

Evaluation of Hepatoprotective Potential of Stem Bark of *Neolamackia Cadamba* Against Chloroform and Over dose of Iron Dextran Induced Hepatotoxicity in Experimental Swiss Albino mice

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Abstract

Background: In general use herbal medicines are considered as the backbone of traditional system of medicines as it has been used for food, flavoring agents and in the form of medicines. Keeping in knowledge the increasing demand of herbal drugs over their synthetic counter parts and to restrain the worldwide problem of multidrug resistance. *Neolamarckia cadamba* that is otherwise called Cadamb is a standout amongst the most significant therapeutic plants having a place with the Rubiaceae family. It is one of such ayurvedic cure that has been referenced in numerous Indian restorative written works. The arrangement of work includes gathering and confirmation of stem bark of cadamb plant pursued by shade drying, granulating, and extraction by twofold cool maceration, phytochemical examination lastly screening of pharmacological exercises as expressed

Aim: The present study aimed to determine the hepatoprotective activity of hydro alcoholic extract of stem bark of *Neolamackia Cadamba* using two established hepatotoxic induced models in Swiss albino mice (Chloroform induced hepatotoxicity and over dose of iron induced hepatotoxicity).

Materials and Methods: In the present study, hepatoprotective effect of *Neolamackia Cadamba* hydro alcoholic extract of stem bark (NCHAE) in experimental mice by giving Corn oil and Chloroform for 7 days period at 0.75mg/kg body weight, orally induced hepatotoxicity.

In the second experimental model over dose of iron dextran hepatic damage was induced in mice at 100mg/kg, i.p on 7 days 3 consecutive periods. Blood sample were collected for biochemical parameters, histopathological changes in liver were investigated. Silymarin (50 mg/kg body wt.) was used as standard hepatoprotective reference drug.

Statistical Analysis Used: The obtained data were analyzed by ANOVA with Dennett's multiple 't' test and level of $p < 0.1$ was considered as statistically significant.

Results: Chloroform and over dose of iron treatment led to elevated levels of liver marker enzymes and disorientation in histological observations which were significantly reversed by treatment with *Neolamarckia cadamba* dependent on dose forms. The results indicated that biochemical changes produced by both the models were restored to normal by NCHAE. Based on the results obtained, NCHAE showed significant hepatoprotective effect ($p < 0.01$) against both inducers, as indicated by an improvement in biochemical parameters. In conclusion *Neolamackia Cadamba* stem bark possessed hepatoprotective activity, which could be linked to their antioxidant activity; this therefore requires further in-deep studies.

Conclusion: The study revealed that the NCHAE have similar hepatoprotective effect.

Keywords: Hepatoprotective, *Neolamarckia cadamba*, chloroform, Iron dextran, hepatotoxicity.

1. INTRODUCTION:

Neolamarckia cadamba usually called as 'Kadamba' in Ayurveda and belongs to the family Rubiaceae. Hindi name kadam is a much loved plant of "Lord Gopal" and one of the vascular plant mentioned in prehistoric Sanskrit manual. (Kumar, 2017). It is a huge deciduous tree between 37.5 -45 meter height. The stem of younger trees appears grayish-green with even bark. As it become older, the bark gets uneven and grey with longitudinally fissured. Leaves glossy, dark green, differing, simple pulpiness base sub sessile to petiolate, broadly ovate to elliptical-oblong, entire, apex and venation pinnate. The flowers that become visible from August to October are orange to yellow. Inflorescences in clusters, terminal globose heads, sub sessile and aromatic. Fruit lets abundant with upper parts contains four void or hard structure. (Devgan et al., 2012). Olden times of Kadamba can be traced back to Vedas, Puranas and Samhita. The Srimad Bhagavata also mentions Kadamba during the time of Kaliya mardana. Pathanjali in his Mahabhasya, mentions the Kadamba, while describing fruit varieties (Acharyya et al., 2018). Liver is the largest organ among the different organs of the body. It is the important site for metabolism and excretion. (Rhitajit & Ward et al., 1999 & 2012). It is also known as the great "chemical factory" of the body since it has a vital role in regulating, synthesizing, storing and secreting

many important proteins, nutrients, chemicals and also in purifying clear toxins or unnecessary substances from the body. The Greek word for liver is "hepar, so medicinal terms related to liver often starts with hepato or hepatic (Sowjanya et al., 2015 & Garba et al., 2009)

