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"Formulation Development and Evaluation of Directly Compressed Polyherbal Tablets for the Management of Infections Caused by Helminthes"

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Abstract

The current study was based on the investigation protective effect of prepared direct compressed poly-herbal tablet formulation in hepatotoxicity using in-vivo animal models. Hepatotoxicity in rats was induced-by an injection of Cisplatin through intraperitoneal route of administration in rat at a dose of 7 mg/kg and DCPT at the dose 100, 300 and 500 mg/kg was injected for assessment of protective properties. AST, ALP, ALT, TB, HP, oxidative stress, pro-inflammatory cytokine, and histological alteration were measured to find the therapeutic activity of DCPT. Rats treated with only group shows the increased levels of Proinflammatory cytokines, altered Oxidative stress, AST, ALP, ALT, TB, HP were increased because of Cisplatin induced hepatotoxicity. After the treatment of DCPT for 7 days the level of cytokines and oxidative stress were reduced in test control group as compared to disease control group. The inhibitory action of DCPT on inflammatory responses was achieved by reducing the expressions of cytokines. Cisplatin-treated rats showed the altered structure of liver tissue; meanwhile, DCPT normalizes the structure of kidney tissue. From our findings, we conclude that DCPT has therapeutic potential in liver complications like hepatotoxicity, by inhibiting the production of Proinflammatory cytokines (i.e. TNF-α) and Oxidative stress as well as protect the rats from hepatotoxicity.

Keyword: Cisplatin; oxidative stress; Cytokine; ALT; ALP; Histopathology.

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Introduction

Polyherbal formulations are mainly applicable for the treatment as well prevention of different disease conditions [1]. Herbs in traditional medicine system have been used from a long period as per special theories as well concepts like Ayurveda, Unani as well Siddha that are accepted by several countries [2, 3]. Modifications in herbal remedies includes modifications in shape, dosage form, mode of route administration, ingredients, method for formulation and medical indications. For the safety as well efficacy of the herbal remedies needs to national regulatory requirements. Imported-products with herbal remedy base covers all imported herbal medicines including raw materials and products [4]. Imported herbal medicines must be registered and marketed in the countries of origin [5]. Cisplatin can induce hepatotoxicity after having administered at high doses [6]. Oxidative stress appears to play an important role in cisplatin induced hepatotoxicity. The mechanism that based on the alteration of liver function due to cisplatin, was not revealed. Researchers accept the true as ROS destruct the membrane lipids, additionally the other cellular components as an imperative influence in Cisplatin induced liver toxicity. Cisplatin causes the variations in GSH (glutathione) as well Lipid Peroxidation (LPO). In Cisplatin induced Liver toxicity Initial target is Mitochondria, a dysfunction in mitochondria leads to loss in mitochondrial protein SH, that obstructing the absorption of calcium as well decreasing membrane potential in mitochondria [7]. Hydrogen peroxide (H2O2), superoxide anion (O2•−) and hydroxyl radicals (OH•) are generated during oxidative stress [8]. The other radical producing mechanism is nitric oxide (NO) which reacts with O2•− to form peroxy-nitrite, a toxic agent to the cellular components. Endogenous antioxidants that prevent the oxidative damage are involved in the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX)[9]. The aim of present study is to evaluate hepato-protective activity of prepared direct compressed polyherbal tablet.

Material and Methods

Preparation and composition of polyherbal tablet

Polyherbal tablet was prepared by using direct compressed methods. The composition of tablet was given in table 1

Table 2: Composition of polyherbal tablet

Sr.No.	Composition	Label Claim	mg/ tablet	Category	Qty Per Batch (g)
1	Standardized Extract 95% Curcuminoids	500mg	500.000	Anti-Anthelmintic Extract	500.000

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2	Ginger Extract	l00mg	100.000	Anthelmintic Extract	100.000
3	Lactose		50.000	Diluent	50.000
4	Microcrystalline Cellulose (PH101		102.000	Diluent	102.000
5	Polyvinylpyrrolidone (PVP K-30)		20.000	Anti-Oxidant	20.000
6	Lycopene 10% Powder DC	2mg	20.000	Antioxidant Extract	20.000
7	Croscarmellose Sodium		50.000	Disintegrant	50.000
8	MCC (Direct Compress)-102		137.000	Diluent	137.000
9	Colloidal Silicon Dioxide (Aerosil-200)		8.000	Glident	8.000
10	Talc		8.000	Lubricant	8.000
11	Magnesium Stearate		5.000	Lubricant	5.000
	Average weight Of Uncoated Tablets		1000.000		1000.000

Animals

In this experiment, adult male Wistar rats were used. Rats were fed a standard diet and water throughout the experiment. Animal were kept at standard laboratory condition, with light and dark cycle of 12 hours each. Experiment was performed according to guidelines provided by CPCSEA, New Delhi. Experimental protocol was approved by IAEC. Protocol approval No-
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Drugs and chemicals

Cisplatin was purchased from local pharmacy vender, ALP,AST, ALT, TB, HP kits were procured from bioscience. Cytokine kits were purchased from bioscience. All other chemical for experiment was analytical grade.

