IJBPAS, February, 2022, 11(2): 828-845

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IN VIVO PROTECTIVE POTENTIAL OF BETULINIC ACID AND ROTUNDIC ACID ON MERCURIC CHLORIDE INDUCED HEPATO-RENAL TOXICITY IN ALBINO WISTAR RATS

REVATHI M[1] AND JAGADEESAN G[2*]

1: Ph.D., Research Scholar, Department of Zoology, Annamalai University, Annamalai nagar, Chidambaram, Tamil Nadu 608002, India

2: Professor and Head, Department of Zoology, Annamalai University, Annamalai nagar, Chidambaram, Tamil Nadu 608002, India

***Corresponding Author: Dr. Ganesan Jagadeesan: E Mail: drjagadeesanganesan@gmail.com; Ph.: Mobile No. 9345421681**

Received 19th March 2021; Revised 20th April 2021; Accepted 9th June 2021; Available online 1st Feb. 2022

https://doi.org/10.31032/IJBPAS/2022/11.2.5911

ABSTRACT

Objective: Mercury is a multidisciplinary global pollutant of environment, mainly accumulating in the liver and kidney inducing hepatorenal toxicity, oxidative stresses, and tissue damages. The current experimental study is developed to scrutinize the protective potential of betulinic acid and rotundic acid against mercuric chloride induced hepatorenal toxicity. **Methods:** $HgCl_2$ was dosed orally about 1.29 mg/kg of body weight upto 7 days and betulinic acid and rotundic acid (5mg/kg per body weight) was nursed for another 7 days on intoxicated rats. **Results:** In the present investigation, there is a tremendous alterations in the volume of Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehyrogenase, albumin, bilirubin, total cholesterol, creatinine and urea amounts were noticed in the mercury intoxicated rats blood serum. Thus, the pursuit of liver marker enzymes namely ALP, AST, ALT and LDH were increased significantly additionally albumin content of rats intoxicated with mercuric chloride was decreased simultaneously. The therapy of Betulinic acid and Rotundic acid on the intoxicated rats shown the normal level of liver marker enzymes as well as maintained their levels near to the normal conditions. **Conclusion:** The present experimental work indicates the detoxify effects and defensive effect of betulinic acid and rotundic acid against $HgCl_2$ induced hepatorenal toxicity.

Keywords: Liver marker enzymes, Total cholesterol, Urea, Creatinine, Mercuric chloride, Betulinic acid, Rotundic acid

1. INTRODUCTION

Heavy metal knocks out numerous virulent reactions on the ecosystems. The rudimentary molecular implements of toxicity by the heavy metal are not understood well still now. Mercury and the compounds of it causes various biochemical modifications through the production of reactive oxygen species in diverse tissues additionally with assorted mechanisms like, lipid peroxidation **[1]**, binding to thiol groups and altering the protein synthesis processes **[2]**. As stated by the Agency for Toxic Substance and Disease Registry (ATSDR), Mercury stands for third place among the threatening heavy metals followed by arsenic and lead **[3]**. It is impossible to avoid the exposure to this metal, specifically in the highly polluted areas of air, soil, food and water. Inorganic salt of Hg appears in monovalent or in divalent form **[4, 76]**.

According to Adams *et al* **[5]** increased levels of mercury may causes significant biochemical and pathological fatalistic effects over the health conditions of the animals **[6, 7]**. Exposure to Hg increased the genesis of oxidative stress and free radicals that insinuated the acute hepatic pathogenesis and kidney dysfunctions. Thus, the intake, accumulations and toxic effects of inorganic Hg binds with endogenous thiol-containing molecules in the liver and kidney **[8]**.

Liver plays as a crucial site for mercury metabolism and kidneys were its accumulation sites, that emerging to severe damages **[9]**. They encourages the building of hydrogen peroxide like various endogenous oxidants **[10]**, depletes defensive antioxidants namely glutathione and diminishes free radicals scavenging system. Liver acts as a major region for synthesizing numerous serum proteins but the amount of serum proteins were reduced through hepatic disease. The oxidative damages of certain amino acids were esteemed as major causes for the metabolic debility of hepatic damages **[11]**. Therefore assessment of mitochondrial enzymes in the liver is regarded as a best tool to understand the presences of hepatic necrosis that which integrated with liver diseases **[12, 13]**. The analysis and perseverance of liver biomarker enzymes in the serum, namely alanine transferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), lactate dehydrogenase (LDH) additionally serum lipid profiles, total cholesterol, lipoproteins and triacylglycerides, gives a better view of the liver function in an animal **[14]**.

Recent days, traditionally used medicines were under research extensively from different species of plant due to their therapeutic ideals throughout the world **[73, 74]**. Phytochemical elements including

herbs of plants and their extracts were used towards centuries for promoting healthy living **[75]**.

Betulinic acid is broadly scatteded globally all over the plant kingdoms especially in birch trees (*Betula* spp., Betulaceae) **[15]**. Betulinic acid (3*β*-hydroxy-lup-20(29)-en-28-oic acid, BA) a natural triterpenoid belongs to pentacyclic lupane- type containing a diversity of biological premises with medicinal properties including anti-bacterial, anti-inflammatory, anti-malarial, anthelmintic, antinociceptive, anti-HSV-1, hindrance of human immunodeficiency virus (HIV) and anti-cancer activities **[16]**.

Rotundic acid (3b, 19a,23-trihydroxy-urs-12-en-28-oic acid, RA) is also one among the pentacyclic triterpenoids, which are derived from the dry bark of *I. rotunda* **[17]**. RA comprises of several biological properties namely anti-inflammatory, neuro-protective, anti-oxidant, *in vivo* hepato-protective, anti-diarrheal, anti-malarial, anti-microbial, anti-hyperglycemic and anti-nociceptive **[18]**.

