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Research Article

Ameliorating property of Morin-5'-Sulfonic acid sodium salt (NaMSA) on mercuric chloride ($HgCl_2$) Induced histological changes in *Albino* rats

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Abstract

Mercury is a divalent metal without any biological functions. It affects every system in the body. Identifying and removing the source of the mercury is very essential. Chelating agents are primarily sulfhydryl-containing compounds. Currently flavonoids have been identified development of therapeutic agent to reduce and prevent harmful effects of heavy metals. Hence; the present investigation was designed to nullify the toxic effect of $HgCl_2$ using a flavonoid (Morin), Morin is found in integral part of the human diet, sparingly soluble in water. Hence, its sulphonation derivative was selected and NaMSA was synthesized with hydrophilic nature. Administration of $HgCl_2$ (1.25 mg/kg p.o.) for continuous 30 days led to its accumulation in the plasma, kidney, liver and testis compared with control rats. At the same time NaMSA(50 mg/kg p.o.) and $HgCl_2$(1.25 mg/kg p.o.) simultaneous administered rats effectively maintained the Hg level similar to the control rats in the plasma, liver, kidney and testis and protects the organs from mercury toxicity than 100mg/kg. Hence, it was determined as optimum dose against $HgCl_2$ (1.25 mg/kg p.o.) toxicity. Mercury causes histopathological and ultra-structural lesions in the liver, kidney, testis and heart. It was evidenced by peripheral fatty degeneration and cell necrosis. Whereas the effect was very negligible due to the simultaneous administration of NaMSA(50 mg/kg p.o.) and $HgCl_2$ (1.25 mg/kg p.o.) and maintains the integrity of the tissues of liver, kidney, testis and heart and the same was proved by the Eosin and Haemotoxylin stains used in this study did not notice any considerable changes in the structure of liver, kidney, testis and heart. From the current investigation it is concluded that administration of mercury in sub-acute doses accumulates in the plasma, kidney, liver and heart,which in turn proportionately damages the liver, kidney, testis and heart, whereas 50 mg/kg NaMSA administerd with $HgCl_2$ protects the organs from mercury toxicity.

Keywords: Morin-5'-sulfonic acid sodium salt, Mercuric chloride, Morin

Introduction

Mercury

Mercury is a heavy metal (Hg) and atomic number 80, occurs as organic mercury, elemental mercuric and inorganic mercury. It is a widespread environmental and industrial pollutant, induces

Sources

Mercuric poisoning can be caused by many ways i) Amalgam dental fillings ii) Eating fish, industrial and work place iii) Hospitals-Thermometers, thermostats, sphygmomanometers, batteries, dental

amalgams and Laboratory chemicals like zenkers solution and histological fixatives. iv) Exposures such as those in the paint industry, cosmetics, air emissions, fungicide industry, v) electrical apparatus, battery production, Electric lamps.

Risks

The concentration of mercury in fish in other sea food consumed in certain coastal areas reported in range of 0.03-10.82 g/g compared to the permissible limit of 0.5 g/g. There is a potential risk to human health and environment due to the entry of mercury in food chain [2.]

Absorption

Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into blood stream [3].

Mechanism

Hg^{2+} has shown a great affinity for endogenous biomolecules-associated with thiol (-SH) group [4] and it is invariably found attached to SH-containing proteins, small-molecular weight pepides (such as glutathione) and amino acid (such as cysteine)[4], leading to a profound deterioration of vital metabolic processes [5,6]. Consequently, the oxidative stress was strongly suggested as one of the crucial mechanisms in Hg-induced pathological aspects [4.] However, biochemical parameters are still more indicative of early physiological changes following sub chronic and chronic Hg exposure [7.] Diagnosis of elemental or inorganic mercury poisoning involves determining the history of exposure, physical findings and an elevated body burden of mercury [8].

Toxic effects

Liver damage

An increase in the reactive oxygen species (ROS) formation by $HgCl_2$ may induce liver cell membrane structural alterations [9.]

Renal damage

Higher levels of urea and, in particular, creatinine clearly reflected progressing renal insufficiency with mercuric chloride[10].

Testis damage

Sperm production may be decreased due to the established inhibitory effects of MeHg on cellular mitosis [11].

Cardiac disease

The clinical consequences of mercury toxicity include hypertension, coronary heart disease, myocardial infarction, atherosclerosis and reduced heart rate [12].

Therapeutic Approaches

Identifying and removing the source of the mercury is crucial, Immediate chelation therapy is the standard of care for a patient showing symptoms of severe mercuric poisoning or the laboratory evidence of a large total mercuric load[13].

Many chelating (therapeutic) agents are available in practice for acute inorganic mercuric poisoning with the following efficiency

DMPS>DMSA>penicillamine>ALA>EDTA.Glutathione and N- Acetylcysteine (NAC) are recommended by some physicians but have been shown to increase mercury concentrations in the kidney sand the brain [14].

