty liver as seen in the case of carbon tetrachloride toxicity [42]. Concurrent depletion of raised bilirubin levels after treatment with *A. indica* aqueous extract suggests the stability of the biliary function during the damage caused due to *L.lunaris* muscle extract. The protein albumin levels also rose suggesting the stabilization of endoplasmic reticulum leading to protein synthesis.

Serum urate concentration is elevated in most of the patients with gout and renal diseases and in conditions where there is increased breakdown of nucleic acid [43-44]. Elevated serum creatinine and uric acid concentrations indicate the risk of impaired renal functions [45]. The elevated serum creatinine and uric acid in the group injected with *L. lunaris* muscle extract can be related to the structural alterations in renal tissues as previously reported by Saoudi et al. [12] and is clearly evident from the histopathological changes in the kidney sections. *A. indica* aqueous extract conferred protection against *L. lunaris* induced nephrotoxicity by maintaining the serum creatinine and uric acid levels.

The increase in level of glucose in the group injected with L. *lunaris* muscle extract may be due to impaired glucose uptake and changes in hepatic glucose metabolism. The impaired glucose tolerance occurs mainly due to insulin resistance of peripheral tissue [46]. *A. indica* aqueous extract showed hypoglycemic effect on L. *lunaris* muscle extract induced metabolic disturbances. This could be due to its insulinotropic action. Additional study is needed to identify the action of *A. indica* extract on glucose regulation.

Moreover, increase in the level of serum triglycerides, total cholesterol and low density lipoproteins indicate there is interference in the metabolism or biosynthesis of lipids. High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage [47]. *L. lunaris* muscle extract increases the LDL cholesterol concentration in the blood, which in turn leads to the buildup of harmful deposits in the arteries, thus favoring coronary heart disease [48]. High levels of serum triglyceride have been strongly linked with low serum HDL cholesterol, and these low HDL levels may contribute to increased risk for cardiovascular diseases [49]. Treatment with *A. indica* aqueous extract lowers the level of serum triglycerides, total cholesterol and low density lipoproteins. The cardioprotective effect of *A. indica* [19,50] is clearly evident from the histopathological changes.

A number of studies have focused on pathogenetic significance of oxidative stress in hepatic, renal and cardiac injury as well as on therapeutic intervention of the process with antioxidant and metabolic scavengers. Excess level of reactive oxygen species and reactive nitrogen species can attack biological molecules such as DNA, protein and phospholipids, which leads to lipid peroxidation, nitration of tyrosine and depletion of antioxidant enzymes (SOD, CAT and GPx) that further results in oxidative stress [51]. In accordance with previous findings [27], in this study a high lipid peroxidation with concomitant decrease in antioxidant enzymes was observed in the group injected with *L. lunaris* muscle extract. *A. indica* aqueous extract was observed to exhibit protective effect as demonstrated by enhanced activities of antioxidant enzymes and diminished amount of lipid peroxidation against *L. lunaris* muscle extract induced toxicity. Previous phytochemical investigations on *A. indica* revealed the presence of acalyphamide (as acetate), aurantiamide and its acetate, succinimide, calypholacetate, 2- methyl anthraquinone, tri o-

methylellagic acid, b- sitosterol and its b-D-glucoside , cyanogenetic glycoside, two alkaloids, viz. Acalyphine and triacetonamine and also n- octasanol, b- sitosterol acetate, kaempferol, quebrachitol, tannin, resin, hydrocyanic acid and essential oil [52-55]. Most of these compounds have been reported to be potent inhibitor of lipid peroxide formation, free radical scavenger and to increase antioxidant enzymes [32,39,56]. Flavanoids, alkaloids and saponins are linked with hepatoprotective, nephroprotective and cardioprotective activity [50, 57-58].

This work reports that *A. indica* aqueous extract is able to counteract the undesirable effects like reduced appetite and diarrhea, oxidative stress, increase in serum aminotransferases, alkaline phosphatase, bilirubin, creatinine, uric acid, triglycerides, total cholesterol and glucose induced by *L. lunaris* muscle extract. Therefore, present results provide strong evidence that *A. indica* aqueous extract inhibits the hepatic, renal and cardiac toxicity induced by *L. lunaris* muscle extract which can be linked to the particular phytoconstituents of *A. indica*. However, further pharmacological evidences at molecular level are required to establish the mechanism of action of the drug which is underway.