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Experimental Protocol

Thirty adult male Wistar rats weighing about 180-220 g were divided into five experimental groups, each group containing six rats.

Group 1- Normal (received vehicle)

Group 2- Diseases control (Received Cisplatin- 7 mg/kg Intraperitonialy)

Group 3- C100 (Received Cisplatin- 7 mg/kg Intraperitonealy + DCPT 100 mg/kg)

Group 4- C300 (Received Cisplatin- 7 mg/kg Intraperitonealy + DCPT 300 mg/kg)

Group 5- C500 (Received Cisplatin- 7 mg/kg Intraperitonealy + DCPT 500 mg/kg)

Cispaltin (7 mg/kg) was injected on 1st day by intraperitoneal route of study in all groups except normal. Group 3,4 and 5 orally administered DCPT at dose 100, 300 and 500 mg/kg/body weight respectively. After last treatment of DCPT, animals were anesthetized by light ether anesthesia and blood sample was collected from each animal for separation of serum. Finally rats were scarified and liver tissues were isolated for Histopathology and liver tissue homogenate in PBS 7.5 pH. Homogenate was prepared by centrifuging at 10000 rpm for 15 min. supernatant was collected and stored at -60^{o}C for further analysis[10]

Measurement of body weight and relative liver weight

Body weight of each animal was measured on 0 day and 24 hour after last treatment. Liver weight was taken after scarifying the animals and percentage relative liver was calculated to examine change in liver weight of animals[11].

Serum aminotransferase levels

To examine the damage on hepatocytes cell membrane, the activity of the liver enzymes markers was determined. Aspartate transaminase (AST) and Alanine transaminase markers (ALT) were measured according to method described by earlier and alkaline phosphatase (ALP) was estimated [12].

Measurement of oxidative stress

Lipid peroxidation assay

The lipid peroxidation in liver tissue homogenate was measured by using method given by Jain et al based on the measurement of total malondialdehyde (MDA) content as the major product of lipid peroxidation in tissue[13].

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Reduced glutathione level

Glutathione (GSH) levels in liver tissue homogenate were measured by using the modified method of shinde et al., 2021. Briefly, 0.5ml of 10% sulphosalicylic acid was added to a mixture of 0.4ml and 0.6ml of distilled water as homogeneous sediment protein. 0.5 ml of the supernatant of the reaction mixture, 4.5 ml and 0.5 ml of 0.5M Tris buffer of 10mm DTNB were mixed and the absorptions were read at 412 nm immediately. GSH content was calculated by using GSH as the standard and expressed as mmol/ of tissue[14, 15].

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was measured according to methods described by shinde et al., 2021. Finally, absorbance was measured at 560 nm and final concentration was expressed as μg/mg of tissue[16, 17].

Determination of cytokine level

The concentrations of cytokine-like IL-6, IL-1β, and TNF-α in kidney tissue homogenate was determined according to the manufacturer's protocol using ELISA kits. The final concentration was determined by using a standard curve [18, 19]

Histopathology

The liver was fixed in 10% neutral buffered formalin solution and embedded in paraffin. Sections of 4 μm thicknesses were cut by using microtone and stained with hematoxylin and eosin for the evaluation of histopathological analysis and images were taken by using motic camera fitted with light microscope[20].

Statistical analysis

Data obtained from experiment were analyzed by using the Graph pad prism version 7.0. by using one-way ANOVA followed by multiple comparison tests. Results were expressed as means ± standard error (SEM). P-Values < 0.05 were considered as statistically significant.

Result

Effect of DCPT on change in body weight

In this experiment, we measured body weight and liver weight to examine change in body and liver weight. Body weight of cisplatin treated animals shows decreased throughout the treatment periods as compared to normal ($P< 0.05$). Rats treated with DCPT 100 and 300 mg/kg shows same pattern of increase in body weight like normal rats. DCPT treated with 100 mg/kg has no effect on change in body weight as compared to Cisplatin group ($P< 0.05$). liver weight was increased Cisplatin treated group as compared to normal group ($P< 0.05$). DCPT treated with 500

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mg/kg showed most prominent effect on liver weight as compared to control group given in table 2.