Chelating agents are primarily sulfhydril-containing compounds such as mono- or dithiol molecules. At the molecular level, the chelation process appears as an inevitable tug of war between the chelating agents and the competing biological ligands [15].

Flavonoids

Currently phytochemicals identified from foodstuffs presenting an exciting opportunity for the development of therapeutic agent to reduce and prevent harmful effects of heavy metals.

Mechanism

Flavonoids act against variety of diseases, such as anti-viral, anti-allergic, anti-platelet and anti-inflammatory, also possibly protective against chronic diseases[16&17]. Anti-tumor promoting activity against human carcinogenesis[18].

Morin (Morin hydrate) - $C_{15}H_{10}O_7$. xH_2O

2', 3, 4', 5, 7- Penta hydroxyl flavone, Molecular Weight: 302.24 (anhydrous basis), Color-Natural yellow 11, Solubility- methanol: soluble 50 mg/ml, Water: Sparingly soluble.

Morin is aubiquitous phenolic secondary metabolite found in orange, almonds, mill, fig, onion, guava and apple and is an integral part of the human diet [19], sparingly soluble in water. Hence, it is necessary to synthesis a derivative of morin, which should have all the qualities of morin and also hydrophilic nature. Hence, sulphonation derivative of morin was selected.

Pharmacokinetic Properties of morin-5'-sulfonic acid sodium salt (NaMSA)

Morin-5'-sulfonic acid sodium salt ismorin derivative, easily soluble in water and keeps the property of the parent compound[20]. Till now no scientific evidence is available for using morin-5'-sulfonicacid sodium salt as
Therapeutic agent against mercuric chloride induced toxicity. Hence, the present investigation was designed to study roleofmorin-5'-sulfonicacidsodiumsaltinamelioratingthemercury induced biochemical changes in *albino* rats using the following objectives i)Accumulation of Hg in Plasma, Kidney, Liver, Testis ii) Histopathological changes in the Kidney, Liver, Testis and Heart.

Materials and methods

Chemicals used

The fine chemicals used for the present study purchased from Merck Company, Mumbai, India. Mercuric chloride and Morin obtained from sigma Aldrich, USA and the rest of the chemicals and biochemical sutilized were obtained from local firms (India) and were of analytical grade.

Methods

Mercury quantified using external calibration from digestion blank (treated batch of tissue samples) and the aqueous samples of digested Analyzer M-6100 [3]**, s**ynthesis of NaMSA[21].

Experimental design

A total number of 24rats were taken for this present study, considered the carefully their age, sex (male) and weight

Group A :(Control): Rats orally administrated with 0.9%saline (at the volume of mercuric chloride/day) for 30days.**Group B:** Rats administered with morin-5'-sulfonic acid sodium salt (50 mg/kg p.o.) dissolved in water for 30 days. **Group C:** Rats administered orally by stomach tube with mercuric chloride(1.25 mg/kg p.o.) dissolved in 0.9%saline for continuous 30 days. The dosage of $HgCl_2$ was determined from the study performed by[22]. **Group D:** Rats orally administered with mercuric chloride (1.25 mg/kg body weight p.o.) dissolved in 0.9%saline for continuous 30 days followed bymorin-5'-sulfonic acid sodium salt (50 mg/kg p.o.) dissolved in water simultaneouslyfor30 days.

Laboratory animals

Wister strain *albino* rats weighing180-220g were used. The rats were acclimatized in animal house for ten days before starting the experiment. This study was approved by CPCSEA, New Delhi and Institutional Ethical Committee of Adhiparasakthi College of Arts and Science. No. APCAS/IAEC/2010/01.

Preparation of $HgCl_2$ and induction of multiple organ failure

Required quantity of $HgCl_2$ was taken and dissolved in 0.9% saline. Rats administered orally by stomach tube with mercury in the form of mercuric chloride at the concentration of 1.25mg/kgb.wt[22].

Morin-5'-sulfonic acid sodium salt (NaMSA)

Morin was purchased from sigma chemicals, not soluble in water but soluble in alcohol 50mg/ml. so, it is necessary to convert insoluble form of morin to water soluble NaMSA form [21].