Acknowledgement

The authors are thankful to the authority of VIT University, Vellore for the facilities and encouragement.

Table 1: Effects of *L. lunaris* muscle extract, *A. indica* aqueous extract and their combination on final body weight and total food consumption of mice.

Parameters	Group A	Group B	Group C	Group D
Final Body weight (g)	20.7±0.82	18.6±1.17***	21.6±1.17$^{###}$	20.7±0.95$^{###}$
Food consumption (g)	52.5±2.7	34.17±3.8***	52.5±2.7$^{###+++}$	43.3±2.6$^{###+++}$

Values are expressed as mean ± SD for 6 mice in each group.
Group B, C, D vs Group A: $^{***}p\leq0.001$
Group C, D vs Group A: $^{###}p\leq0.001$
Group C vs Group D: $^{+++}$ p≤0.001

Table 2: Effects of *L. lunaris* muscle extract, *A. indica* aqueous extract and their combination on serum biochemical parameters

Parameters	Group A	Group B	Group C	Group D
ALT	82.51±9.1	26.52±9.7***	79.56±9.7$^{###}$	64.83±14.4$^{###}$
AST	306.45±14.4	111.97±9.1***	306.45±14.4$^{###}$	194.48±11.2$^{***###+++}$
ALP	49.72±11.1	27.12±0.0*	45.2±14.1	36.16±14.1
T-BIL	0.26±0.02	0.51±0.02***	0.26±0.03$^{###}$	0.4±0.002$^{***###+++}$
D-BIL	0.04±0.01	0.25±0.01***	0.07±0.02$^{*###}$	0.22±0.01$^{***###+++}$
CRE	0.43±0.1	1.390.04***	0.37±0.1$^{###}$	0.77±0.2$^{**###+++}$
UA	5.8±0.2	8.68±0.2***	5±0.1$^{***###}$	7.95±0.4$^{***###+++}$
TP	7.49±0.1	5.97±0.1***	7.34±0.2$^{###}$	6.4±0.5$^{###++}$
ALB	4.12±0.03	3.3±0.09***	4.2±0.06$^{###}$	3.82±0.14$^{***#+++}$
GLB	3.38±0.3	2.67±0.2*	3.15±0.3$^{###}$	2.59±0.4$^{##}$
A/G	1.23±0.2	1.24±0.1	1.35±0.1	1.51±0.3
GLU	87.37±0.5	195.15±0.7***	80.97±2.4$^{###}$	139.12±8.9$^{***###+++}$
TC	64.29±1.9	115.17±3***	53.35±4.5$^{***###}$	78.02±2.6$^{***###+++}$
TG	73.46±7.2	155.3±17.4***	72.35±7.2$^{###}$	100.48±15.2$^{**###++}$
HDL-C	30.94±1.6	9.71±2.1***	31.47±2.6$^{###}$	19.92±0.7$^{***###+++}$
LDL-C	18.67±2.6	74.47±5.7***	8.91±2.4$^{***###}$	38±2.9$^{***###+++}$

Values are expressed as mean ± SD for 6 mice in each group.
Group B, C, D vs Group A: $^{***}p\leq0.001$; $^{**}p\leq0.01$; $^{*}p\leq0.05$.
Group C, D vs Group A: $^{###}p\leq0.001$; $^{##}p\leq0.01$; $^{#}p\leq0.05$.
Group C vs Group D: $^{+++}$ p≤0.001; $^{++}p\leq0.01$.