Table 1: Effect of DCPT on change in body weight and relative liver weight

Group	Body weight of 0 day	Body weight of 10^{th} day	Relative liver weight/ 100 g of body weight
Normal	168.3 ± 1.65	170.8 ± 3.01	1.92 ± 0.02
Control	171.8 ± 1.70	163.8 ± 2.21	3.64 ± 0.17
C100	168.3 ± 1.88	165.0 ± 2.27	2.95 ± 0.08
C300	171.8 ± 1.70	174.0 ± 2.12	2.41 ± 0.03
C500	168.8 ± 0.85	173.0 ± 1.08	2.07 ± 0.05

Effect of DCPT on serum aminotransferase levels

Rats were treated with cisplatin that had markedly elevated level of the serum ALT, AST and ALP compared to the normal Rats, induction of liver damage. The effects of the treatment with different doses of DCPT on serum ALT, AST and ALP activities after the injection of cisplatin are shown in **figure 1.** The treatment with DCPT was continued for seven days after receiving the cisplatin reduced serum activities of ALT, AST and ALP. The maximum decrease in serum ALT, AST and ALP activities was observed in rats treated with 500 mg/ kg b.w. of DCPT.

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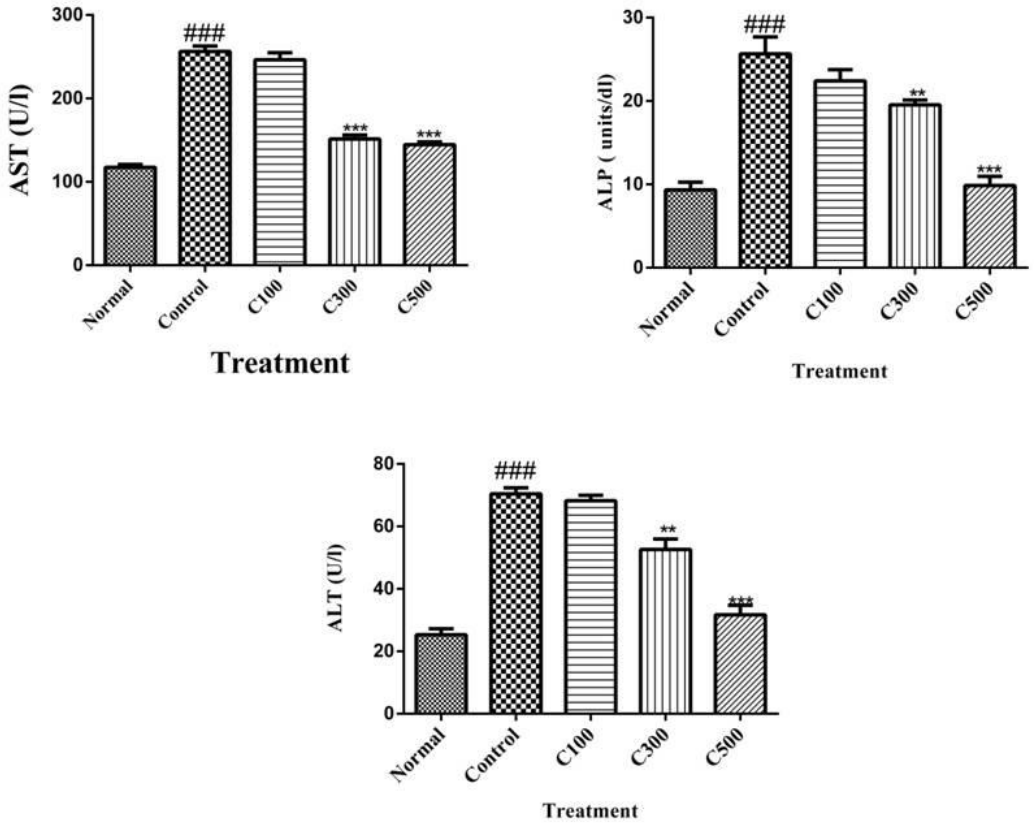

Figure 1- Effect of DCPT on serum aminotransferase levels

Effect of DCPT on serum total bilirubin and Hydroxyproline level

Rats treated with toxic dose of Cisplatin showed dramatically increases in total bilirubin and Hydroxyproline level as compared to the normal group ($P < 0.05$). Treatment with DCPT 100 and 500 mg/kg shows decrease in total bilirubin, hydroxyproline level, when compared with control group. 500 mg/kg DCPT treated group showed highest effect on total bilirubin and hydroxyproline level as compared to the normal group shown in figure 2.