Preparation of serum and tissue

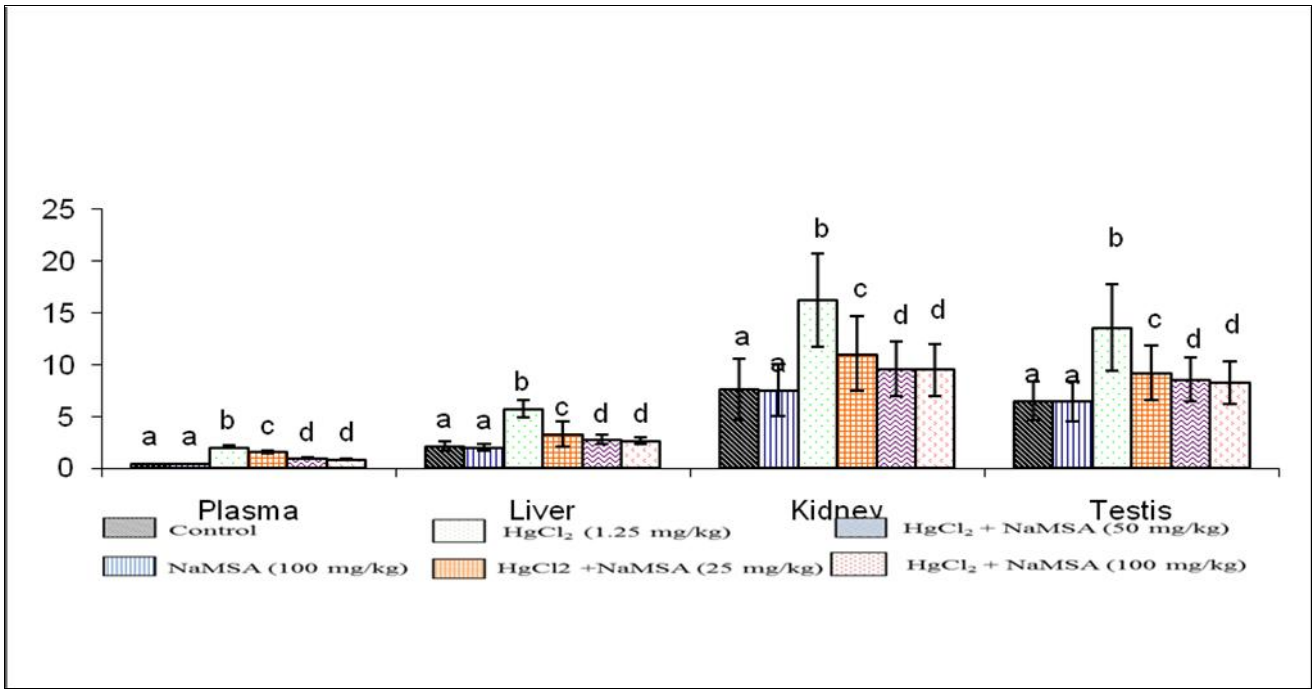
At the end of the experimental period (30days), all the animals were anesthetized with intramuscular injection of ketamine (75 mg/kgb.wt.) and sacrificed by cervical decapitation. Blood was collected with anticoagulant and centrifuged (2000 xg for20 min)to separateplasma. The tissues were dissected out, weighed, minced and homogenized(10%w/v) in Tris-HCL buffer(0.1M;pH 7.4) and centrifuged at 3000xg for 20min at 4°C. The resultant supernatant was used for the analysis.

Histopathological study

Haematoxylin and Eosin staining: A portion of liver, kidney, testis and cardiac tissue were fixed in 10 % formalin. The washed tissue was dehydrated in descending grades of isopropanol and cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5-µm thickness and stained with haematoxylin and eosin. The sections were then viewed under light microscope for histopathological changes in the Heart. The assessment of cardiac injury was carried out in a blinded fashion. Heart section with histopathological alterations like vacuolation, hydropic degenerative changes was recorded.

Results

Figure - 1: Effect of NaMSA on $HgCl_2$ induced Changes on the Hg level of control and experimental rats



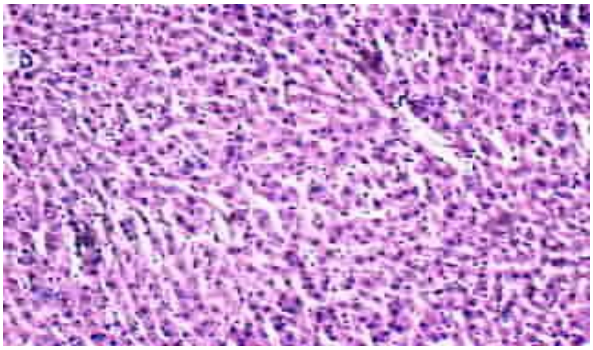
$HgCl_2$ - Mercuric chloride; NaMSA – Morin -5'-sulfonic acid sodium salt
Values are means ± S.D for six rats.
Hg Values in liver, kidney and testis are expressed in µ moles/g tissue
Hg plasma value expressed in µg/dl
Values not sharing a common superscript and differ significantly at $p< 0.05$ (DMRT)

Administration of $HgCl_2$ brought the mercury level to abnormal level significantly ($p<0.05$) in the plasma, kidney, liver and testis compared with control rats and the accumulation of mercury in organs in the following order, kidney>liver>testis. At the same time NaMSA and $HgCl_2$ simultaneously administration in rats keeps the Hg level similar to the control rats in the plasma, liver, kidney and testis. Significant accumulation of mercury was shown in the present work in plasma, liver, kidney and testis as the time progresses. According to [23], Hg content increases in the liver dose dependent manner. Administration of $HgCl_2$ (1.25 mg/kg p.o.) for continuous 30 days led to its accumulation in the plasma, kidney, liver and testis compared with control rats. At the same time NaMSA(50 mg/kg p.o.) and $HgCl_2$(1.25 mg/kg p.o.) simultaneous administered rats effectively maintained the Hg level similar to the control rats in the plasma, liver, kidney and testis and protects the organs from mercury toxicity than 100mg/kg. Hence, it was determined as the optimum dose against $HgCl_2$ (1.25 mg/kg p.o.) toxicity