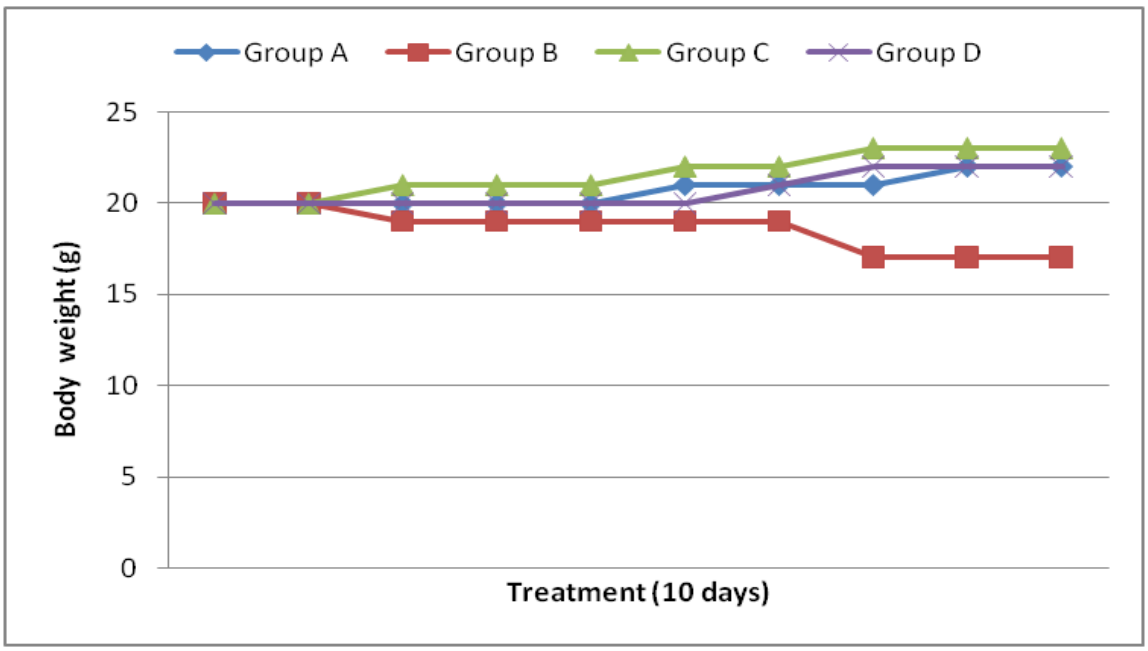
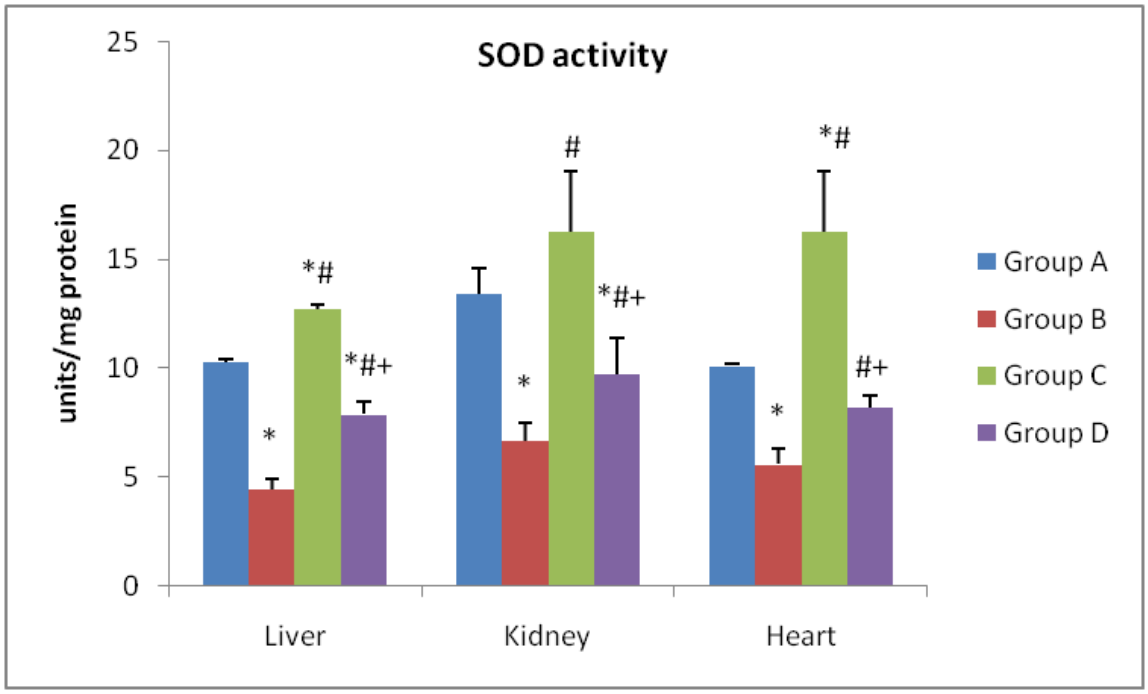
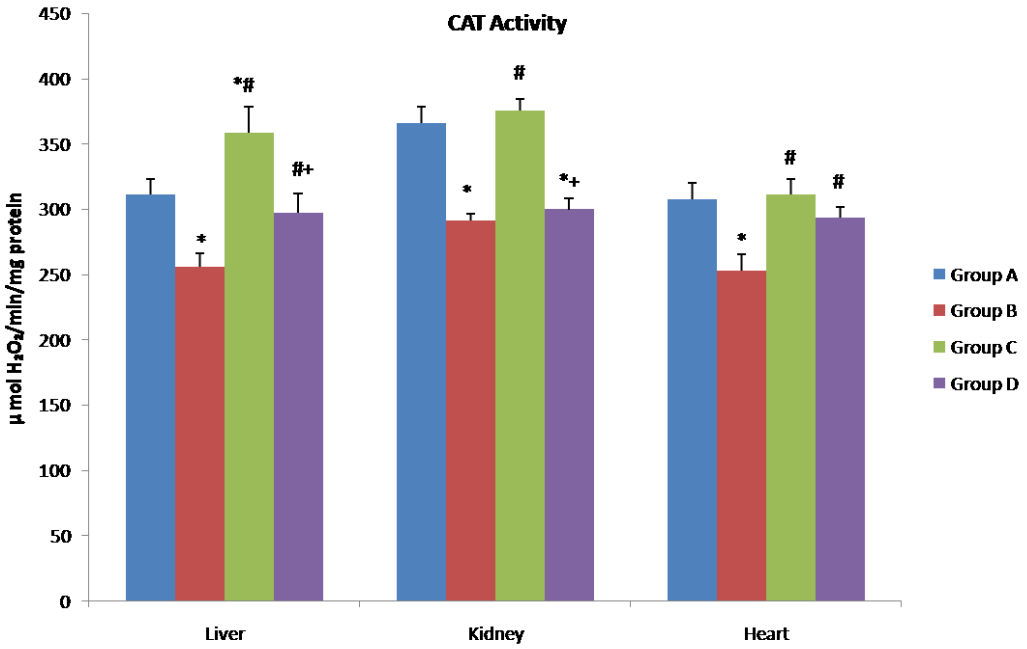