In patients with iron overloading, hepatotoxicity is the mostly observed since liver is the main storage site of iron (Richa et al 2013 & Papanastasiou et al 2000). Hepatic damage affects the normal metabolic functions which may lead to severe health problems (Wolf., et al 1999). Liver problems like cirrhosis, hepatitis and alcoholic liver diseases can be caused by continuous exposure of environmental toxins, drug abuse, and alcohol abuse and over the counter drug use.

Table 1. Chemical constituent of *Neolamackia Cadamba*

S.No	Part	Chemical Composition
1	Heartwood	Dihydrotectochoyrisin, Dihydrowogonin, pinocembrin
2	Stem	Narigenin, apigenin, β -sitosterol, sakuranetin.
3	Leaves	Quercetin-3-rhamnoglucoside, Kaempferol
4	Seed	Narigenin-5-O- α -L-rhamnopyranoside
5	Branches Substitute	hydrocyanic acid, amygdalin

MATERIAL AND METHODS

Selection of plant and authentication

Dried stem bark of plant *Neolamarckia cadamba* were assembled from the campus of NIET, Greater Noida, during the long stretch of September 2018 and it was authenticated by Dr. Anjula Pandey (Taxonomist), National department of plant and hereditary assets (NBPGR), Pusa grounds, New Delhi.

Preparation of the drug

Experimental animal

Swiss albino mice weighing 25-30gm of both the sex were used for hepatoprotective activity. They were obtained from Central Animal House of pharmacy institute NIET, Greater Noida. The animals were housed in standard conditions such that temperature was maintained at $25 \pm 2^{\circ}\text{C}$ with 12 hrs light and 12 hrs dim cycles.

Plant extraction process

The dried stem bark of the *Neolamarckia cadamba* were subjected to pounding into coarse powder. The obtained powder was then sieved through a mesh of sieve number 80 into fine powder. The fine powder was preserved in airtight container. Double cold maceration was done in pharmaceutical lab. The shade dried stem bark of *Neolamarckia cadamba* plant were mixed in the ratio of (1:3) and extraction was carried out by double cold maceration process with hydroalcoholic extract solvent for 7 days with occasionally shaking every 4 hours and placing them in dark condition so as to minimize the light entrapment and maintaining the proper temperature 35°C . After 3 days filtration was carried out and marc was re-macerated with the solvent for another 3 days. The filtration was concentrated in rotatory evaporator and water bath by maintaining the temperature not exceeding 35°C with continuous stirring. The extract was concentrated to a liquid form and placed in a container. The dried extract was found to be 43.34% w/w with regard to air dried drug.

Chemicals

All the chemicals and reagents used in the experimental study were procured from standard and reputed firms and are of analytical grade regularly used in the laboratory. The reference standard drug used for hepatoprotective evaluation is Silymarin. It was purchased from the market with the trade name Silybon manufactured by micro labs limited.

The toxicants used to induce hepatic injury in respective protocol were chloroform and iron.

Evaluation of hepatoprotective activity

Chloroform Induced Hepatotoxicity

Hepatoprotective study using chloroform induced hepatotoxicity model in Swiss albino mice was carried out using method followed by Ghosh et al. with slight modification. Swiss albino mice of either sex weighing 25-35 g were selected and divided into 5 groups of 6 animals in each group (n=6). Treatment was given as described below for 16 days.

Group I: Negative Control (Saline), 0.2ml/kg body weight was administered p.o.