The target of the ongoing investigation is designed to prove the protective potency of betulinic acid and rotundic acid against hepatorenal toxicity induced via mercuric chloride in the wistar rats.

2. MATERIALS AND METHODS

2.1. Chemicals

In the present experimental study mercuric chloride ($HgCl_2$) and further more mandatory reagents for analytical assessment were obtained from Hi-Media laboratories Ltd, Mumbai, India. Betulinic acid and rotundic acid were obtained from Sigma Aldrich laboratories Pvt. Ltd, Bangalore, India.

2.2. Adaptations of Animals

Healthy male albino wistar rats, *Rattus norvegicus* (180–200 g), were acquired from the Central Animal House, Department of Experimental Medicine, Raja Muthiah Medical College and Hospital Annamalai University, and sustained in a room with air condition about 25 ± 3◦C with a 12-hrs of light followed by 12-hrs of dark cycles. Feed, water and *ad libitum* were allocated for every single animal. The experimental protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (IAEC, Proposal Number: AU-IAEC/1228/1/19), Annamalai University, Annamalai nagar.

2.3. Experimental design

Sum of 36 animals were acclimatized within the animal cages for 7 days. They were divided randomly as six groups, respective group contains six rats each. The mercuric chloride toxic dosage was determined (sub-lethal dose of $HgCl_2$ 1.29 mg/kg bodyweight) out of our earlier

investigation done within our laboratory. Thus, it has adequate to evoke moderate or mild oxidative stresses on wistar rats (Manju and Jagadeesan, 2019) **[19]**.

- Group I: Untreated control- only vehicle (0.9% NaCl) was provided to these animals and observed for 7 days.
- Group II: Mercuric chloride treatment- the animals were administered 1.29 mg of $HgCl_2$ per kg body weight in 0.9% NaCl intraperitoneally for 7 days.
- Group III: Mercuric chloride followed BA treatment- the animals were administered betulinic acid (5 mg per kg body weight) after the intoxication of mercuric chloride for 7 days.
- Group IV: Mercuric chloride followed RA treatment- the animals were administered rotundic acid (5 mg / kg body weight) after the intoxication of mercuric chloride for 7 days.
- Group V: BA treatment alone- the animals were given betulinic acid (5mg / kg body weight) alone for 7 days.
- Group VI: RA treatment alone the animals were given rotundic acid (5mg per kg body weight) alone for 7 days.

Towards the end of the experimental period, the rats were anaesthetized with intramuscular injection of ketamine hydrochloride (24 mg per kg body weight) and sacrificed through cervical displacement. The entire liver and kidney tissues were immediately isolated from the animals and kept within an ice-cold saline and furthermore used for the estimation of biochemical analysis and additionally for histological studies.

a. Sample preparation

Towards the end of the experimental program, blood sample were collected in clean dry test tube through the sinoauricular punch of the animal and permitted for coagulate at normal room temperature about 40min. Then the serum were segregated via centrifugation for 2000 rpm about 10 min. The samples of serum were used for liver marker assays namely ALT, AST, ALP, LDH, bilirubin, albumin and cholesterol, urea, and creatinine analysis.

b. Biochemical analysis

The ALP assay was evaluated through the method of King and Armstrong **[20]**. The AST and ALT activity of was estimated with the help of King's method **[21]**. The LDH activity was analyzed through the method of King **[21]**. Serum albumins were determined by Reinhold's Biuret method **[22]**. The volume of serum bilirubin was assayed by adopting the method of Malloy and Evelyn **[23]**. Total

cholesterol present in the tissues and plasma were evaluated through the Allain's enzymatic method **[24]**. Serum urea was determined by using the diagnostic kit method assumed by Fawcett and Scott **[25]**. Creatinine in the serum was analyzed by Bonsnes and Taussly method **[26]**.

i. Estimation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity

The AST and ALT activity was analyzed by using the method described by King **[21]**. 0.1 ml of serum was taken in a dry clean test tube and 0.5 ml of buffered substrate was added to it and placed in an incubator at 37°C for 10 min. Exactly later an hour, two drops of aniline-citrate reagent and 0.5 ml of DNPH reagent was added and allowed for 60 min at normal room temperature. Towards the end, 5.0 ml 0.4 N sodium hydroxide was added. A series of standards also treated in the same manner and read after 10 min at 520 nm. To the blank tubes, 0.1 ml of distilled water was added instead of the sample. The results were expressed as IU/L for serum.

ii. Estimation of serum ALP activity

The ALP was analyzed by adopting King and Armstrong method **[20]**. 2.0 ml of mixed buffered substrate was taken into cleaned test tubes and it was incubated at 37°C for 5 min, to that 0.1 ml of serum was added in the tubes and incubated for another 15 min. After completing the incubation period, the kinetic reaction was arrested by adding of 0.8 ml of 0.5N NaOH and then 1.2 ml of 0.5M sodium carbonate was added and thoroughly mixed. Again 1 ml of amino antipyrine was added in the above mixture and then 1.0 ml of potassium ferricyanide was added, the color developed was read at 520 nm against the reagent blank in an UV-spectrophotometer. The activities of serum ALP were expressed as IU/L of serum.

iii. Estimation of LDH activity in serum

The LDH activity was determined by the method determined by King **[21]**. 0.5 ml of serum was taken in a clean, dry test tube, and 1.0 ml of buffered substrate was added, and then the mixed content was incubated at 37°C for 15 min in an incubator. After 15 min, 0.2 ml of coenzyme solution (NAD) was then added to the above contents and again incubated for 15 min. After completing the incubation period, the reaction was stopped by the addition of 1.0 ml of 2, 4-dinitrophenylhydrazine. The contents were incubated at 37°C for another 15 min and 5 ml of 0.4 N NaOH was added to the contents and mixed well. The color was developed. The intensity of the color was measured at 520 nm in an UV-spectrophotometer. A control was performed simultaneously as like a test, but serum was added after the addition of

