

Effects of *Newbouldia laevis* (P. Beauv) Aqueous Leaf Extract on Liver Function and Histology in Mercury Chloride-Induced Wistar Rats

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Abstract

Mercury is an environmental toxicant that has been proven to induce organ damage on prolonged exposure. The essence of this study is to determine the hepatoprotective efficacy of *Newbouldia laevis* leaves against mercury chloride exposure by assessing the changes in the body weight, hepatosomatic index, and liver function markers and on the histopathology of the liver in adult wistar rats. Twenty five male wistar rats were randomly divided into five groups of five rats each. Group 1 served as the control while groups 2-5 were induced with 10mg/kg body weight mercury chloride. Groups 2 and 3 were then treated with 200 and 800mg/kg body weight of *Newbouldia laevis* respectively. Groups 4 and 5 were the untreated rats and positive control rats respectively. After the 28 days of treatment with the extract, blood samples and liver tissue were collected for liver function tests and histological examination. Significant decreases ($p \leq 0.05$) in liver enzymes (Aspartate amino transferase, Alanine aminotransferase, and Alkaline phosphatase) were observed for the extract treated animals when compared to the untreated ones. Non-significant ($p \geq 0.05$) changes were observed for serum total protein, albumin, globulin and bilirubin concentration. In the histological evaluation, although the treatment restored the normal hepatocyte structure of the liver, characteristic periportal infiltrates of inflammatory cells and vascular congestion were evident. The present study has shown that aqueous leaf extract of *Newbouldia laevis* can be useful for management of liver damage resulting from mercury chloride exposure.

Keywords: Hepatotoxicity, Histology, Liver markers, Mercury chloride, *Newbouldia laevis*

1.0 Introduction

Mercury is a heavy metal present in the environment in different forms. Inorganic mercury and organic mercury are the natural forms. Mercury toxicity has been investigated and in recent times, there has been increased usage of the metal in agricultural, industrial and in medical sectors [1]. Mercury toxicity affects many organs, causing hypertension, hepatotoxicity, nephrotoxicity, and neurotoxicity [1]. Increased exposure to mercury has been shown to affect the brain, nerves, kidneys, muscles of adults, and the developing fetus [2-4]. It accumulates in these organs producing major biochemical and physiological changes in the blood [5]. Hence,

measurement of some blood markers can help determine the extent of its toxicity.

The liver is the largest organ in the body located in the upper right quadrant. It weighs about 1.5 kg and is estimated at about 2.5% of the body weight of an adult [6]. The liver basically is the major site for the metabolism of chemical substances in the body, hence, it is involved in the metabolism of mercury which can eventually accumulate in the liver causing severe hepatic damages [7]. Previous studies have shown that mercury chloride (HgCl) caused histopathological and ultra-structural lesions in the liver which was depicted by periportal fatty degeneration and cell necrosis [8].

Newbouldia laevis is an angiosperm belonging to the Bignoniaceae family [9]. It found in the tropical Africa, Guinea savannah and dense forest [10]. It is referred to as “Aduruku”, “Ogirisì” and “Akoko” in Hausa, Igbo and Yoruba languages respectively. Many nutritional and biochemical benefits have been reported on various parts of the plant *Newbouldia laevis*. Extracts of its leaves have been employed in the management of cough, diarrhea, and dysentery [11]. The leaf also has aphrodisiac properties and is used by women to enhance lactation. Nwaka and Ezeabuchi, [12] have demonstrated the lipid lowering effects of leaf extracts of *Newbouldia laevis*. The ameliorative effect of *Newbouldia laevis* has been reported on alloxan-induced anaemia [13]. Preliminary assessment of various parts of the plant have revealed the presence of bioactive agents and important phytochemicals such as alkaloids, saponins, flavonoids, phenols, tannins etc. [14].

Chelating agents/drugs have been used to eliminate mercury from tissues but owing to the side effects and ineffective elimination of mercury from tissues by these drugs, better and less cheap alternatives have been sought for. Several literatures are available to show the protective role of medicinal plants on the adverse effects caused by toxic metals on humans and animals [15-17]. This study was therefore conducted to determine the ameliorative effects of aqueous leaf extracts of *Newbouldia laevis* on mercury chloride induced liver damage in wistar rats.

