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Enhanced Therapeutic Potential of Boldine – Phospholipid Complex in Carbon Tetrachloride Induced Hepatotoxicity in Rats

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Abstract

Boldine is naturally occurring aporphine alkaloid, possessing wide spread therapeutic activity such as, antioxidant, anti-inflammatory, hepatoprotective, antidiabetic etc. Because of its, unfavorable pharmacokinetics associated with a lower plasma half-life, poor oral bioavailability and rapid clearance from body, the clinical application of boldine is restricted. Hence frequent dosing is required to maintain effective plasma concentration. The objective of present research work was to develop and evaluate hepatoprotective potential of boldine–phospholipid complex in carbon tetrachloride – induced hepatotoxicity model and compared with free drug component. The boldine phospholipid complex was formulated by refluxing followed by solvent evaporation method the various hepatic biochemical parameters such as ALT, AST, ALP and total bilirubin (TB) evaluated. The result obtained from the investigation showed that BOL-PC formulation restored the elevated levels of liver enzymes, which was found to be significant with respect to carbon tetrachloride treated group at $p < 0.05$ and < 0.01. So the study revealed the superiority of boldine–phospholipid complex over the free drug boldine in terms of hepatoprotective potential and prolonged duration of action, which might be helpful in reducing the fast elimination of the drug molecule from body.

Keywords: Boldine–Phospholipid Complex (BOL-PC), Hepatoprotective activity, boldine physical mixture (BOL-PM), Carbon Tetrachloride,

1. Introduction

Boldine (1, 10-dimethoxy-2, 9-dihydroxyaporphine, (Figure1.) an aporphine alkaloid commonly found in the bark and leaves of the boldo tree (Peumus boldus). The majority of the health-promoting properties of boldo extract are due to this alkaloid. [1,2]. Boldine posseses a wide spared pharmacological activity such as antioxidant, cytoprotective, antitumor, anti-inflammatory, immunomodulatory, hepatoprotective, and antipyretic properties [3–6].

Despite having promising pharmacological activity of boldine, the clinical application has been hampered by unfavorable pharmacokinetics associated with low plasma half life and extremely poor oral bioavailability. The basic reason for low oral bioavailability might be due to poor water solubility, low dissolution rate and rapid clearance from the body [7]. Hence the frequent administration of drug is required to maintain effective plasma concentration of boldine in blood for longer period of time. Therefore, it is required to develop a novel drug delivery system of boldine, which can enhance the absorption as well as biological activity.

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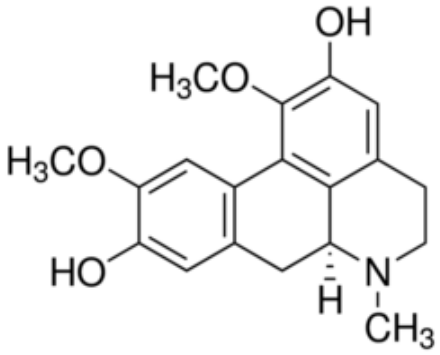

Figure 1. Structure of boldine

So to overcome these problems and make more effective herbal therapy, the novel drug delivery system for herbal drugs (NHDDS) have been developed. Many of the novel approaches have been utilized to improved bioavailability of phytoconstituents like nanoparticulate system, lipid based system such as liposomes, niosomes or phytophospholipid complex, microparticulate delivery in the form of micro emulsions or microsphere, modification in chemical structures of parent compounds, pro-drugging and complexation with cyclodextrins [8-9].

The phospholipid molecule have unique structural configuration that resembles human mammalian cell membrane attributed to attain excellent biocompatibility. It also plays a important role as carrier system for those drug or phytocompounds which require sustained/controlled release in vivo because of rapid clearance from body [10].

Hence the phytophospholipid complexation techniques are chemical interaction of natural phyto compound or extract, specially polyphenolic compound with phospholipid molecules containing phosphatidylcholine to form phospholipid compatible molecular complex which may improve the bioavailability and therapeutic potential of a number of poorly absorbed naturally occurring phyto-constituents and their derivatives [11-14].

Thus the present study was undertaken to ascertain the superiority of value added boldine phospholipid complex as drug delivery, over free boldine and its physical mixture in term of hepatoprotective potential in albino wistar rats by carbon tetra chloride induced hepatotoxicity.

2. Material and method-

Chemicals

Boldine drug was procured from Sigma-Aldrich, Mumbai (India). lipid (Phospholipon 90H) was obtained as a gift sample from Lipoid, Ludwigshafen, Germany. All other chemicals and reagents were used of analytical grade.