DNPH. The enzyme activity was expressed as IU/L for serum.

iv. Estimation of serum total bilirubin

The volume of serum bilirubin was estimated by using the method described by Malloy and Evelyn **[23]**. 0.5 ml at serum was taken in a clean test tube and added 2 ml of the Protein free filtrate and 0.8 ml of distilled water and the solution was mixed well with constant rotation, and then 0.8 ml of diacetyl monoxime solution was added. The contents were thoroughly mixed, and 0.8 ml of arsenic sulfuric acid solution was added and mixed thoroughly and then kept in an incubator at 100°C for exactly 20 min. The test tubes were allowed against a reagent cooled gradually in ambient air at least for 15 min and then cooled in a 25°C water bath for at least another 15 min. The test tubes were kept apart from direct light during the periods of heating and the cooling. The yellow color developed was read at 475 nm in an UV spectrophotometer against a reagent blank, urea nitrogen was used to build the standard graph. The level of serum bilirubin was exposed as IU/L.

v. Estimation of serum albumin

Albumin in the serum was analyzed by Reinhold method **[22]**. 0.5 ml of sample was grabbed in a clean, dry test tube, and 9.5 ml of sodium sulfite solution was added and mixed thoroughly. To that 3 ml of the mixture was transmitted into a tube for total protein estimation to which 5 ml of biuret reagent was added. To the remaining mixture, 3 ml of ether was added, stoppered, shaken well for 20 sec and then centrifuged for 5 min. 3 ml of the clear supernatant was utilized for the estimation of albumin and managed with 5 ml of biuret reagent. Concurrently, 2.0 ml of standard egg albumin was mixed with 1.0 ml of water and treated with 5.0 ml of biuret reagent. The purple color emerged was read at 540 nm after 15 min using reagent blank. Values were expressed as g/dL.

vi. Estimation of total cholesterol

The total cholesterol within the plasma and tissues was assayed by the method described by Allain *et al*. **[24]**. 0.1 ml of serum was grabbed in a clean dry test tube and to this 4.9 ml of ferric chloride-acetic acid reagent added then centrifuged for 5 min at 3000 rpm. After centrifugation, 2.5 ml taken in a cleaned test tube and to this 1.5 ml of concentrated H_2SO_4 was added and the absorbance was read after 30 min at 560 nm in an UV spectrophotometer against a reagent blank (2.5 ml of ferric chloride-acetic acid reagent and 1.5 ml of con. H_2SO_4). The cholesterol concentration was expressed as mg/dL of plasma or mg/g of tissue.

vii. Estimation of serum urea

Urea in serum was analyzed using the diagnostic kit implemented by the Fawcett

and Scott method **[25]**. 10 µL of serum was taken in a clean dry test tube and 1 ml of buffered enzyme was added and thoroughly mixed well and allowed for 5 min at 37°C. 10 µL of standard and 10 µL distilled water (blank) were also prepared at the same time. 1 ml of color developing reagent was introduced to all the tubes and mixed well and incubates the contents for 5 min at 37°C. After completing the period of incubation, 1 ml of distilled water was added and the color evolved was read at 600 nm in an UV-spectrophotometer against a reagent blank. The results are expressed as mg/dL of serum.

viii. Estimation of serum creatinine

Serum creatinine was analyzed using the method described by Bonsnes and Taussly **[26]**. Diluted 1 ml of 10% of serum was grabbed in a clean, dry test tube, and 1 ml of 2N sulfuric acid was added. The contents were mixed well and filtered with the help of Whatman No. 1 filter paper. From this filtrate, 3 ml of content was taken in a test tube and 2 ml of picric acid solution and 2 ml of 0.75 n sodium hydroxide were added to it. The developed yellow color was read at 540 nm in the UV-spectrophotometer against a reagent blank. Creatinine concentration was used to construct the standard graph. The results are expressed as mg/dL.

c. Statistical analysis

Values are stated as mean ± S.D. for six rats within each group. The data for various biochemical parameters were analyzed by using T-test analysis and the group means were compared by Duncan's multiple range test (DMRT) **[27]**. Values were assessed statistically significant when $p < 0.05$ and the values sharing a common superscript did not differ significantly.

3. RESULTS

The amount of ALT, AST, ALP, LDH and bilirubin were increased significantly in the blood serum of rat intoxicated with mercury when compared to control group that illustrated in **Table 1**. On the other hand dosage of betulinic acid and rotundic acid to the animals decreased the ALT AST, ALP, LDH and bilirubin levels significantly nearer to the normal one when compared to the rat induced with mercury chloride.

The level of cholesterol, urea and creatinine were increased significantly in the blood serum of rat induced with mercuric chloride when compared with control group as illustrated in **Table 2**. The betulinic acid and rotundic acid post-treated rats shows significant restoration in cholesterol, urea and creatinine seems near to the normal levels when compared to rats intoxicated with mercuric chloride. There was a significant reduction in the albumin activity in the rats intoxicated with mercuric chloride. Therapy of betulinic

acid and rotundic acid when dosed orally to the rats significantly increased the level of albumin activity nearly to the normal levels when compared with blood serum of rat intoxicated with mercuric chloride.