Group II: Positive Control (chloroform+corn oil) initially corn oil was given at the dose of 0.75mg/kg for 5 days and after 2 days 1:1 ratio of chloroform and corn oil was given at the dose of 0.75mg/kg)

Group III: Mice received standard drug treated with silymarin at the dose of 50mg/kg for 5 days and 2 days ratio of chloroform and corn oil was given at the dose of 0.75mg/kg)

Group IV: Mice received hydro alcoholic extract (*Neolamarckia cadamba* 250mg/kg) 5 days test drug was given and after 2 days chloroform was administered.

Group V: Mice received hydro alcoholic extract (*Neolamarckia cadamba* 500mg/kg) 5 days test drug was given and after 2 days chloroform was administered.

Liver abnormality was influenced in mice by orally giving corn oil and chloroform for 7 days period. In initial 5 days the combination of corn oil and chloroform in the ratio of 1:1 was given at the dose of 0.75mg/kg. In last 2 days only, chloroform was given at the dose of 0.75mg/kg. This dose was given in control and standard group. In test group only chloroform was administered for 2 days and then test drug was administered for 5 days period. On day 16th, mice groups were sacrificed after 48 hr of Chloroform administration, liver & blood sample was collected; Serum was separated from blood sample to estimate the serum biochemical parameters like AST, ALT, ALP, total bilirubin and total protein. From histopathology liver necrosis, fatty change, blooming of cells was estimated. (Ghosh et al 2006)

Over Dose of Iron Dextran

Hepatic damage was induced in mice by administration iron dextran at 100mg/kg, i.p on 7 days 3 consecutive period, *Neolamarckia cadamba* extract (test drug) and silymarin that was served as standard the dose was given at the same day of administrating iron dextran for continuous 3 days in control, standard, and test groups. On 21st day blood was taken from every group to detect biochemical parameters. Mice that showed maximum hepatotoxicity were dissected and liver was extracted from histopathology to find out the liver necrosis, total fatty change, blooming of liver cells (Sarkar 2012).

Hepatoprotective Experimental Model

Over dose of iron dextran

The investigational mice were isolated into five gatherings each group containing six mice and that were filled in as pursues:

Group I: Negative control (Saline) 0.2ml/kg body weight was administered p.o.

Group II: Positive control (iron dextran, administered at 100mg/kg for 10 alternate days)

Group III: Mice received standard drug treated with silymarin at the dose of 50mg/kg at the same day on iron dextran administration.

Group IV: Mice received hydro alcoholic extract (*Neolamackia cadamba* 250mg/kg) was given at the same day on the iron dextran administration.

Group V: Mice received hydro alcoholic extract (*Neolamackia cadamba* 500mg/kg) was given at the same day on the iron dextran administration.

Parameters employed for assessing the extent of liver injury

Ponderal changes

Organ weight – liver. The organ weight was presented in terms of relative weight.

Serum biochemical parameters

The blood was collected and sent to biochemistry laboratory for biochemical investigations involving serum parameters. Serum biochemical parameters were estimated. The following biochemical parameters were estimated according to standard laboratory procedure; serum glutamic oxaloacetic transaminase (SGOT) activity, Serum glutamic Pyruvate transaminase (SGPT) activity, alkaline phosphatase (ALP) activity, total protein, albumin, total bilirubin.

Histopathological analysis

Mice were fasted during the night after the testing finished. They were anaesthetised with ethyl ether. Liver section was dissected out and washed with saline to abolish the blood cells and processed individually for histological determination. Early the liver was set in 10% buffer neutral formalin for 48 hours. A paraffin implanting strategy was passed and areas were taken at 5 μ m thickness, set apart with hematoxylin and eosin, and performed minutely for histopathological changes.

Statistical analysis method (SEM)

The collected data of all the groups was examined by using one way ANOVA with Dunnett comparison testing. Readings were calculated as Mean \pm SEM where n= 6. The level of significance will be denoted by ‘P’ values.