Preparation of boldine-phospholipid complex-

The boldine phospholipid complex was prepared by refluxing followed by solvent evaporation method. The molar ratio (1:1.5) of boldine to phospholipid was taken in round bottom flask containing 30 ml of alcohol. The mixture was refluxed and clear resultant solution was evaporated under reduced pressure and sufficient amount of n-hexane was added with continuous stirring to obtain the complex as precipitate. The prepared complex was filtered, dried under vacuum for 12 h to remove the traces of solvent and stored in amber colored air tight container for further use [15-16].

Carbon tetrachloride induced hepatoprotective activity:

The *in-vivo* hepatoprotective activity of boldine and its formulations were evaluated by carbon tetra chloride induced hepatotoxicity model. Carbon tetra chloride is used as inducing agent for liver damage in rats. The CCl_4 in liquid paraffin (1:1) administered intraperitoneal route at the dose of 1 ml/kg body weight, once in every 72 hours for 14 days. The hepatoprotective effect of drug and its formulations were compared with standard drug Silymarin at the dose of 25 mg/kg [17, 18]. The boldine dose was selected on the basis of previous research studies [19, 20]. On the other hand the higher dose of boldine elicits the pro-oxidant property [21]. Thus we preferred the lower dose (20mg/kg body. wt) of boldine for current research study.

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Animals-

The albino wistar rats weighing between 150–200 g were used and the animals were housed under standard conditions, room temperatures 28^0C and a 12 hrs light: dark cycle and 45-50% RH. They were provided with food and water *adlibitum.*The experiment was performed prior obtaining the approval from Insitutional Animal Ethics Committee, Department of Pharmaceutical Sciences, Kumaun University, Bhimtal Campus, Nainital, Uttarakhand and the experimental protocol for in vivo studies fulfill the requirements of national guidelines of CPCSEA.

Experimental protocol-

Rats were divided into six groups containing six animals each.
Group I- (Normal Control): This group received only vehicle.
Group II- (Disease Control): received a mixture of carbon tetra chloride in liquid paraffin (1:1) (1.0 ml/kg i.p.) once in every 72 hours for 14 days.
Group III- (Standard Control): received Standard drug silymarin (25 mg/kg) orally for 14 days and carbon tetrachloride in liquid paraffin (1:1) (1.0 ml/kg i.p.) once in every 72 hours for 14 days.
Group IV- received boldine (20 mg/kg p.o) for 14 days and carbon tetra chloride in liquid paraffin (1:1) (1.0 ml/kg i.p.) once in every 72 hours for 14 days.
Group V- received boldine PM (dose equivalent to (20 mg/kg of boldine p.o) for 14 days and mixture of carbon tetra chloride in liquid paraffin (1:1) (1.0 ml/kg i.p.) once in every 72 hours for 14 days.
Group VI-This group received boldine phospholipid complex (dose equivalent to (20 mg/kg of boldine p.o) for 14 days and mixture of carbon tetrachloride in liquid paraffin (1:1) (1.0 ml/kg i.p.) once in every 72 hours for 14 days.

Collection of serum and Assessment of hepatoprotective activity-

After 24 hours of administration of last dose of carbon tetra chloride, the animals were anaesthetized under light ether and blood samples were withdrawn by puncturing the retro-orbital plexus. Further blood sample was collected and allowed to stand for 30 min at 37°C to coagulate. Serum was separated by centrifugation process at 2500 rpm for 15 min at room temperature for further estimation of hepatic biochemical parameter like serum enzymes aspartate aminotransferase (AST,), serum alanine aminotransferase (ALT,) [22], serum alkaline phosphatase (ALP) [23] , and total bilirubin [24] by using liver biomarker enzyme assay kit.

Histopathological studies-

The visual assessment of the influence of different treatments on CCL_4 live damage was analyzed by histopathological studies on rat liver tissues. After killing the animals, the liver was isolated and preserved in buffered formalin solution. Further the liver was serially sectioned, staining with haematoxylin and eosin and examined microscopically.

Statistical analysis:

For the liver function test, the data were expressed as mean ± SEM. The statistical significance was determined one way analysis of variance (ANOVA) followed by Dunnett's test. The p values less than 0.05 and 0.01 were considered statistically significant.

3. Result and discussion

Hepatic damage caused by carbon tetra chloride is a widely used model for the assessment of hepatoprotective potential of phytochemical drugs or herbal extract [25]. Firstly the CCl4 is metabolized by the cytochrome P450 to produce the trichloromethyl free radical ($CCl3^+$), and further a highly reactive trichloromethyl peroxy radical ($CCl3OO^+$) was produced by reaction with oxygen. This trichloromethyl peroxy radical ($CCl3OO^+$), covalently binds to cell membranes or organelles which leads to the lipid peroxidation and disturbs Ca2+ homeostasis, and overall results in cell death [26-28].