Table 1: The protective potency of betulinic acid and rotundic acid on hepatic metabolic enzymes of ALT, AST, ALP, LDH, Bilirubin activity in blood serum of rats induced with mercuric chloride

Groups	ALT(IU/L)	AST(IU/L)	ALP(IU/L)	LDH(IU/L)	Bilirubin(IU/L)
Control	26.58±1.44	36.75±0.86	88.42±0.64	119.74±3.32	0.918±0.60
$HgCl_2$	48.06±1.62	54.69±0.17	154.34±0.67	294.96±2.02	1.351±1.25
$HgCl_2$+Betulinic acid	31.15±1.83	42.86±0.28	99.61±0.71	128.64±2.31	0.983±2.03
$HgCl_2$+Rotundic acid	34.42±1.04	48.51±0.99	112.53±0.69	137.06±2.06	1.022±3.67
Betulinic acid	23.66±1.26	34.66±0.65	85.96±0.75	116.14±3.56	0.910±4.01
Rotundic acid	25.19±0.98	35.77±0.67	86.28±0.63	114.62±2.52	0.914±2.81

Each value is mean±SD of six rats in each group. The data for various biochemical parameters were analyzed using t-test analysis and the group means were compared using Duncan's multiple range tests. Values were appraise statistically significant at P<0.05. ALT- Alanine transferase, AST- Aspartate transaminase, ALP- Alkaline phosphatase, LDH- Lactate dehydrogenase

Table 2: The protective potency of betulinic acid and rotundic acid in level of urea, creatinine, cholesterol, and albumin activity against mercuric chloride induced rats

Groups	Urea(mg/dL)	Creatinine(mg/dL)	Cholesterol(mg/dL)	Albumin(g/dL)
Control	25.11±0.45	2.24±0.04	136.92±0.42	5.66±0.27
$HgCl_2$	51.31±0.62	3.81±0.31	289.35±0.27	3.54±0.31
$HgCl_2$+Betulinic acid	32.46±0.88	2.46±0.09	164.43±0.24	4.52±0.21
$HgCl_2$+Rotundic acid	36.52±0.57	2.52±0.12	186.02±0.36	4.27±0.26
Betulinic acid	23.32±0.64	2.16±0.11	133.61±0.16	5.62±0.32
Rotundic acid	23.46±0.72	2.12±0.42	135.21±0.34	5.60±0.28

Each value is mean±SD of six rats in each group. The data for various biochemical parameters were analyzed using t-test analysis and the group means were compared using Duncan's multiple range tests. Values were appraise statistically significant at P<0.05.

4. DISCUSSION

The toxic impact of mercury and their compounds involves in various interactions between the numerous numbers of cellular operations includes complex formations between the groups of protein thiols and free thiols might prompt oxidative stress which was revealed in the present experimental study. Those developed oxidative stress leads to tissue injuries and cellular damages. These injuries of tissues therefore causes functional disability that indicated by hepatic function tests, such as uplifted AST, ALT and ALP of serum activities illustrate the seriousness of tissue damages generated by mercury **[28]**. At normal conditions, based on the concentration gradients between an organ and the blood receptacle are the enzymes were released **[12, 29, 30]**. In the liver, the persistent deliverance of mitochondrial enzymes indicates the origination and stimulation of the hepatic necrosis. Divergent secretion of liver marker enzymes level may illustrate the liver damages and alteration in the bile flow of the liver organ **[31]**.

The liver is known as a major organ of sensitive for pre-oxidative damages that which is greater with oxidizable radicals. The higher release of the hepatic enzymes indicates severe liver damages **[32]**. These biomarker enzymes performs a major role in the liver functions of animals and they

are considered as catabolic enzymes namely ALT (alanine aminotransferase), AST (aspartate aminotransferase), and ALP (alkaline phosphates) **[33, 34]**. ALT performs a vital role in sustaining the gluconeogenesis process and amino acid metabolism, remarkably during stressful conditions **[35]**. When the hepatocytic cell membrane was damaged, a collection of hepatic enzymes like AST, ALT and ALP were distributed through cytosol into the bloodstream **[11, 36]**. The elevated level of serum transaminase is a diplomatic signal of cell membranes damage of hepatic cells even though there was no evidence on hepatic disability. Mercury induced substantial liver damages was evidenced by the rise in the level of ALT, AST and ALP activities which were perceived in the group treated with mercuric chloride. In our present experimental analysis, liver injury was remitted through various biochemical analyses. Separately hepatic enzymes, which includes AST and ALT were togetherly known to be transaminases, were as ALP is a chloestatic enzyme of liver which are authentic indicator connected with liver functions **[37, 38, 41]**.

AST and ALT are censorious enzymes meant for biological mechanisms. The rise in AST and ALT of serum in the mercury treated rats illustrated that exposure to mercury assist hepatic necrosis that revealed in the liver. From the injured hepatic cells AST was released in the plasma. The increased level of serum AST indicates that the existence of this enzymes in large proportions within the liver prompt liver diseases and also followed by hepatocytes degradation or devastation. Enhanced level of enzymatic serum proposed liver damages and transformations in the functions of liver **[39, 40]**.

The protein catabolism process occurred in the mercury intoxicated animal, ALT plays important role to fulfill the kinetic reactions to get additional energy from the process of generating pyruvate and glutamate products. ALT catalysis alanine and alpha-ketoglutarate and transfers them into appropriate products. It also performs a vital role on the intermediate metabolism of amino acid and glucose process **[42]**. In normal condition, only a limited amount of ALT is released into the bloodstream, when the level of ALT activity increased in the blood proposes that the liver gets injured due to the stress or intoxication **[43-45]**. Consequently, ALT is assessed as one of the important indicator for the diagnosis of liver function in animals and also in human beings **[46]**.