2.0 Materials and Methods

2.1 Collection and Identification of Plant Material

The leaves of *Newbouldia laevis* were collected from a field around Oluku, Benin City. Identification, authentication and deposition (Voucher number- UBH-N425) was done in the department of Plant Biology and Biotechnology, University of Benin.

2.2 Preparation of Plant Extract

The collected leaf samples were chopped into fine portions and air-dried (at room temperature) for about a week, then pulverized into powder using a mechanical grinder. Pulverized plant sample (500g) was macerated in 2L of distilled water for 48 hours after which it was filtered using cheese cloth. The obtained

extracts were then concentrated *in vacuo* using a rotary evaporator. The crude extracts gotten were reconstituted to obtain a stock solution using distilled water as solvent. Each reconstituted crude extract was stored in small-capped plastic containers in a refrigerator at -4°C until used.

2.3 Acquisition of experimental animals

Twenty-five (25) adult male wistar rats of average weight 130g were procured from the animal House, Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria, and were utilized for this experimental research. The rats were acclimatized for two (2) weeks before commencement of the experiment. During this period, the animals were allowed free access to standard animal feed (Top feeds grower mash from Bendel Feed and Flour Mill, Ltd, Ewu, Nigeria) and clean water *adlibitum*. The weight of each animal in each group was measured weekly using a Scientech weighing balance (model no. SL 3100D). Approval for this study was gotten from the Research Ethics Committee of the College of Medical sciences, University of Benin with Research ethics number (CMS/REC/2023/339).

2.4 Experimental design

The rats were randomly divided into five groups consisting of five rats each

Group 1 served as the normal control

Group 2 was induced with mercury chloride (10mg/kg body weight) and treated with low dosage *Newbouldia laevis* extracts (200mg/kg body weights)

Group 3 was induced with mercury chloride (10mg/kg body weight) and treated with high dosage *Newbouldia laevis* extracts (800mg/kg body weight).

Group 4 Rats induced with mercury chloride (10mg/kg body weight) only- untreated animals

Group 5 was induced with mercury chloride (10mg/kg body weight) and treated with standard drug (silymarin)

2.5 Blood collection and Liver function tests

At the end of the treatment (28 days), the rats were weighed and then sacrificed under

chloroform anaesthesia. Blood samples were collected with heparin bottles for liver function tests.

Standard laboratory kits procured from Randox laboratory (Randox® UK), were used for the liver function test. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the procedures described by Reitman and Frankel [18], bilirubin (BIL) was determined using the procedure of Jen-Drassik and Grof [19], total proteins by Gornall et al. [20], albumin using the Doumas et al. [21] procedure. TECO (TECO Diagnostic, USA) was used to determine the Alkaline phosphatase (ALP) activity. Hepatosomatic index was calculated using equation 1.

$$\text{Hepatosomatic index (HIS)} = \frac{\text{Liver weight}}{\text{Body weight}} \times 100 \quad (1)$$

2.6 Histopathological analysis

During sacrifice, the liver of each rat was harvested and immediately fixed in 10% formalin, processed, and stained using hematoxylin and eosin staining. Tissue section photomicrographs were obtained and examined under the Leica DM750 research microscope with a digital camera (LeicaCC50) attached, with magnifications of x400.

2.7 Data analysis

Data were subjected to statistical analysis using the SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were obtained. Final and initial weights of each group were compared using independent Sample T-test. One-way analysis of variance (ANOVA) was carried out and values were presented as mean $\pm$ SEM. LSD post-hoc test was used. Values of $P < 0.05$ were considered significant.

3.0 Results

3.1 Weight of the animals

Table I shows the effect of *Newbouldia laevis* on initial and final body weight of the animals. A significant increase ($p \leq 0.05$) in final body weight was observed for all the groups when compared with the initial body weights of the rats.

Table I: Effects of *Newbouldia laevis* extract on body weight of mercury chloride induced rats

Groups	Initial body weight	Final body weight
Group 1	110.60 $\pm$ 2.29	174.00 $\pm$ 7.80*
Group 2	126.80 $\pm$ 7.12	169.80 $\pm$ 12.48*
Group 3	143.80 $\pm$ 8.35	200.40 $\pm$ 9.98*
Group 4	134.67 $\pm$ 11.57	182.00 $\pm$ 7.81*
Group 5	122.75 $\pm$ 3.09	169.25 $\pm$ 5.66

*Significantly different from the initial body weight

3.2 Hepatic weight and hepatosomatic index

Table II shows the effects of *Newbouldia laevis* on hepatic weight and hepatosomatic index of the rats. A non-significant ($p \geq 0.05$) increase in hepatic weight and hepatosomatic index was observed for all the groups when compared with the control rats.