RESULTS AND DISCUSSION

There are several models available for screening of potential hepatoprotective drugs. The method chosen here is chloroform and over dose of iron induced hepatotoxicity to assess the hepatoprotective potential of *Neolamarkia cadamba*, iron, although, a safe drug at therapeutic dose, it may likely to produce fatal reactions and cause death in human and experimental animals in overdose.

Chloroform which is used as an agent to produce hepatotoxicity, has produced substantial hepatotoxicity by resulting in significant fall in the levels of total serum proteins and albumin globulin ratio and a significant increase in ALP, AST and ALT. Increased levels of ALT, AST and ALP in serum of the Swiss albino mice indicate

liver damage as these enzymes leak out from liver in to the blood at the instance of tissue damage which is always associated with hepatonecrosis.

Assessment of liver cell necrosis is most frequently done by estimation of SGOT and SGPT activity. Serum levels of SGOT and SGPT are increased on damage to the tissues producing them. In the present, study also similar elevation was observed. Thus, the observed elevation in transaminase activity can be considered indicative of liver inflammation and injury due to the toxic effect of chloroform and over dose of iron. Transaminase estimations are useful in the early diagnosis of viral hepatitis. Very high levels are seen in extensive acute hepatic necrosis such as in severe viral hepatitis and acute cholestasis. Alcoholic liver disease and cirrhosis are associated with mild to moderate elevation of transaminases.

Effect of test drugs *Neolamarckia cadamba* on liver biochemical parameters

Among all biochemical parameters in serum, significant increase was observed in SGOT, SGPT, and total bilirubin, direct bilirubin indicating hepatotoxicity in chloroform and over dose of iron. Thus, elevation in the above parameters can be considered as an index of chloroform and over dose of iron induced hepatic injury and its reversal as sign of expression of hepatoprotection. Significant reversal in the elevation of parameters like SGOT, SGPT, direct bilirubin, and serum cholesterol were observed in T1, while in T2 group significant reversals were seen in SGPT. Direct bilirubin level was significantly decreased in test drug T2 group. Test drug T4 showed significant reversal in elevation of SGOT, SGPT.

The elevated level of SGOT was reversed by all four test drugs in therapeutic doses and reference standard administration. There was significant decrease observed in standard drug and T1 and T4 test drugs groups. SGPT was significantly decreased by the administration of reference standard group and test drugs T1, T2 and T4 at therapeutic doses. There was moderate decrease in alkaline phosphatase activity observed after hepatotoxins. In the present study total bilirubin and direct bilirubin were markedly elevated in chloroform and over dose of iron control group indicating hepatocellular damage. Elevation of total bilirubin was significantly decreased by standard reference drug and mild decrease was observed in test drug.

In the present study serum total protein non-significant increase was observed in chloroform and over dose of iron. Test drugs and standard reference drug mildly decreased total protein which is not significant. Hence its estimation did not contribute to the determination of the hepatoprotective potential of the test formulations and reference standard. Estimation of total serum cholesterol, triglyceride and lipoprotein fraction are frequently done in liver disease.

Calculated values of Hepatoprotective activity of NCHAE

Table 2 Hepatoprotective activity of stem bark of NCHAE by Chloroform induced hepatotoxicity

Group	Drug	Dose mg/kg	AST (IU/L)	ALT (IU/L)	Total Protein	ALP (IU/L)	Total Billirubin mg/dL
1	Saline	0.2ml	27.81±1.98	28.18±0.84	3.04±0.19	81.16±0.59	0.77±0.05
2	Chloroform+corn oil	0.75	36.29±0.81	35.94±0.93	5.44±0.21	88.85±0.27	0.81±0.01
3	Silymarin	50	16.11±0.63***	22.35±0.62***	6.99±0.04***	76.04±0.85***	0.70±0.04***
4	NCHAE	250	19.34±0.71*	25.32±0.53*	6.47±0.32*	78.50±0.86*	0.79±0.01*
5	NCHAE	500	17.20±0.54**	23.37±0.74**	7.04±0.35**	75.88±0.02**	0.75±0.07**

Values are expressed in MEAN ± SEM; ***p<0.01 and *p<0.1, when compared with control group (chloroform+corn oil), highly significant but comparability less than standard group. (One-way ANOVA followed by Dennett's t test) N=6: NCHAE represented as Neolamackia Cadamba hydro alcoholic extract, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphate.