ALP is known as a membrane-bound enzyme present in all internal organs such as liver and kidney. The major role of ALP enzymes are catalysation of specified

chemical reaction through the process of hydrolyzing the phosphate groups of an organic molecules at an alkaline or basic pH **[47]**. It also used as diagnostic indicator to find out the intensity of liver damages evolved in an animal when exposed to stressful condition **[48]**. During the normal condition liver plays a major role in draining the fluid through the bile duct bearing ALP along with some more substances. When these bile ducts were damaged or blocked the accumulated ALP and other substances were leaked or escaped into the bloodstream. The bile ducts cell lining is naturally responsible for the production of ALP enzyme (Cholestatic) in the liver **[49]**. The extent of increase in the serum levels is directly equivalent to the degree of liver damages. It was observed that there was a conspicuous elevation in the liver marker enzymes of serum (AST, ALT, ALP, and LDH) and the total bilirubin content when compared with standard control groups. During the present study, it was also found that the reduction in the amount of enzyme transaminases, ALT and LDH due to the post-treatment of rats indicated repair of hepatic tissue **[50]**.

Lactate dehydrogenase is almost present in all tissues with meagre amount. The main role of LDH is utilization of the glucose molecule for energy production during the stressful condition, to overlook the energy demand. The enhancement of LDH in the bloodstream may indicate the cellular damage of distinct tissues of skeletal muscles, cardiac muscles, livers and kidneys. LDH isoenzyme also involves in energy metabolism of muscle tissues, during the oxygen demand or energy demand facilitating the production of ATP through the process of glycolysis. The high levels of LDH available in bloodstream may be due to hypoxic conditions or stressful situation or energy demand which leads to stimulate the LDH secretion as an alternative anaerobic pathway to enhance the ATP production **[51]**. The formation of cell necrosis caused by heavy metal treatment is also a reason for the enhancement of LDH levels. Constant enhancements of LDH are as well as revealed the causes of megaloblastic anemia, shocks, renal infarctions, hemolytic complications, leukemia and liver diseases **[52]**. The conversion of lactate to pyruvate is a catalytic process carried out through LDH in a stressful condition. Therefore, it is scrutinized as a chief enzyme for generating energy in cells during the requirement of energy demand endured by the internal organs **[53]**.

During the mercury intoxication an enhanced level of LDH activity was noticed in circulation because of hepatic necrosis or alterations in the cell membrane permeability which leads to hepatic damage. The present investigation initiate

that intoxication of mercury produces enhanced activity of lactate dehydrogenises **[54, 55]**. The regulations of liver markers most virtually by betulinic acid and rotundic acid indicates clearly the improvements from the utilitarian reputation of the hepatic cells through restoration of above mentioned enzymes activity in the serum and it might secure against the dysfunction of acute organs and cellular damages influenced by the antioxidative impact which is promoted by mercury toxicity.

Bilirubin contents were also analyzed to subsidize the stated hypothesis. An elevated amount of bilurubin was observed in the serum of rat intoxicated with mercury supports the tissue injuries present in treated rats. Mercuric chloride treated rat's exhibits an enhanced level of bilirubin that is one among the fragile consideration which reflects the prediction of acute liver diseases **[56]**. Normally bilirubins are enzymes present in cytoplasm at higher concentration. In an animal, during hepatopathy the bilirubin enzymes come into the bloodstream in conformance accompanied by the proportion of hepatic damage. Assessment of bilirubin in the bloodstream is the conservative criterion of liver diseases or damages caused by the toxicants **[57-59]**. The measurement of bilirubin and its components in the blood reflects the liver function or hemolysis in animals **[60]**. Bilirubin is formed across the breakdown of heme content in the liver **[61]**.

Albumin, the diversely generous plasma protein, exhibiting about 55 to 65 percentage of the total proteins **[62, 63]**. It performs a principle role in carrying endogenous ligands and also xenobiotics. Albumin is the chief and major protein content in the bloodstream, and it also synthesized by liver. Under the stressful condition, the synthesis of albumin content is decreased may be due to hepatotoxicity in animals. The normal range of albumin present in the bloodstream is abundantly modified through the exposure to heavy metal so that the albumin content present in the bloodstream can be utilized as a supplementary test for hepatic function of an animal **[64]**. The important feature of albumin in the bloodstream is the genesis of non-covalent complexes at specific binding sites, stimulating the regulation of plasmatic concentration **[65, 66]**. The liver synthesized albumin based on the intake proteins, which is synchronized through the level plasma albumins. The noticed level of reduced albumin in rats treated with mercury might be because of alteration in the synthesis of protein or protein metabolisms within the livers **[67]**. During present experimental study, the level of albumin content was decreased incredibly

in the rat's blood when it was intoxicated with mercury.

Integral part of cell membranes was formed with a help of cholesterol, it provides peculiar substantial character of the membranes promotes cellular functions **[68]**. The absorption of cholesterol is based on the biliary discharge and the hydrolytic enzyme activity of pancreatic lipase. In spite of the absence of fat hydrolysis during pancreatic inadequacy, some cholesterol retention actually happens because of bile salts emulsification **[69]**. The measure of cholesterols from the hepatic fusion and dietary sources is under intimate homeostatic controls with the pace of synthesis symmetrically related to assimilation. The liver crucially utilized the dietary cholesterol ester and it is evade in the cast of bile acids, free cholesterols or its derivatives of bile **[70]**. Thus the level of cholesterol was terrifically elevated in the serum of mercury chloride intoxicated rat and lowered nearer to the control when they were post-treated with betulinic acid and rotundic acid subsequently.