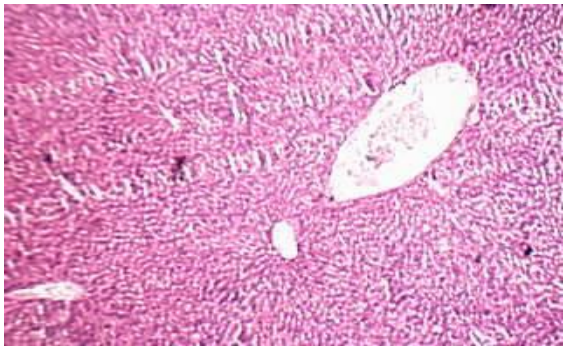
Figures - 2, 3, 4 & 5, Shows the histopathological changes in the tissues of liver, kidney, heart and testis

Figures 2, 3, 4 & 5 reveals respectively the mercuric chloride caused ultra-structural lesions in the liver, kidney, testis and heart. it was evidenced by peripheral fatty degeneration and cell necrosis whereas the effect was very negligible due to the simultaneous administration of NaMSA and $HgCl_2$ and maintains the integrity of the tissues of liver, kidney, testis and heart and the same was proved by the Eosin and Haemotoxylin stains used in this study did not notice any considerable changes in the structure of liver, kidney, testis and heart.

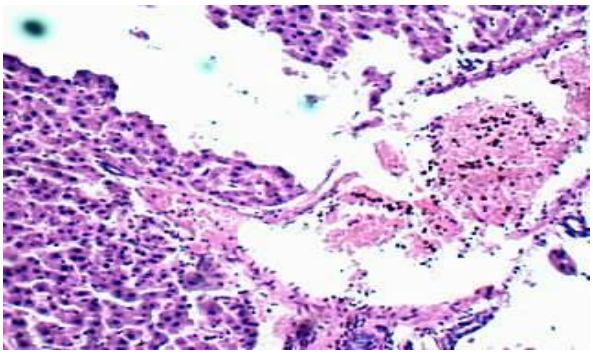
Figure- 2: Effect of NaMSA on $HgCl_2$ influenced liver of control and experimental rats



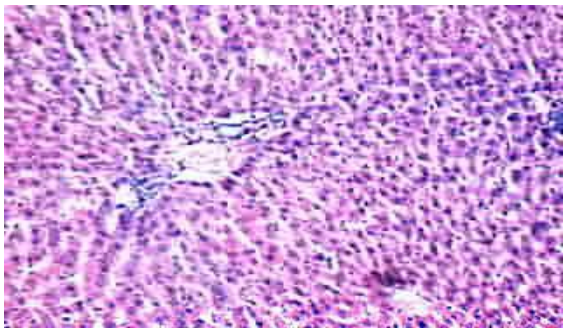
Control
H and E 100 of control liver (0.9 % saline) - appears with normal hepatocyte.



Control + NaMSA
H and E 100 of positive control liver (0.9 % saline + 50 mg / kg NaMSA) – exhibits normal hepatocyte lobules.

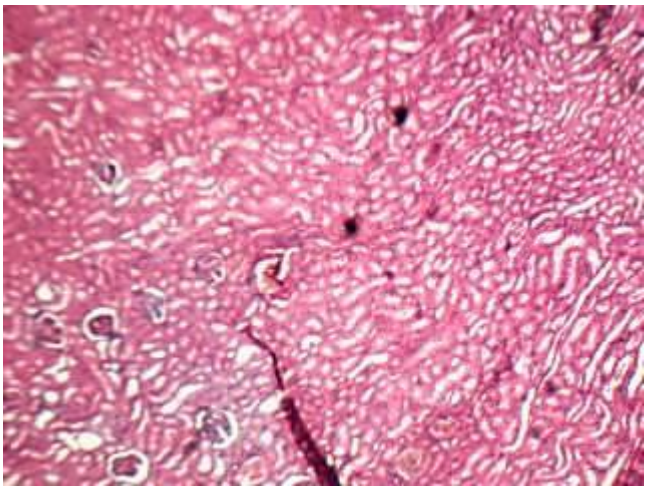


Control + $HgCl_2$
H and E 100 of negative control liver (0.09 % saline + 1.25 mg / kg $HgCl_2$) – section shows dilated central vein and congestion of portal trial.