Fig. 1 Effects of *L. lunaris* muscle tissue extract, *A. indica* aqueous extract and their combinations on mice body weight and food consumption.



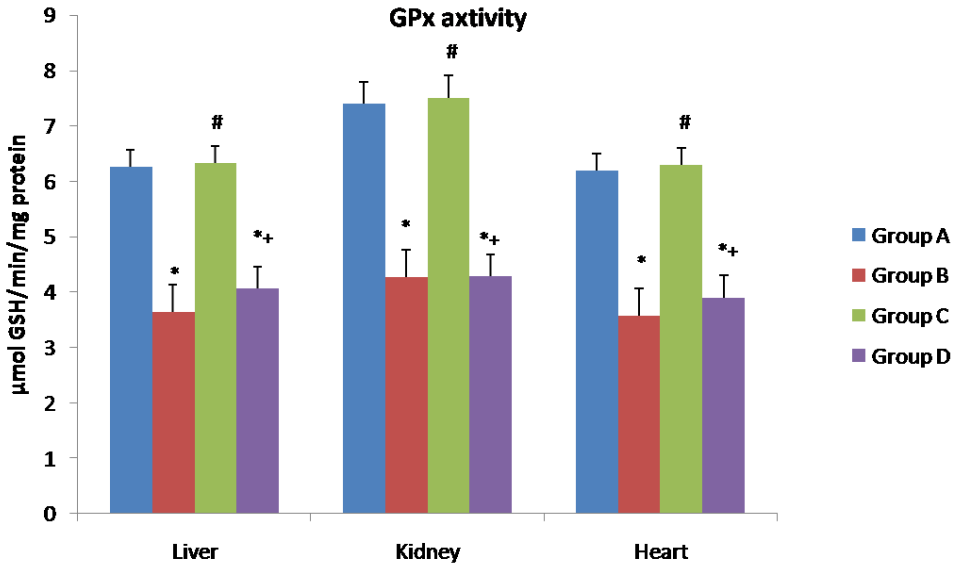
Values are expressed as mean ± SD for 6 mice in each group. *p≤0.05: Group B, C, D vs Group A; #p≤0.05: Group C, D vs Group A; + p≤0.05: Group C vs Group D.

Fig. 2 Effects of *L. lunaris* muscle tissue extract, *A. indica* aqueous extract and their combinations on Superoxide dismutase activity in liver, kidney and heart tissues of mice.