Mercury chloride intoxication might also alter the kidney functions. The rise of LDH may also accompanied with the dropping of brush borders in the cells of proximal tubules during the early stages of necrosis in the epithelial cells of kidneys, these enzymes visibly indicates the damages in the plasma membranes **[41]**. Mostly the kidney glomeruli filter the albumins and they are reabsorbed through the proximal tubular cells where the enzymes of lysosomes degenerates the albumins as fragments which are replaced into the circulation process **[67]**. Therefore the intoxication of mercury chloride results in the reduction of albumin content.

Renal injury perceived in rats manifest to mercuric chloride was additionally revealed through increase urea level in the plasma and creatinine that were assumed as renal markers **[63, 71]**. Creatinine and urea were considered as clinical markers for renal function even now. Hematological alterations like hemolysis of red blood cells have been described as a cause mercury **[28]**. Serum urea and creatinine content in the present investigation were remarkably increased in the rats dosed with mercuric chloride indicating restricted renal functions. Concerning renal injury, biochemical discoveries in this exploratory investigation are in concurrence with bioenzymological examines **[63]**. The current outcome exhibiting that the oral organization of mercury brought about a huge expansion in serum urea and creatinine substance. Altogether, the above analysis indicates that the aggregation of mercuric chloride in the kidneys could develop nephrotoxicity through the degradation of renal tubular cells **[67, 71, 72]**.

5. CONCLUSION

In the course of reclamation period when betulinic acid and rotundic acid dosed orally to the rat intoxicated with mercury, the serum indicates a depleted shift. The raised levels of both together the bio-enzymological and biochemical properties were extent nearer to the normal (control) levels in the rats intoxicated with mercury when treated with betulinic acid and rotundic acid sequentially. Thus, the betulinic acid and rotundic acid followed against mercury significantly ameliorates the health conditions of the animals and additionally repairs the liver and kidney tissues injury implemented by toxicant. On the whole, our result recommends that betulinic acid and rotundic acid were trusted compounds for toxicity induced through mercury chloride.

6. DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest regarding this article.

7. ACKNOWLEDGEMENTS

The authors are thankful to the Department of Zoology, Annamalai University for furnishing all the mandatory laboratory facilities to complete this work successfully.

REFERENCES

[1] Woods JS, Calas CA, Aicher LD, Robinson BH, Mailer C. Stimulation of por-phyrinogen oxidation by mercuric ion. I. Evidence of free radical formationin the presence of thiols and hydrogen peroxide. Mol Pharm 1990; 38: 253–60.

[2] Bashandy SA, Alhazza IM, El-Desoky GE, Al-Othman ZA. Hepatoprotective andhypolipidemic effects of Spirulina platensis in rats administered mercuric chlo-ride. Afr J Pharm Pharmacol 2011; 5(2): 175–82.

[3] Emsley, J., Nature's Building Blocks: An A–Z Guide to the Elements. Oxford University Press, Oxford, 2001.

[4] Johnson, C.L., Mercury in the environment: sources, toxicities, and prevention of exposure. Pediatr. Ann. 2004; 33, 437–442.

[5] Adams, D.H., Sonne, C., Basu, N., Dietz, R., Nam, D.H., Leifsson, P.S., Jensen, A.L., Mercury contamination in spotted seatrout, Cynoscion nebulosus: an assessment of liver, kidney, blood, and nervous system health. Sci. Total Environ. 2010; 408, 5808–5816.

[6] Flora, S.J., Mittal, M., Mehta, A., Heavy metal induced oxidative stress & it's possible reversal by chelation therapy. Indian J. Med. Res. 2008; 128, 501–523.

[7] Magos, L., Clarkson, T.W., Overview of the clinical toxicity of mercury. Ann. Clin. Biochem. 2006; 43, 257–268.

[8] Gado, A.M., Aldahmash, B.A., Antioxidant effect of Arabic gum against mercuric chloride-induced nephrotoxicity. Drug Des. Devel. Ther. 2013; 7, 1245–1252.

[9] Wadaan MAM. Effects of mercury exposure on blood chemistry and liver

histopathology of male rats. J Pharmacol Toxicol 2009; 4:126–31.

[10] El-Shenawy SMA, Hassan NS. Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercuricchloride in the rats. Pharmacol Rep 2008; 60: 199–208.

[11] Murali A, Ashok P, Madhavan V. Protection against CCl4 induced hepatotoxicityby pretreatment with methanol extract of Hemidesmus indicusvar. Pubescens leaf in Wistar rats. Intl J Appl Res Nat Prod 2012; 5(2):5–13.

[12] Schmidt E, Schmidt FW. Aspekte der enzym-diagnostik. Med Welt 1970; 21: 805-16.

[13] Frederiks WM, Vogels IM, Fronik GM. Plasma ornithine carbamyl transferase level as an indicator of ischaemic injury of rat liver. Cell Biochem Funct 1984; 2: 217-20.

[14] Reuben A. Hy's law. Hepatology 2004; 39:574-8.

[15] Cichewicz R.H., and Kouzi, S.A., "Chemistry, Biological Activity, and Chemotherapeutic Potential of Betulinic Acid for the Prevention and Treatment of Cancer and HIV Infection." Medicinal Research Reviews, 2004; 24 (1), 90-114.

[16] Moghaddam, M.G., Ahmad, F. B. H., Kermani, A. S., Biological Activity of Betulinic Acid: A Review. Pharmacology & Pharmacy, 2012; 3, 119-123.

[17] Sun, H., Zhang, X.Q., Cai, Y., Han, W.L., Wang, Y., Ye, W.C., Study on chemical constituents of *Ilex rotunda* Thunb. Chem. Indus. Forest Prod. 2009; 295, 111–114.

[18] Parvez, M.K., Al-Dosari, M.S., Arbab, A.H., et al., The in vitro and in vivo anti-hepatotoxic, anti-hepatitis B virus and hepatic CYP450 modulating potential of Cyperus rotundusin vitro and in vivo anti-hepatotoxic, anti-hepatitis B virus. Saudi Pharm. J. 2019; 2, 1319-0164.