Table II: Effects of *Newbouldia laevis* extract on hepatic weight and hepatosomatic index of mercury chloride- induced rats

Groups	Hepatic weight (gram)	Hepatosomatic index (%)
Group 1	6.18 $\pm$ 0.39	3.56 $\pm$ 0.21
Group 2	6.40 $\pm$ 0.35	3.79 $\pm$ 0.13
Group 3	7.40 $\pm$ 0.56	3.68 $\pm$ 0.13
Group 4	7.00 $\pm$ 0.62	3.84 $\pm$ 0.26
Group 5	7.18 $\pm$ 0.45	4.02 $\pm$ 0.20

There were no statistically significant differences ($P > 0.05$) in hepatic weight and hepatosomatic index in all groups

3.3 Liver function parameters

Table III shows the effects of *Newbouldia laevis* on liver function parameters of the treated and untreated animals. A significant increase ($p \leq 0.05$) was recorded for Alkaline phosphatase (ALP), Aspartate amino transaminase (AST) and Alanine transaminase (ALT) activities in all groups when compared with the control (Group 1). Treatment with extracts resulted in a significant decrease in AST, ALP and ALT activities when compared with untreated group. Non-significant ($p \geq 0.05$)

changes were observed for serum total protein, albumin, globulin and bilirubin concentration

when compared with the normal control and untreated rats.

Table III: Effects of *Newbouldia laevis* extract on liver function markers on mercury chloride - induced rats

Markers	Group 1	Group 2	Group 3	Group 4	Group 5
ALP (U/L)	293.00±33.78**	369.67±70.83**	348.67±24.63**	553.33±72.90*	382.336±1.66**
AST (U/L)	97.00±6.00**	114.33±17.19**	96.33±18.84**	121.00±14.47*	118.67±15.33**
ALT (U/L)	117.00±12.65**	96.67±10.33**	119.33±3.84**	167.33±5.49*	119.00±20.55**
Total protein (g/dl)	7.43±0.43	6.60±0.06	7.10±0.15	6.80±0.32	6.83±0.12
Albumin (g/dl)	3.43±0.07	3.30±0.06	3.37±0.03	3.43±0.03	3.17±0.09
Globulin (g/dl)	4.00±0.36	3.30±0.10	3.73±0.12	3.37±0.30	3.67±0.07
Total bilirubin (g/dl)	0.30±0.06	0.27±0.03	0.23±0.03	0.30±0.06	0.27±0.03
Conjugated bilirubin (g/dl)	0.13±0.01	0.13±0.01	0.11±0.02	0.11±0.02	0.13±0.01