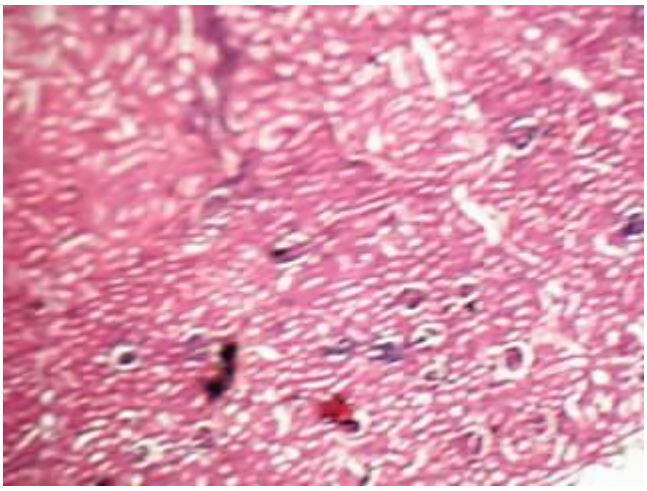
$HgCl_2$+ NaMSA
H and E 100 of treatment control liver (1.25 mg / kg $HgCl_2$ + 50 mg / kg NaMSA) - section shows congested liver lobules with inflammation.

Figure - 3: Effect of NaMSA on $HgCl_2$ influenced kidney of control and experimental rats



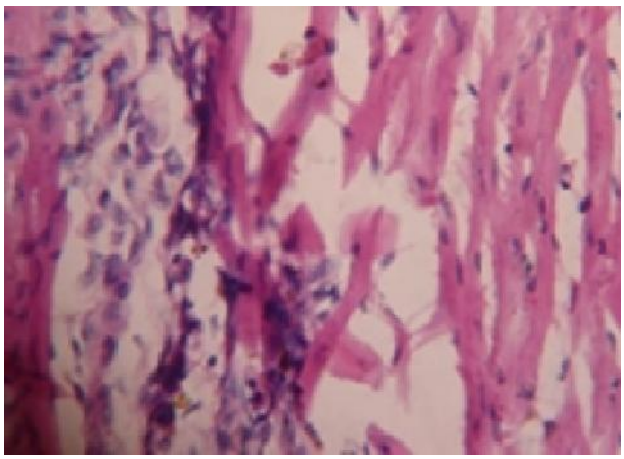
Control

H and E 100 of control kidney (0.9 % saline) - glomeruli appears normal



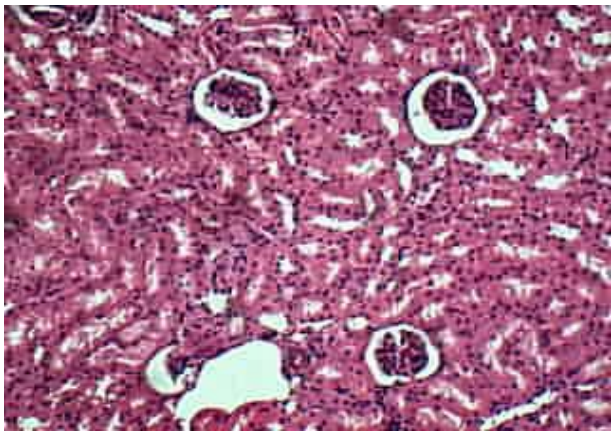
Control + NaMSA

H and E 100 of positive control kidney (0.9 % saline + 50 mg / kg NaMSA) - glomeruli appears normal architecture



Control + $HgCl_2$

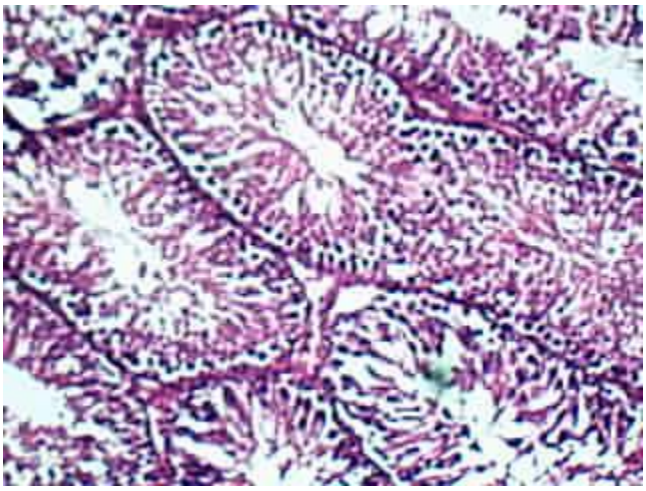
H and E 100 of negative control kidney (0.09 % saline + 1.25 mg / kg $HgCl_2$) – section appearing glomeruli with dilated tubules.