[19] Manju, M., Jagadeesan, G., In vivo hepatoprotective effect of caffeic acid on mercuric chloride-induced biochemical changes in albino wistar rats. Asian J. Pharm. Clin. Res. 2019; 12 (4), 119-124.

[20] King EJ, Armstrong AR. Calcium, phosphorus and phosphatases. In: Practical Clinical Biochemistry. New Delhi: CBS Publishers; 1988; 458.

[21] King J. The dehydrogenases or oxido reductase-lactate dehydrogenase. In: Practical Clinical Enzymology. London: Van D. Nostrand Co. Ltd.; 1965; 83-93.

[22] Reinhold JG. Manual determination of serum total protein albumin and globulin fractions by buiret method. In: Reiner M, editor. Standard Method of Clinical Chemistry. New York: Academic Press; 1953; 88.

[23] Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937; 119:481-90.

[24] Allain S, Poon CC, Chan LS, Richmond W. Enzymatic determination of total serum chlosterol. Clin Chem 1974; 20: 470-5.

[25] Fawcett JK, Scott JE. A rapid and pricise method for the determination of urea. J Clin Pathol 1960; 13:156-9.

[26] Bonsnes RW, Taussky HN. On the calorimetric determination of creatinine by the Jaffe reaction. J Biol Chem 1945; 158: 851-91.

[27] Duncan BD. Duncan's multiple range tests for correlated and heteroscedastic mean. Biometrics 1957; 13: 359-64.

[28] Neciba Y, Bahi A, Zerizer S. Argan oil (*Argania spinosa* L) provides protection against mercuric chloride induced oxidative stress in rat Albinos Wistar. Int J Basic Appl Sci 2013; 2(1):73–80.

[29] Batt AM, Ferrari L. Manifestations of chemically induced liver damage. Clin Chem 1995; 41:1882-7.

[30] Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB, et al. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem 2000; 46: 2027-49.

[31] Giannini EG, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. CMAJ 2005; 172: 367-79.

[32] El-Khayat Z, Ahmed RE, Mahmoud SA, Wafaa IR, Tahany RE. Potential effects of bee honey and propolis against the toxicity of ochratoxin A in rats. Maced J Med Sci 2009; 2:311-8.

[33] Stojević Z, Piršljin J, Milinković-Tur S, Zdelar-Tuk M, Ljubić BB. Activitie activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. Vet Arhiv 2005; 75:67-73.

[34] Šamanc H, Danijela K, Stojić V, Dragica S, Vujanac I, Prodanović R, et al. Application of the metabolic profile in the prediction and diagnosis of fatty liver in holstein cows. Acta Vet Beograd 2011; 61:543-53.

[35] Felig P. The glucose-alanine cycle. Metabolism 1973; 22:179-207.

[36] Brzoska MM, Moniuszko-Jakoniuk J, Pilat-Marcinkiewicz B, Sawickl B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol. 2003; 38(1):2–10.

[37] Jagadeesan G, Sankarsamipillai S. Hepatoprotective effects of taurine against mercury induced toxicity in rats. J Environ Biol 2007; 28(4):753–6.

[38] Mohamed ET, Mahran HA, Mahmoud MS. Hepato ameliorative effect of *azadirachta indica* leaves extract against mercuric chloride

environmental pollution. J Am Sci 2010; 6(9): 735–51.

[39] Abdella E, Gad M. Protective role of diallyl disulphide compound (from garlic extract) against mercuric chloride–induced genotoxicity and cytotoxicity in albino rats. Iran J Can Preven 2008; 3:95–109.

[40] Kumar M, Sharma MK, Kumar A. A food supplement against mercury induced hepatic toxicity. J Health Sci., 2005; 51(4):424–30.

[41] Tounes M, Abdennour C, Houaine N. Influence of *pistacia lentiscus* oil on serumbiochemical parameters of domestic rabbit *oryctolagus cuniculus* in mercury induced toxicity. Eur J Sci Res 2008; 24(4):591–600.

[42] Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC, Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008; 47:1363-70.

[43] Anderson FH, Zeng L, Roch NR, Yoshida EM. An assessment of clinical utility of serum ALT and AST in chronic hepatitis C. Hepatol Res 2000; 18:63-71.

[44] Puoti C, Bellis LB, Martellino F, Guarisco R, Dell-Unto O, Durola L, et al. Chronic hepatitis C and normal ALT levels: Treat the disease not the test. J Hepatol 2005; 43:534-5.

[45] Lai M, Hyatt BJ, Nasser I, Cyrry M, Fdhal NH. The clinical significant of persistently normal ALT in chronic hepatists B infection. J Hepatol 2007; 47:760-7.

[46] Kaplwitz N. Mechanism of liver injury. J Hepatol 2000; 32:39-47.

[47] Rankin SA, Christiansen A, Lee W, Banavara DS, Lopez-Hernandez A. Invited review: The application of alkaline phosphatase assays for the validation of milk product pasteurization. J Dairy Sci 2010; 93:5538-51.

[48] Pillai SS, Jagadeesan G. Influence of taurine on phosphatases acitivity in selected tissues of mercuric chloride into xicagted mice. J Exp Zool India 2005; 8:295-300.

[49] Beaussier M, Wendum D, Schiffer E, Dumont S, Rey C, Lienhart A, *et al*. Prominent contribution of portal mesenchymal cells to liver fibrosis in ischemic and obstructive cholestatic injuries. Lab Invest 2007; 87:292-303.