*indicates statistical significant difference when compared to all groups

**indicates statistical significant difference when compared with the untreated group

3.4 Results of histopathology of liver tissues of treated and untreated rats

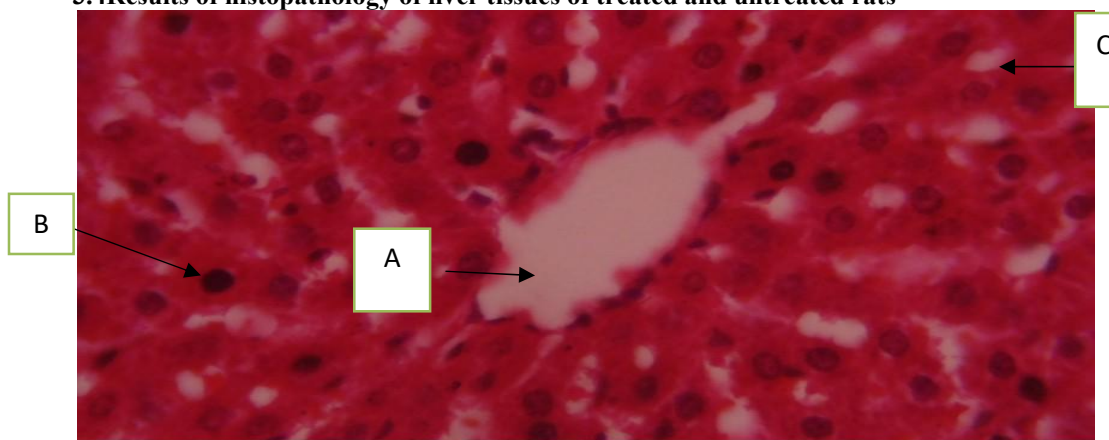


Plate 1: photomicrograph of control rats showing: A. hepatocytes, B. sinusoids, C. central vein (H&E x 400)

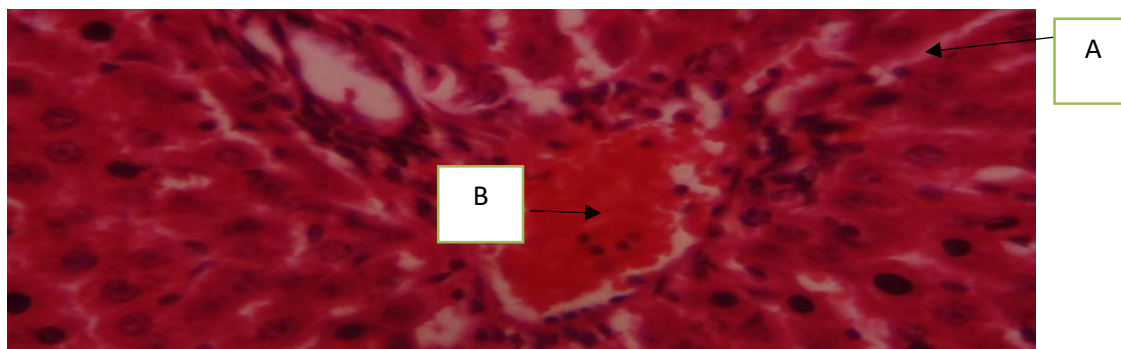


Plate 2: photomicrographs of rats treated with 200mg/kg body weights *Newbouldia laevis* extracts showing A. vascular congestion, B. mild periportal infiltrates of inflammatory cells, C. normal hepatocytes (H&E x 400)

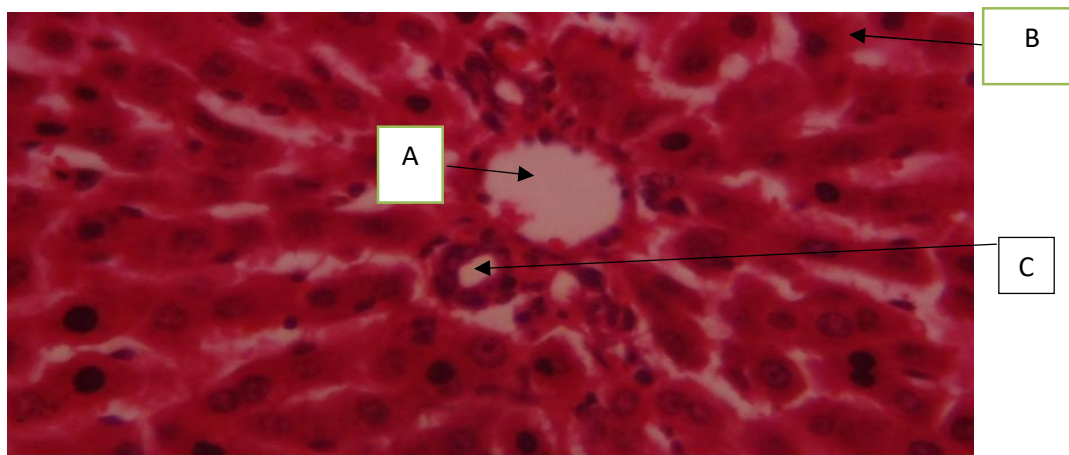


Plate 3: photomicrographs of rats treated with 800mg/kg body weights *Newbouldia laevis* extract showing A. hepatocytes, B. portal vein, C. bile duct (H&E x 400)