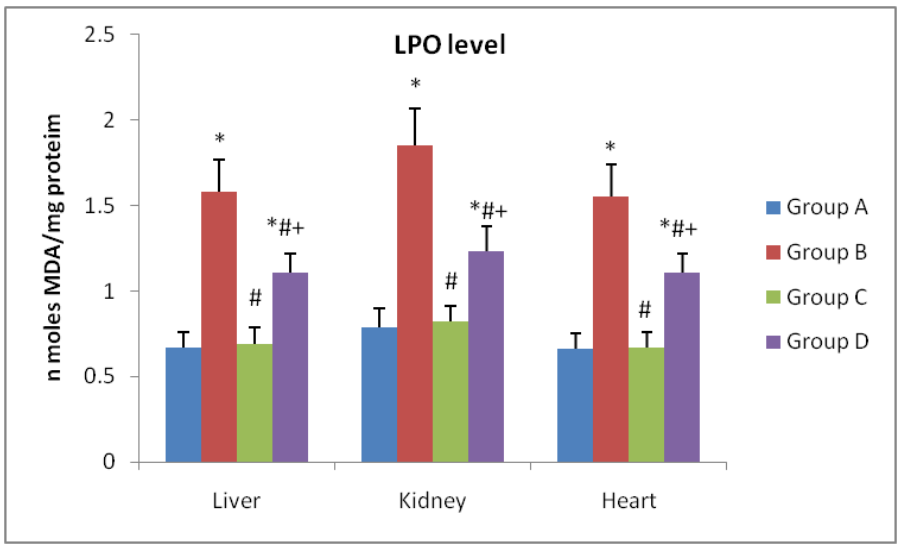
Values are expressed as mean ± SD for 6 mice in each group.*p≤0.05: Group B, C, D vs Group A; #p≤0.05: Group C, D vs Group A; + p≤0.05: Group C vs Group D.

Fig. 3 Effects of *L. lunaris* muscle tissue extract, *A. indica* aqueous extract and their combinations on Catalase activity in liver, kidney and heart tissues of mice.



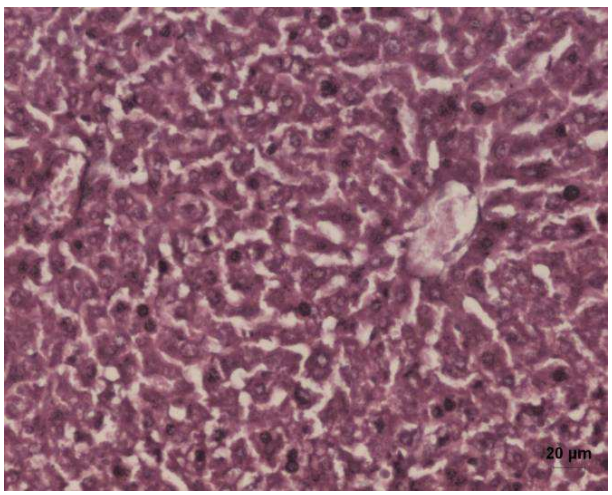
Values are expressed as mean ± SD for 6 mice in each group.*p≤0.05: Group B, C, D vs Group A; #p≤0.05: Group C, D vs Group A; + p≤0.05: Group C vs Group D.

Fig. 4 Effects of *L. lunaris* muscle tissue extract, *A. indica* aqueous extract and their combinations on Glutathione peroxidase activity in liver, kidney and heart tissues of mice.

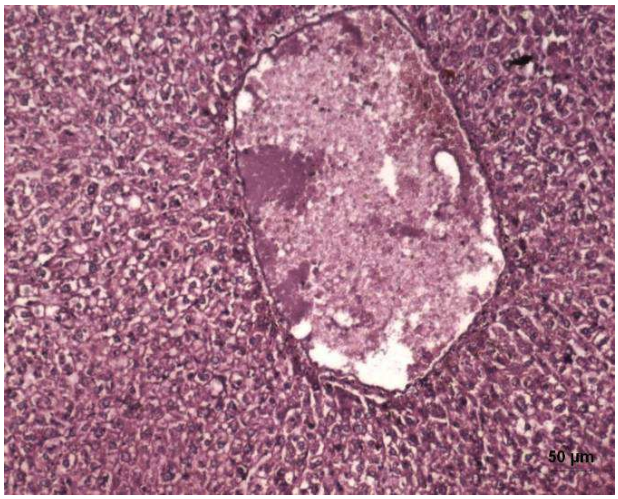


Values are expressed as mean ± SD for 6 mice in each group.*p≤0.05: Group B, C, D vs Group A; #p≤0.05: Group C, D vs Group A; + p≤0.05: Group C vs Group D.