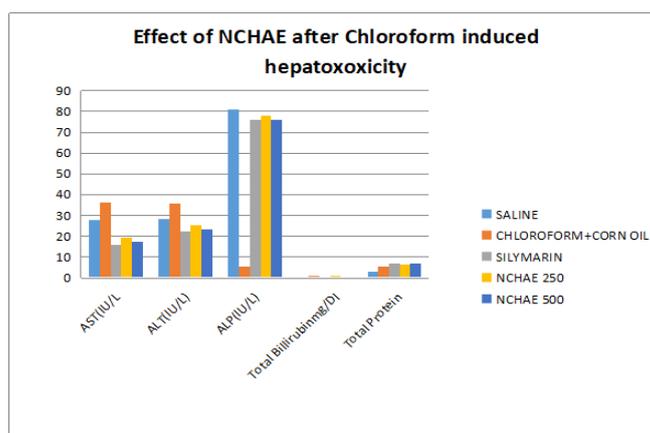


Figure 1: Efficacy of methanol extract from the stem bark of *Neolamackia Cadamba*: after the administration of chloroform+corn oil serum enzyme AST, ALT, ALP increased, levels of serum enzymes decreased after administration of NCHAE when compared with standard (Silymarin) at the dose of 50mg/kg.

Histopathological findings:

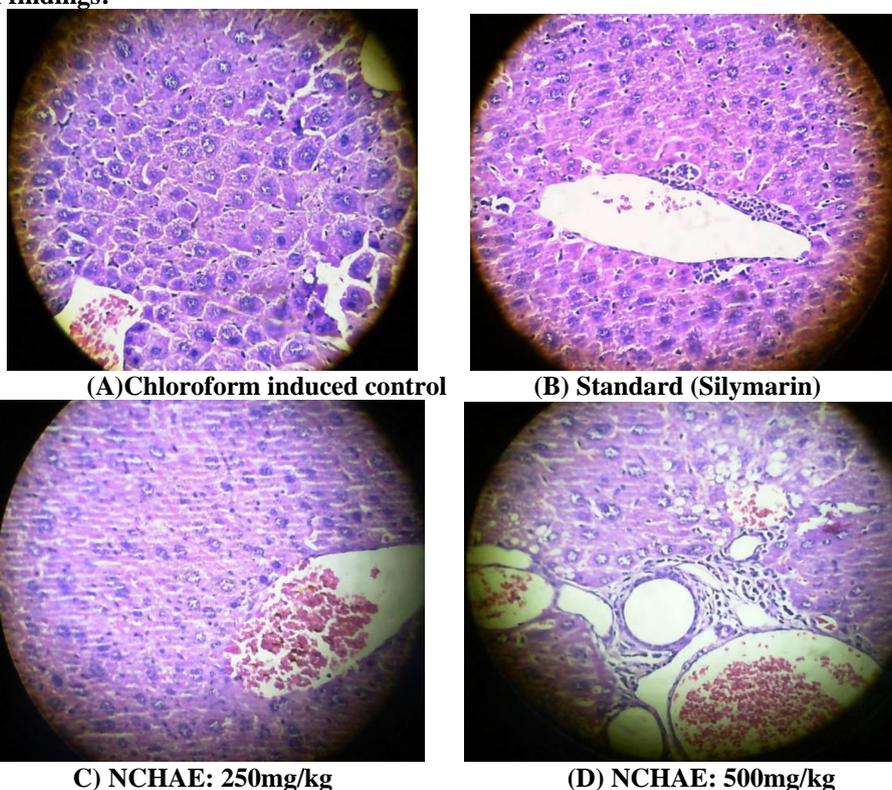


Figure 2 Effect of NCHAE on histopathology of liver in chloroform induced hepatotoxicity