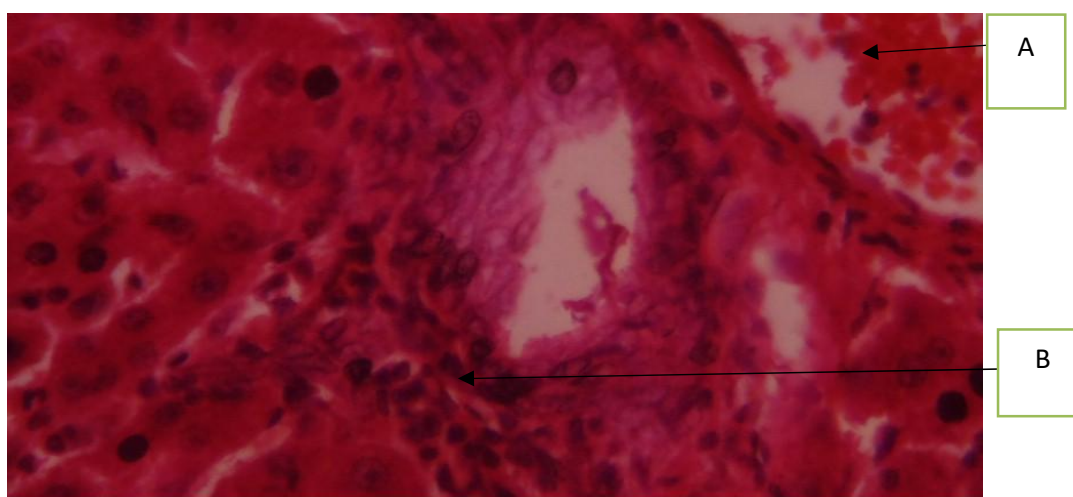


Plate 4: Rat liver given mercury chloride only showing: A. vascular congestion, B. bile-duct epitheliosis, C. periportal infiltrates of inflammatory cells (H&E x 400)

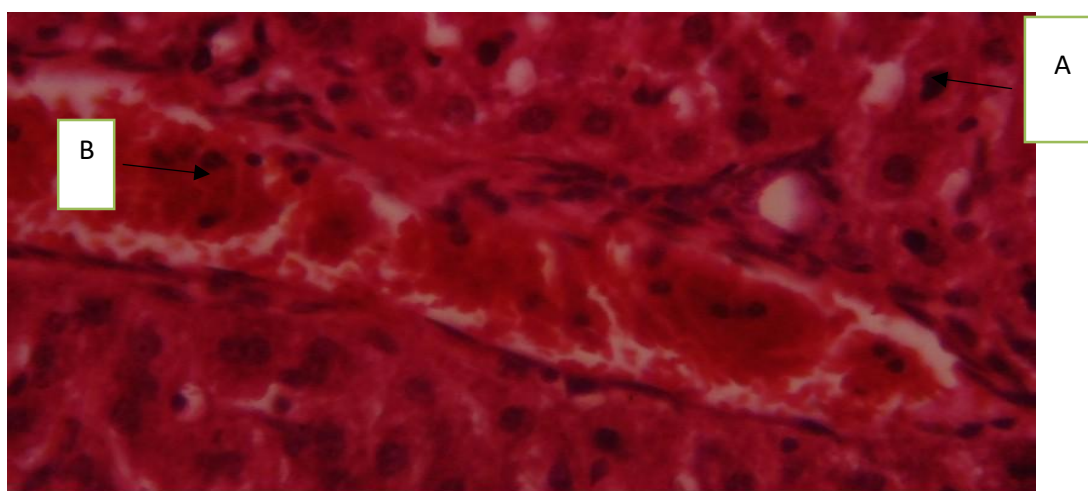


Plate 5: Rat liver given Silymarin + mercury chloride showing normal architecture: A. normal hepatocytes, B. active vascular congestion (H&E x 400)

4.0 Discussion

Mercury causes toxicity using several mechanisms of action, one of which is oxidative stress induced pathological damage [22, 23]. The Hg^{2+} form of mercury has been reported to have great affinity for endogenous biomolecules-associated thiol (-SH) groups [24], and it is invariably found attached to SH-containing proteins, small-molecular weight peptides (like glutathione) and amino acids (e.g. cysteine), thereby affecting several important metabolic processes [25, 26]. Mercury chloride in particular exerts its action by activating many enzymes resulting in the blockage of functional sites through binding to sulfhydryl groups, which make up the catalytic or binding domains [27]. Mercury (II) chloride ($HgCl_2$) has been reported to cause hepatotoxicity, because it generates free radicals which can simultaneously increase the oxidative stress in the liver, eventually causing hepatotoxicity [28]. In this study we assessed the hepatic weight, hepatosomatic index, liver function parameters, and histological examination of the liver tissues as a way of determining the ameliorative effects of *Newbouldia laevis* (p. beaur) aqueous leaf extract on mercury chloride induced liver damage in wistar rats.