[50] Ramadan A, Soliman G, Mahmoud SS, Nofal SM, Abdel-Rahman RF. Hepatoprotective and hepatotheraputic effects of propolis against D-galactosamine/lipopolysaccharride-induced liver damage in rats. Int J Pharm Pharm Sci 2015; 7:372-8.

[51] Holman RR, Paul SK, Bethel MA, Neil HA, Matthews DR. Longterm follow-up after tight control of blood

pressure in Type 2 diabetes. N Engl J Med 2008; 359:1565-76.

[52] Joseph J, Mazza MD. Manual of Clinical Hematology. 3rd ed. USA: Lippincott Williams and Wilkins; 2002. p. 461.

[53] Shahsavani D, Mohri M, Gholipour Kanani H. Determination of normal values of some blood serum enzymes in Acipenser stellatus Pallas. Fish Physiol Biochem 2010; 36: 39-43.

[54] Brandão R, Borges LP, Nogueira CW. Concomitant administration of sodium 2,3-dimercapto-1-propanesulphonate (DMPS) and diphenyl diselenide reduces effectiveness of DMPS in restoring damage induced by mercuric chloride inmice. Food Chem Toxicol 2009; 47:1771–8.

[55] Oda SS, El-Ashmawy IM. Protective effect of silymarin on mercury-induced acute nephro-hepatotoxicity in rats. Global Vet 2012; 9(4):376–83.

[56] Batra Y, Acharya SK. Acute liver failure: prognostic markers. Indian J Gastroen-terol 2003; 22:66–8.

[57] Girish SA, Sudhir A, Avinash WG, Dorle K. Evaluation of hepatoprotective effect of amalkadi ghrita against carbon tetra chloride induced hepatic damage in rats. J Ethanopharmacol 2004; 90:229-32.

[58] Margarat A, Jagadeesan G. In vivo Recovery of Some Oxidative Parameters (SDH and LDH Activities) in Mercury Intoxicated Mice, Mus musculus. Madras: Proceeding, Environment and Bioethics LIFE, Loyola College; 2000. p. 280-9.

[59] Rajamanikandan S, Sindhu T, Durgapriya D, Sophia D, Ragavendran P, Gopalakrishnan VK, et al. Protective effect of Mollugo nudicaulis Lam. On acute liver injury induced by perchloroethylene in experimental rats. Asian Pac J Trop Med 2012; 5: 862-7.

[60] Limdi JK, Hyde GM. Evaluation of abnormal liver function tests. Postgrad Med J 2003; 79:307-12.

[61] Fevery J. Bilirubin in clinical practice: A review. Liver Int 2008; 28: 592-605.

[62] Al-Hashem F. Camel's milk protects against aluminum chloride-induced tox-icity in the liver and kidney of white albino rats. Am J Biochem Biotech. 2009; 5(3): 98–108.

[63] Hazelhoff MH, Bulacio RP, Torres AM. Gender related differences in kidney injury induced by mercury. Int J Mol Sci. 2012; 13:10523–36.

[64] Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. J Clin Toxicol. 2011; S4:1.

[65] Xiang GH, Tong SL, Liu HZ. Study of interaction of mercuric chloride with bovine serum albumin by multi-spectroscopic method. J Fluoresc. 2007; 17:512.

[66] Silva PR, Neto OC, Laurentiz AC, Junqueira OM, Fagliari JJ. Blood serum components and serum protein test of hybro-pg broilers of different ages. Rev Bras Ciên Avícola Camp 2007; 9:229-32.

[67] El-Shenawy SMA, Hassan NS. Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats. Pharmacol Rep. 2008; 60:199–208.

[68] Dietschy JM, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res. 1993; 34: 1637-59.

[69] Peretti N, Marcil V, Drouin E, Levy E. Mechanisms of lipid malabsorption in cystic fibrosis: The impact of essential fatty acids deficiency. Nutr Metab (Lond) 2005; 2:11.

[70] Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. J Lipid Res. 1990; 31:1149-72.

[71] Necib Y, Bahi A, Zerizer S. Protective role of sodium selenite on mercuric chloride induced oxidative and renal stress in rats. J Stress Physiol Biochem. 2013; 9(2):159–72.

[72] Sundberg A, Appelkwist EL, Dallner G, Nilsson R. Glutathione transferase in theurine: sensitive methods for detection of kidney damage induced by nephro-toxic agents in humans. Environ Health Perspect 1994; 102(3):293–6.

[73] T. Preeti, K. P. Rakesh Estimation of Total Phenolics and Flavonoids and Antioxidant Potential of Ashwagandharishta Prepared by Traditional and Modern Methods. Asian J. Pharm. Ana. 2014; 4(1) 05-10.

[74] Amit Roy, Dayananda Bhoumik, Ram Kumar Sahu, Jaya Dwivedi. Phytochemical Screening and Antioxidant Activity of *Sesbania grandiflora* Leaves Extracts. Asian J. Res. Pharm. Sci. 2014; 4(1) 16-21.

[75] Ahmad Zubaidi Bin A. Latif, Mainul Haque, C. Shanmugasundaram, U.S. Mahadeva Rao. Clinical Study of Preventive Potentials of Consumption of Buah naga [Cactaceae] Against Paracetamol–Induced Hepatotoxicity as well as the Other Associated Biological Effects. Asian J. Res. Pharm. Sci. 2012; 2(1): 16-23.

[76] Bayader F. Abbas, Wessal M. Khamis Al-Jubori, Ahmed M. Abdullah, Hanan Kd. Sha`aban, Mustafa Taha Mohammed. Environmental Pollution with the Heavy Metal Compound. Research J. Pharm. and Tech 2018; 11(9): 4035-4041. DOI: 10.5958/0974-360X.2018.00743.6