Histopathology. Photomicrograph of mice liver section (staining with haematoxylin and eosin, X 40) by the effect of methanol extract of stem bark of *Neolamaria Cadamba* (NCHAE) in Chloroform induced hepatotoxicity. Histopathological observation of experimental mice after 16 days of treatment. (A) Marked increase in aggregation of hepatocytes and necro inflammatory changes after chloroform administration. (B) Liver section after

administration standard drug (silymarin) at the dose of 50mg/kg showing reduced hepatocellular necrosis, ballooning degeneration and inflammatory, (C) Liver sections after adding extract (250 mg/kg) showing moderate improvement of inflammatory cells and cell necrosis. (D) Liver sections after adding extract (500 mg/kg) showing reduced necrosis area and increased number of hepatocytes.

Calculated Value of Hepatoprotective Activity of NCHAE

Table 3 Hepatoprotective activity of Stem Bark of NCHAE by Over Dose of Iron Induced Hepatotoxicity.

Group	Drug	Dose mg/kg	AST (IU/L)	ALT (IU/L)	Total Protein	ALP (IU/L)	Total Billirubin mg/dL
1	Saline	0.2	20.75±0.87	23.10±1.01	9.58±0.44	71.75±1.21	0.81±0.02
2	Chloroform + corn oil	0.75	21.86±0.97	24.86±1.15	6.31±0.21	74.81±2.33	0.89±0.02
3	Silymarin	50	17.55±0.85***	20.84±0.74***	7.50±0.35***	69.07±0.48***	0.76±0.03***
4	NCHAE	250	19.73±0.69	22.62±0.74	8.65±0.45	70.57±2.14	0.77±0.01
5	NCHAE	500	18.79± 0.75**	21.03± 0.90**	8.15± 0.41**	68.70± 2.11**	0.73± 0.02**

Values are expressed in MEAN ± SEM; **p<0.01 and *p<0.1, when compared with control group (Iron dextran), highly significant but comparability less than standard group. (One-way ANOVA followed by Dennett's t test) N=6: NCHAE represented as Neolamackia Cadamba hydroalcoholic extract, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphate.

Effect of NCHAE after overdose of iron induced hepatotoxicity model

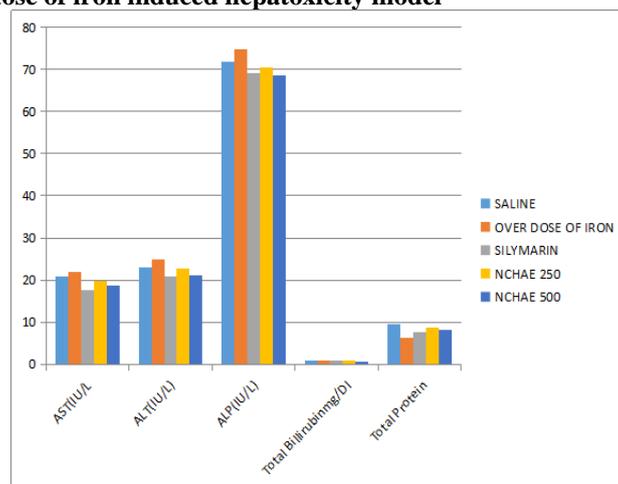


Figure 3: Efficacy of methanol extract from the stem bark of *Neolamackia Cadamba*: after the administration of over dose of iron serum enzyme AST, ALT, ALP increased, levels of serum enzymes decreased after administration of NCHAE when compared with standard (Silymarin) at the dose of 50mg/kg.