Our results showed that administration of mercury chloride did not significantly alter the hepatic weight and hepatosomatic index of the wistar rats (Table II). This finding is in line with the results obtained by Ajibade et al. [29] on the hepatotoxic effects of mercury chloride in adult wistar rats.

In this study, the effects of aqueous extract of *Newbouldia laevis* on serum enzymatic and non-enzymatic liver markers in mercury chloride induced wistar rats was ascertained. The estimation of activities of ALP, AST and ALT activities in serum has been used to determine the extent of liver damage in both clinical and experimental settings.

In this study we confirmed that administration of *Newbouldia laevis* extract was capable of reducing serum activities of alkaline phosphatase, aspartate amino transferase, and alanine amino transferase that were increased by mercury induction (Table III). Nwaka and Ezeabuchi [30] in their study reported that administration of *Newbouldia laevis* leaves did not cause any significant change in the levels of the liver enzymes, implying that the observed increase in the liver enzymes from this study, resulted from

mercury induction. Several studies have demonstrated that mercury induction in rats leads to significant increase in liver enzymes [31]. Osigwe et al [32] in their studies on the “biochemical and haematological effects of the leaf extract of *Newbouldia laevis* in alloxan induced diabetic rats” demonstrated that leaves of *Newbouldia laevis* extract significantly reduced the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The plant’s rich phytoconstituents namely flavonoids [33] and apigenin [34] were linked to the observed beneficial effects.

Protection of cellular integrity of organs by reduction of oxidative stress induced damages are characteristic features of plants having high antioxidant contents of which *Newbouldia laevis* is one. Studies done by Habu and Ibeh [35] on the in vitro antioxidant capacity and free radical scavenging ability of active metabolite constituents of *Newbouldia laevis* showed that the leaves of *Newbouldia laevis* are rich in antioxidants and have high free radical scavenging potential. It was also reported to contain important antioxidant vitamins like vitamin E and C, which may have helped protected the livers from mercury induced oxidative stress damage thus leading to the significant decrease in the liver enzymes in this study.

This study also shows that treatment with mercury chloride did not alter the synthetic ability of the liver as evident by the non-significant changes in the total protein, albumin, and globulin levels (Table III). However, Kumar et al. [15] obtained a different result on their study on the ameliorative effect of *Bryophyllum pinnatum* against induced mercuric chloride toxicity in rat

The photomicrograph of control rats showed normal liver histo-architecture having well characterized central vein, sinusoids and hepatocytes. However the photomicrograph of the groups induced with mercury chloride showed abnormal architecture with vascular congestion, bile-duct epitheliosis, and periportal infiltrates of inflammatory cells. Although, treatment with *Newbouldia laevis* restored the normal hepatocyte structure, characteristic periportal infiltrates of inflammatory cells and vascular congestion were evident (plates 2 and 3). Several reports are available to show that exposure of wistar rats to mercury chloride resulted in mild to severe

histopathological damage to various organs. Ajibade, [29] in his study on “the hepatotoxic effects of mercury chloride on the liver of adult wistar rats” presented several finding sinhistological distortions of the liver tissues ranging from loss of liver parenchyma, disorganization hepatocellular profile, cell death, dilation of the central vein, fibrosis etc. Ajibade et al. [31] in their study also reported gross morphological changes which include congestion, severe haemorrhage, necrosis, degenerative changes in the kidneys when treated with mercury chloride.

5.0 Conclusion

This study has therefore demonstrated that aqueous leaf extract of *Newbouldia laevis* (p. beaur) has ameliorative effect on mercury chloride induced liver damage in wistar rats.

6.0 Acknowledgement

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7.0 Conflict of Interest

The authors declare no conflict of interest

8.0 References

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