Cl_2+ NaMSA

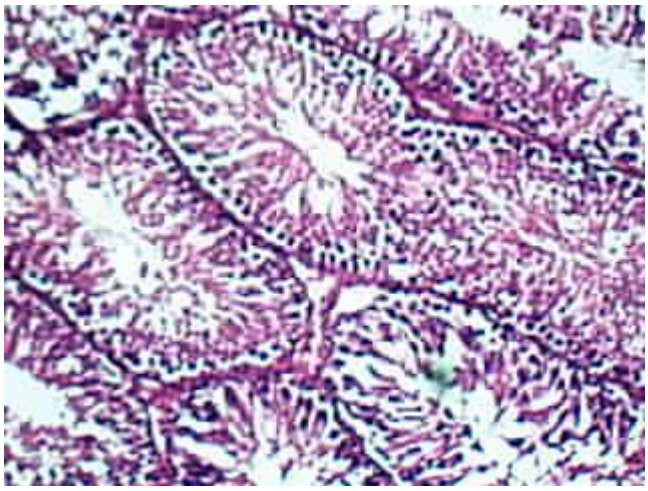
H and E 100 of treatment control kidney (1.25 mg / kg $HgCl_2$ + 50 mg / kg NaMSA) - section shows mild hyper cellular glomeruli and congested tubules.

Figure - 4: Effect of NaMSA on $HgCl_2$ influenced testis of control and experimental rats



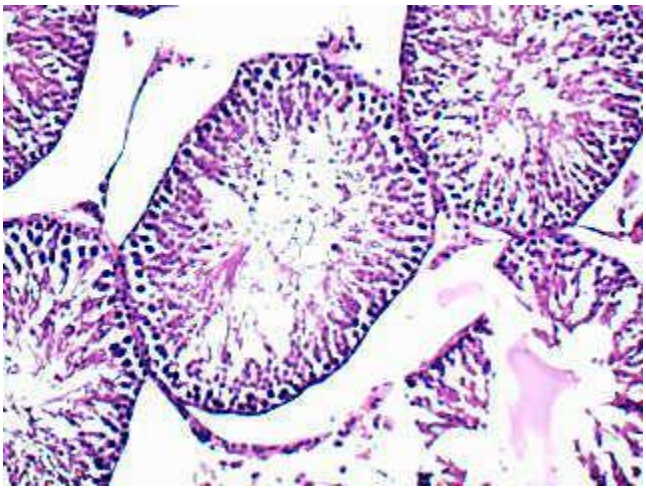
Control

H and E 100 of control testis (0.9 % saline) – shows normal seminiferous tubules.



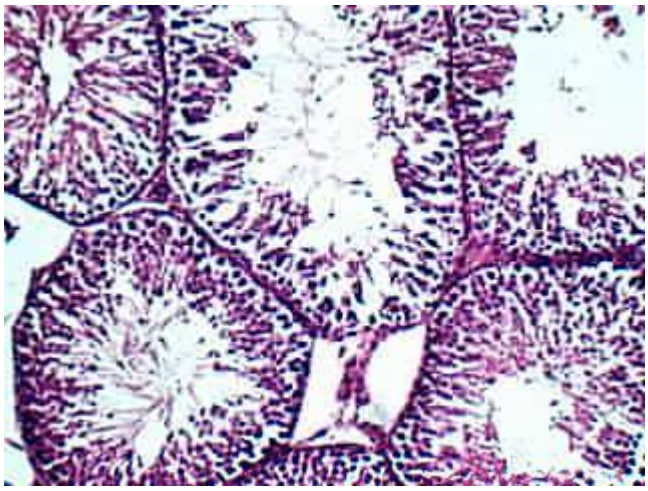
Control + NaMSA

H and E 100 of positive control testis (0.9 % saline + 50 mg / kg NaMSA) – appears with normal spermatogenesis tubules.



Control + $HgCl_2$

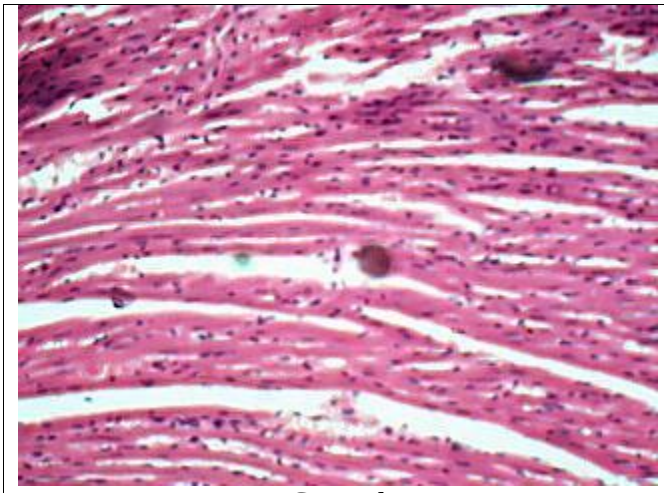
H and E 100 of negative control testis (0.09 % saline + 1.25 mg / kg $HgCl_2$) – section shows cut surface of seminiferous tubules with hypospermatogenesis and interstitial oedema.