Histological findings:

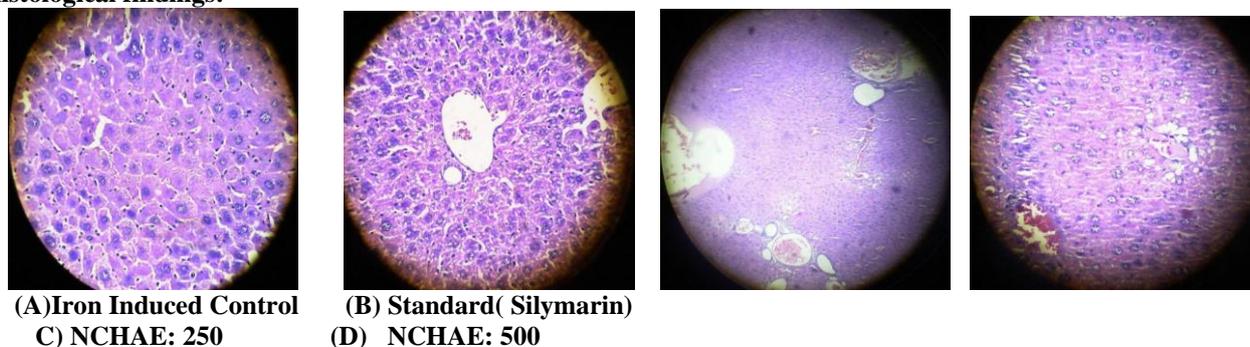


Figure 4 Effect of NCHAE on histopathology of liver in over dose of iron induced hepatotoxicity

Histopathology. Photomicrograph of mice liver section (staining with haematoxylin and eosin, X 40) by the effect of hydroalcoholic extract of stem bark of *Neolamackia Cadamba* (NCHAE) in over dose of iron induced hepatotoxicity. Histopathological observation of experimental mice after 21 days of treatment. (A) Liver segment appearing, greasy swelling degeneration, aggravation and cell boundaries, (B) Liver segment after administration standard drug (sylimarin) at the dose of 50mg/kg showing decreased hepatocellular necrosis, ballooning degeneration and inflammatory, (C) Liver sections after adding extract (250 mg/kg) showing improved histology with portal inflammation, (D) Liver sections after adding extract (500 mg/kg) showing reduced necrosis area and increased number of hepatocytes.

Discussion

Expanded levels of serum transaminase, in investigational mice, is seen in the present examination, can be credited to the harmed structural integrity of the liver because these are cytoplasmic in nature and are free into the course after cell hurt. Decrease in the absolute serum protein was assessed in mice treated with chloroform and over portion of iron and might be connected with the lessening in the quantity of hepatocytes, which thus may impact in the hepatic ability to deliver protein and therefore diminish liver burden. Organization of methanol concentrate of *Neolamackia cadamba* indicated critical hepatoprotective action, which was contrasted and the standard medication (silymarin). Hepatocellular putrefaction or on the other hand membrane harm led to extremely abnormal amounts of serum AST, ALT discharged from liver to flow, abnormal state of billirubin respectively.

CONCLUSION

The present study demonstrated that the hydro alcoholic extract of stem bark of *Neolamackia cadamba* possessed hepatoprotective activity against chloroform and over load of iron induced hepatotoxicity. We can conclude that the hepatoprotective activity of our plant part rationalize the conventional state of the plant for the hepatoprotective

activity. It was observed from our study that stem bark of *Neolamackia cadamba* has a defensive action against both the models (chloroform induced hepatotoxicity; over dose of iron induce hepatotoxicity. The methanol extract of stem bark of *Neolamackia cadamba* has revealed the capability to regulate the normal function of the liver.

From the above fundamental examination, it very well may be reasoned that the methanol concentrate is demonstrated to be one of the herbal remedies for liver disorder.

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