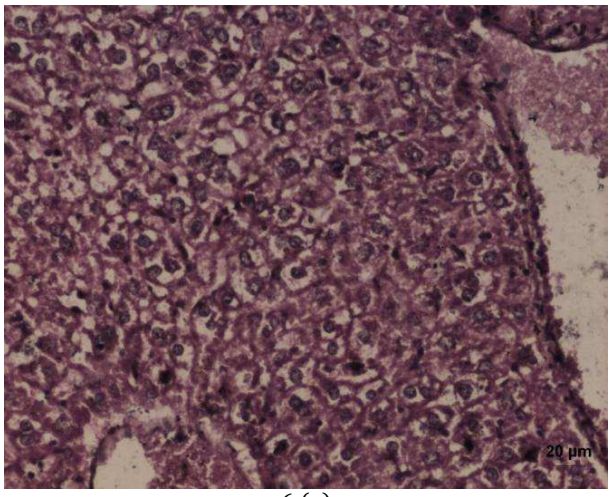
Fig. 5 Effects of *L. lunaris* muscle tissue extract, *A. indica* aqueous extract and their combinations on Lipid peroxidation level in liver, kidney and heart tissues of mice.



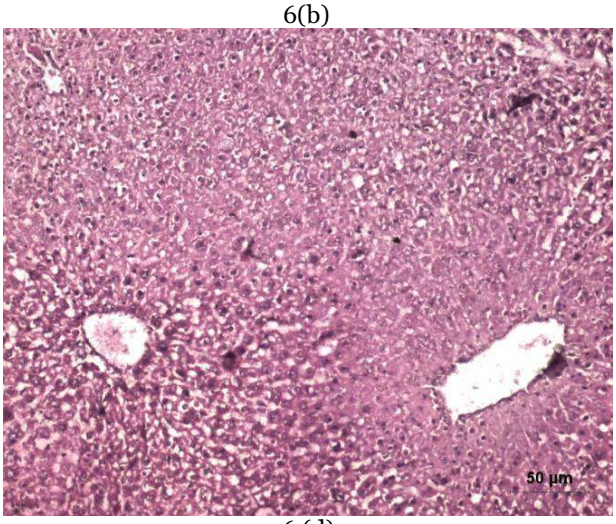
6(a)



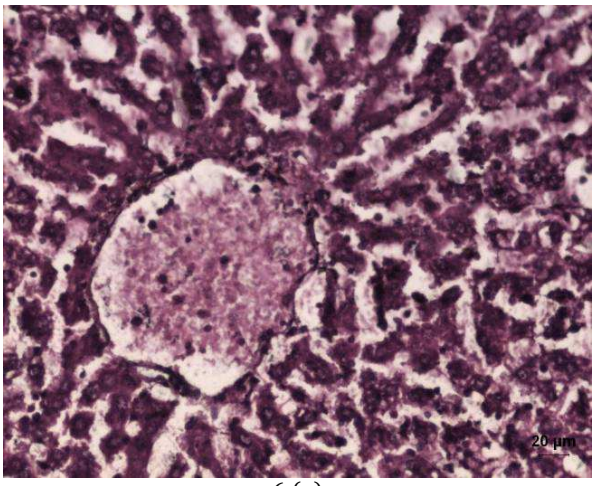
6(b)



6 (c)

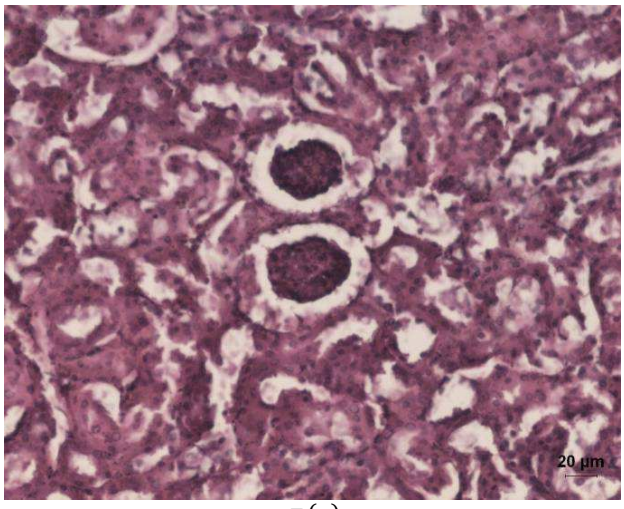


6 (d)