The serum marker enzymes, like ALT, AST and ALP are an important tool for assessment of liver function. Due to lipid peroxidation, hepatic cell plasma membrane is damaged and the liver enzymes present in cytosol are released into the blood. Therefore the quantitative estimation of serum enzymes is a useful method to know the extent and type of hepatocellular damage [29].

4. Estimation of Bio chemical parameters

The liver damage caused by administration of CCl_4 to the animals resulted in significant increase of liver marker enzymes such as ALT, AST, ALP and serum total bilurubin. The animal groups treated with boldine, boldine

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phospholipid -physical mixture (BOL-PM), boldine phospholipid complex formulation (BOL-PC) and standard drug silymarin showed the reduction in elevated enzyme level. The results of different biochemical parameters are given in Table 1, and Figure.2, respectively.

The result obtained on the basis of biochemical evaluation showed that the serum bilirubin, ALT, AST and ALP levels significantly increased in CCl_4 treated group of animals, as compared to normal control groups. The treated group with standard silymarin reduces the elevated level of serum bilirubin, ALT, AST and ALP as compared to disease control groups of animals at $p< 0.05$ and 0.01.The complex formulation BOL-PC was found to be significantly reduced the elevated serum bilirubin, ALT, AST and ALP levels in comparison to that of pure boldine and its physical mixture form, when it compared to disease control groups of animal at $p< 0.05$ and 0.01. So the overall result clearly indicated that Silymarin and phospholipid complex formulation were able to restoration of elevated liver marker enzymes and reversal of liver injury caused by CCl4.

Table no 1. Effect of different treatments on serum ALT, AST, ALP levels (IU/ml) and Total bilurubin (TB) content (gm/dl)in rats.

GROUPS	TREATMENTS	ALT(IU/ml)	AST(IU/ml)	ALP(IU/ml)	TB (gm/dl)
I	Normal Control	46.39 ± 3.55**	79.89 ± 4.08**	127.37 ± 2.79**	0.51±0.028**
II	Disease Control (CCl_4 treated)	163.25 ± 4.99	157.83 ± 4.26	194.57±2.14	1.74±0.023
III	Standard Control (Silymarin)	53.57± 4.54**	89.27 ± 3.76**	134.57±4.75**	0.63±0.025**
IV	Boldine	144.87 ±3.14*	141.48 ± 2.57*	177.48±2.74*	1.58±0.053*
V	Boldine -PM	85.57±3.04**	117..09 ± 2.92**	149.46 ±4.02**	1.02±0.034**
VI	BOL-PC (formulation)	61.18±3.79**	102.79 ± 2.79**	142.49± 4.72**	0.69±0.033**

The data are expressed as mean ± SEM (n=6). The statistical significance was determined by one way ANOVA followed by Dunnett's test.* $p< 0.05$ and ** $p< 0.01$ as compared to disease control group.

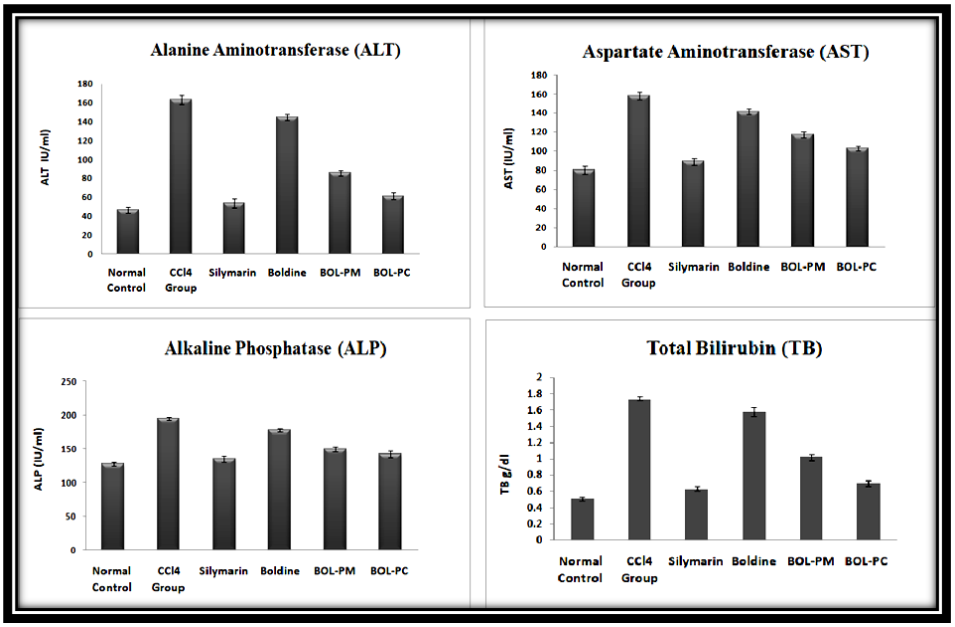

Figure 2. Effect of different treatments on serum ALT, AST, ALP and Total bilurubin level in rats.