$HgCl_2$+ NaMSA

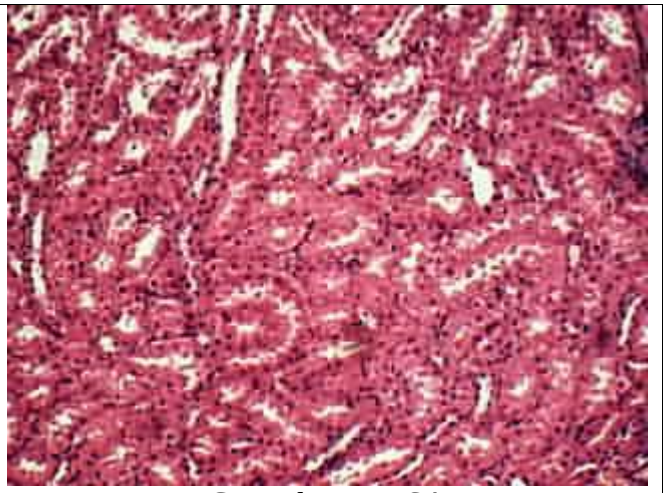
H and E 100 of treatment control testis (1.25 mg / kg $HgCl_2$ + 50 mg / kg NaMSA) - shows very minute disturbance normal spermatogenesis tubules..

Figure - 5: Effect of NaMSA on $HgCl_2$ influenced heart of control and experimental rats



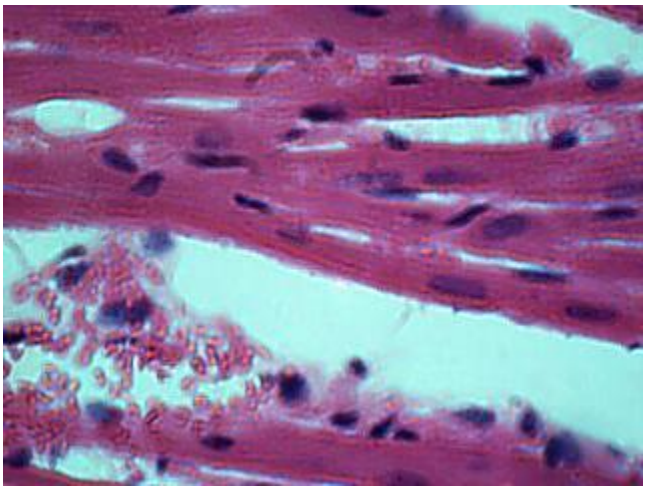
Control

H and E 100 of control heart (0.9 % saline) – shows normal cardiacmuscles.



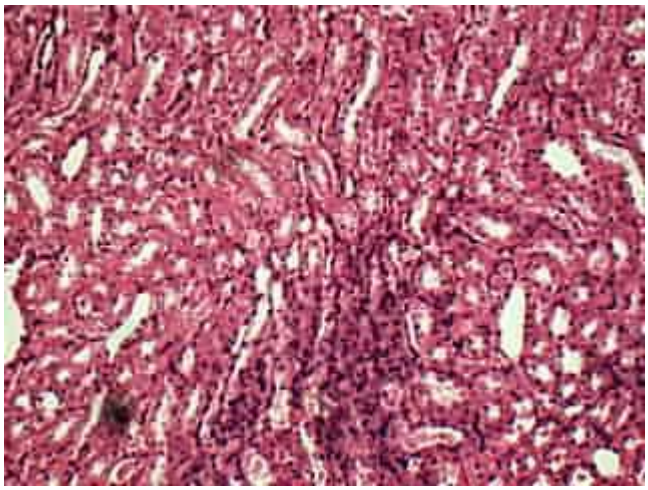
Control + NaMSA

H and E 100 of positive control heart (0.9 % saline + 50 mg / kg NaMSA) - shows normal architecture of heart muscle



Control + $HgCl_2$

H and E 100 of negative control heart (0.09 % saline +1.25 mg / kg $HgCl_2$) – shows dilated cirrhosis with necrosis of heart muscles.



$HgCl_2$+ NaMSA

H and E 100 of treatmentcontrol heart (1.25 mg / kg $HgCl_2$ +50 mg / kg NaMSA) shows very minute disturbance in the architecture in the heart muscles.

Discussion

Figure-1 Depicts the level of mercury in different organs

The detrimental effect of Hg may attribute mainly to the accumulation of mercury in the kidney and functional impairment probably resulted from both vasoconstriction and a direct cytotoxic effect of mercury [24] explained heavy metals induces oxidative stress,renal and testicular injuries.

Injury of the kidney is caused by selective mercury ion accumulation [25] and rats exhibited macroscopic sign of gastroenteritis at the same time animal death is observed mainly in the second post poisoning week due to renal failure.