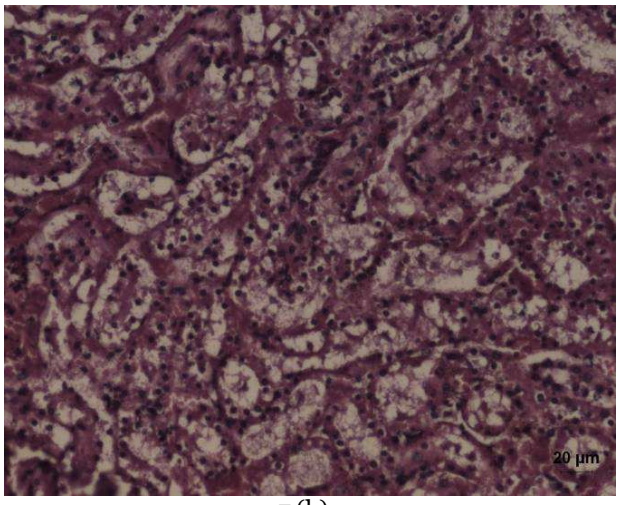


6 (e)

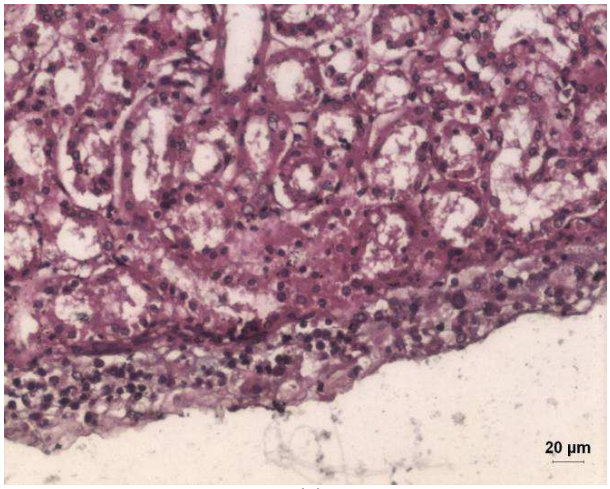
Fig. 6 Pictomicrographs of liver sections taken from different groups. (a) control group showing normal lobular architecture and normal hepatic cells (b) *L. lunaris* muscle extract injected group showing liver damage in the form of congestion (c) degeneration (d) hepatocellular necrosis (e) group injected with *L. lunaris* muscle extract and then treated with *A. indica* aqueous extract showing improvement as there was no necrosis however congestion was observed.



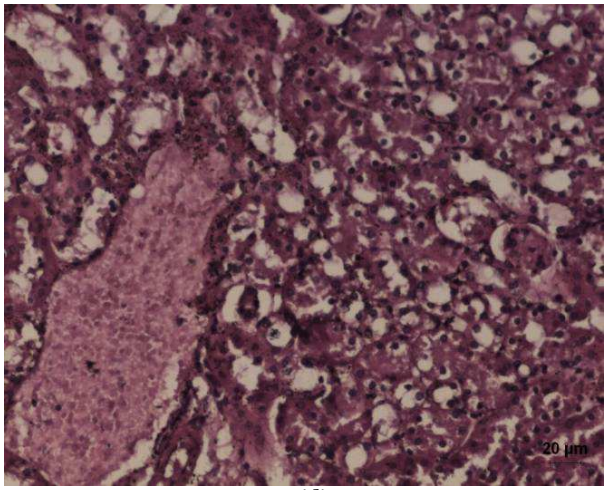
7 (a)



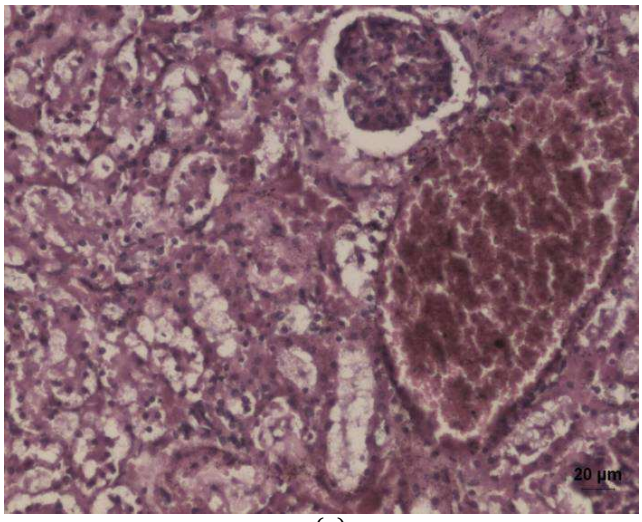
7 (b)