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5. Histopathological studies

The histological investigation of liver tissues through electron microscopy showed that in control group of animal, the normal architecture of hepatic cells was observed. In CCl_4 treated group showing hepatic degeneration with necrosis, vacuolar degeneration, space formation, with inflammatory cells. The standard group showing complete regeneration where as boldine drug showing mild regeneration of the hepatocyte, lesser inflammatory cell, and vacuolization but higher level of regeneration was observed in case of physical mixture as compared to pure boldine alone. The complex formulation (BOL-PC) showing the complete regeneration and hepatocyte are found to be almost normal. The morphologic change caused by carbon tetrachloride in a dose dependent manner was given in figure 3.(A-F).

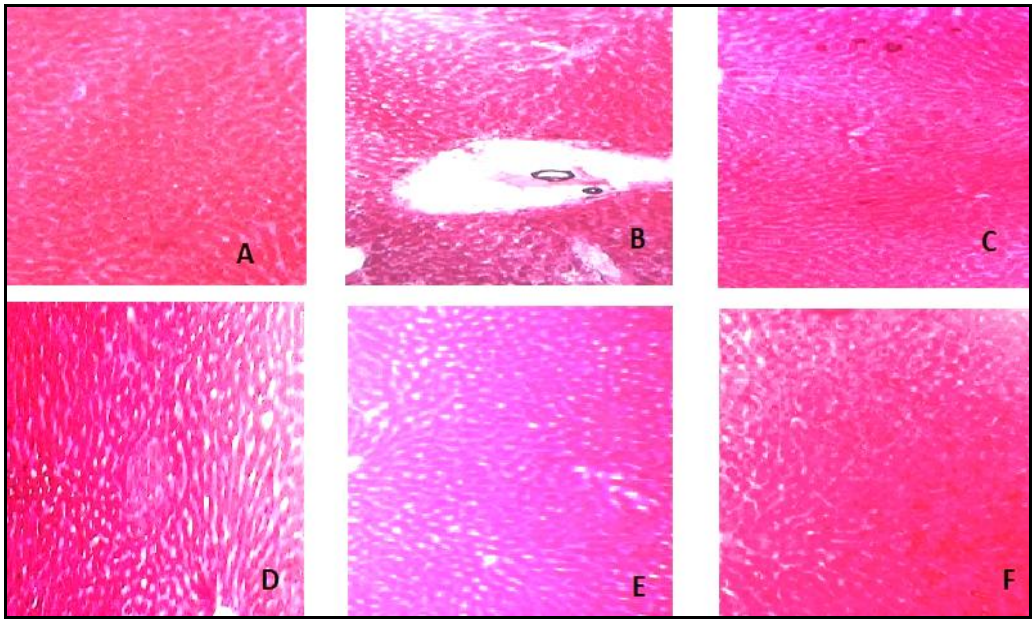

Figure 3. Histopathological Changes of Hepatic Tissue

A. Control group (administered vehicle daily) showing the normal architecture of hepatic cells.
B. Disease control, CCl_4 treated group showing hepatic degeneration with necrosis, vacuolar degeneration, Space formation and inflammatory cells.
C. CCl_4 + Silymarin showing complete regeneration of the hepatocytes .
D. CCl_4 + Boldine showing reduced inflammation, lesser degree of fatty degeneration and reduced hepatic cells necrosis.
E. CCl_4 + BOL-PM showing lesser degree of inflammation and lesser degree of hepatic cell necrosis, higher level of fatty regeneration..
F. CCl_4 + BOL-PC showing the almost complete regeneration and hepatocyte were almost normal.

6. Conclusion

The present research study was to develop boldine phyto phospholipid complex formulation to enhance the biological activity of boldine. The result revealed that free boldine reduces the elevated serum enzyme level in rats after ccl_4 treatment. The complextation of boldine with phospholipid molecule showed marked decrease in the elevated serum enzyme level, resulted in reversal of hepatic injury caused by CCl_4 as compared to free drug boldine at the same dose level. So the conclusion is withdrawn from the present research that the boldine phospholipid complex (BOL-PC) formulation showed better therapeutic activity as compared to drug molecule alone. This enhanced therapeutic potential of boldine as in complex form may be because of better *in –vivo* absorption of drug molecules from the complex.

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Conflict of interests there is no conflict of interest between the authors.

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