One of the harmful effects of mercury action during its accumulation in a body region leads to excessive release of reactive oxygen species and increased lipid peroxidation in the cells [26] and in a multitude of body systems [27].

Morin has a convenient position of the 5OH and 4C=0 as well as 3OH and 4C=0 groups ion a molecule, morin can easily form the chelate complex with ions of p-, d-, and f-electron metals [28], the morin complex is considered to have a better biological activity due to its cooperative effectiveness, which results in the appealing studies of its antitumor activity.

From the earlier studies it is known that mercury in the form of $HgCl_2$ releases Hg^{2+} ion and has natural affinity towards the sulphur and -OH groups present in the NaMSA. The results obtained in the present study showed very positive indication for NaMSA and $HgCl_2$ complex formation and protected the rats from mercury direct action on the organs. These findings also supported by our histopathological observations.

Figures - 2, 3, 4 & 5, Shows the histopathological changes in the tissues of liver, kidney, heart and testis

Free radicals and intermediate products of peroxidation are capable of damaging the integrity and altering the function of bio-membranes, lead to the development of many pathological processes [29]. Mercuric chloride generated Hg^{2+} induces severe alterations in the tissues of both animals and men [30] and increases the intracellular level reactive oxygen species, oxidative stress [31] resulting in tissue damage [32].

Mercury exposure causes peri portal hepatocyticvacuolations and dilatation of hepatic sinusoids with subsequent atrophy of hepatic cords in the focal areas of hepatic necrosis. Similar changes were also reported by [33], in liver tissue after mercury exposure[34]. $HgCl_2$ caused histopathological and ultra-structural lesions evidenced by preiportal fatty degeneration and cell necrosis in the liver [35]. It is a major site of metabolism for mercury and it can accumulate in the liver, resulting in severe hepatic damage[36] evidenced by [37]Histochemical findings confirmed the mercury-intoxicated hepatocytes concomitant with the ultra-structural changes noticed in the rough endoplasmic reticulum [38] explained that mercuric chloride caused histopathological and ultra-structural lesions in the liver. Also, evidenced by peripheral fatty degeneration and cell necrosis. [27] observed that mercury impairs the integrity of hepatocytes. Mercury-induced oxidative stress; make an important contribution to molecular mechanism for liver injury [9].The observations of [39] recorded that $HgCl_2$ causes swollen hepatocytes as well as foci inflammation and apoptosis on the other hand in kidney proximal tubular cells swollen, vacuolation, degeneration, inflammation.

The detrimental effect may attribute mainly to the accumulation of mercury toxic metal in kidney. Such functional impairment probably resulted from both vasoconstriction and a direct cytotoxic effect on mercury[40]. [24]found heavy metals induce oxidative stress, renal and testicular injuries [41].

It is unknown whether cardiovascular effects of mercury are due to direct cardiac toxicity or to indirect toxicity caused by effects on the neural control of cardiac function. Inorganic mercury induced testicular damage is a known fact in experimental animals [42]. In humans

hypospermia,asthenospermia and teratospermia[43] owing to the relative spermiotoxicity of $HgCl_2$.

$HgCl_2$-treated rats exhibited hepatic failure, renal failure and testis damage,indicating wide spread deleterious toxic effect of mercury in the body systems [27]. Severe histopthological changes observed in all organs: tubular degeneration of the kidney, hepatocyte degeneration, hyperemia and hemorrhage in the liver, heart, kidney [44]. The sub chronic toxicity of mercury induces severe alterations in the structural rigidity and also histological changes in the liver, kidney and testis [7].

Morin decreases the oxidative damage in several biological systems such as cardiovascular, lung, fibroblasts, hepatocytes and neurons [45]. It also has antioxidant, cytoprotectiveand free radical scavenging capability [46].

NaMSA proved its antidote efficiency for $HgCl_2$ toxicity in lowering kidney damage and decreased the death rate of the poisoned animals by protecting them from renal damage. Also, indicated by a rise in urea and creatinine concentrations almost to the control level [47].

In the present study histopathological examination results of the liver, kidney, testis and heart have drawn a conclusion that Hg damages all the organs due to oxidative stress. The severity of the necrosis was based on the quantity and accumulation of Hg present in the particular organ present; this was confirmed with earlier findings. In our study the most damaged organ was kidney because it contains more mercury than any other organ analysed. On the other hand least affected organ was heart due to less quantity of Hg present in it.

Conclusion

NaMSA has biochemical examination evidences for its antioxidant and chelating activity. Whereas the present study proved the ameliorating effect of NaMSA on $HgCl_2$ induced histopathological changes in the liver, kidney, testis and heart. The ameliorating effect of NaMSA could be due to the formation of metal and NaMSA complex, which in turnmay led to the excretion of the complex in urine and faeces there by prevents the Hg metal exposure to the organs. From the current study results we conclude that NaMSA is recommended for treating mercury toxicity. In the feature prospects, the therapeutic effect of NaMSA against mercury extended to molecular analysis.

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