7 (c)

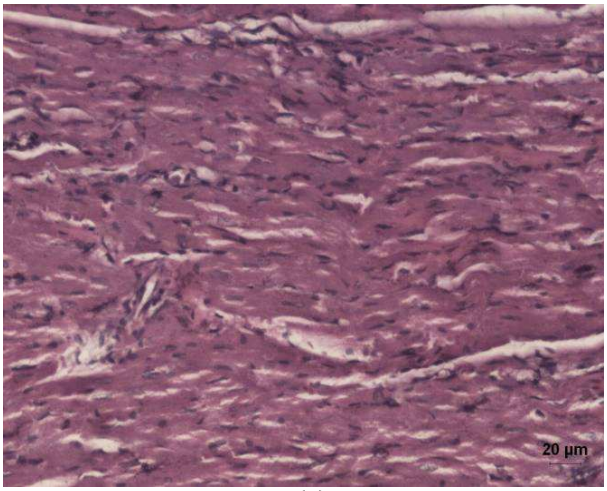


7 (d)

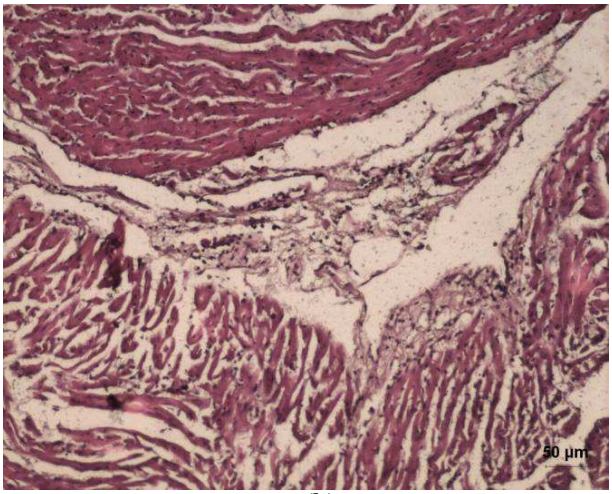


7 (e)

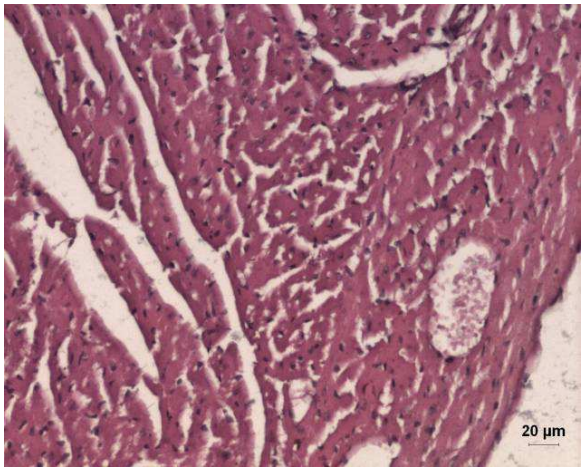
Fig. 7: Pictomicrographs of kindey sections taken from different groups. (a) control group showing normal renal tubules and Bowman's capsule (b) *L. lunaris* muscle extract injected group showing renal damage in the form of tubular epithelial cell degeneration (c) capsule showed mononuclear cell infiltration (d) congestion (e) group injected with *L. lunaris* muscle extract and then treated with *A. indica* aqueous extract showing improvement with mild tubular epithelial cell degeneration.



8 (a)



8 (b)



8 (c)

Fig. 8 Pictomicrographs of heart sections taken from different groups. (a) control group showing normal features (b) *L. lunaris* muscle extract injected group showing cardiac damage in the form of mononuclear cell infiltration, myocardial degeneration and necrosis (c) group injected with *L. lunaris* muscle extract and then treated with *A. indica* aqueous extract showing improvement as there was no necrosis however congestion was observed.

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