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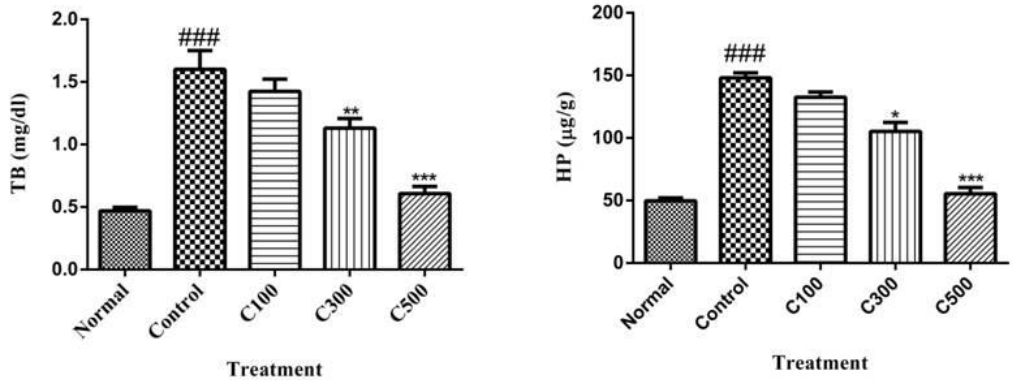

Figure 2- Effect of DCPT on serum total bilirubin and Hydroxyproline level

Effect of DCPT on anti-oxidant enzyme activity

Tissue levels of MDA were significantly high in the cisplatin-treated group in comparison with the control group. On the other hand, DCPT reduced the level of MDA, and the best dose was 15 mg/ kg. GSH and SOD levels in the liver of the treated rats with only one injection of cisplatin showed a significant reduction compared to the normal group. The treatment with DCPT after the injection of a single dose of cisplatin significantly inhibited the reduction of the GSH and SOD level in the liver tissue, and the maximum effective dose was of 500 mg/ kg of DCPT shown in figure 3.

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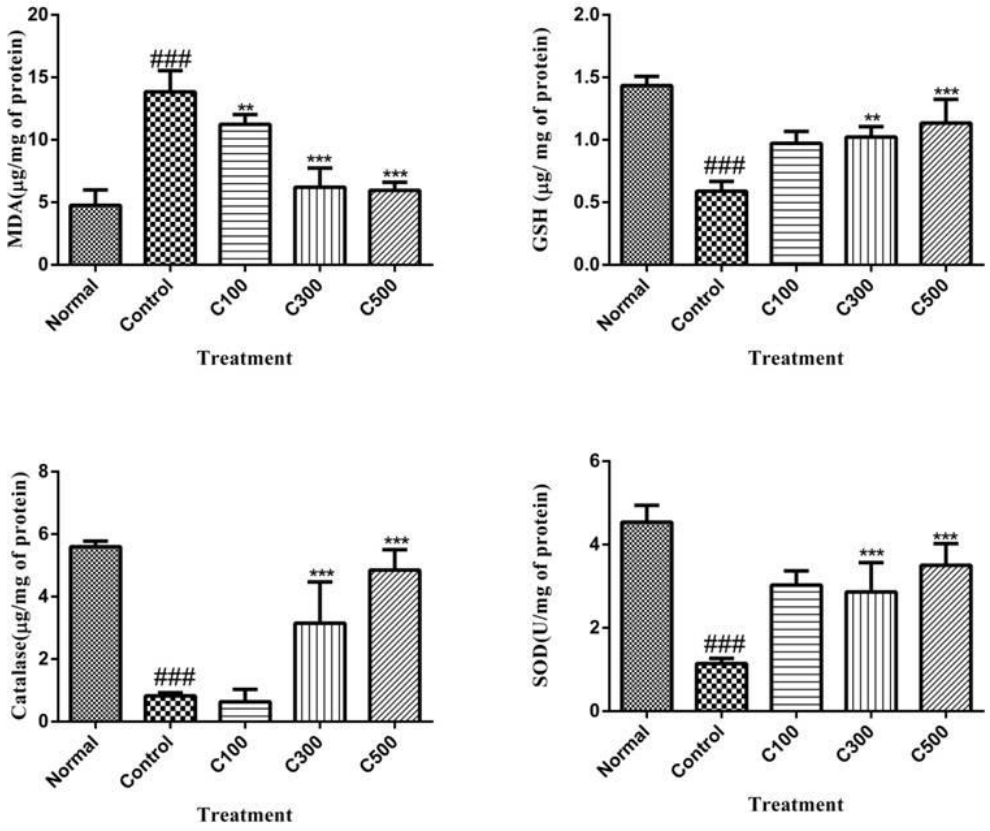

Figure 3- Effect of DCPT on anti-oxidant enzyme activity

Effect of DCPT on serum cytokine levels

The Effect of DCPT was determined on rise in pro- inflammatory cytokine expression of IL-6, TNF-alpha and IL-1β in the serum. It was found that increased level of pro-inflammatory cytokines in toxic dose of cisplatin treated rats at the end of study as compared to normal group. Treatment with DCPT at dose 300 and 500 mg/kg for 7 days significantly decreased the level of IL-6, TNF-alpha and IL-1β levels almost to the normal levels indicating that DCPT exerts an inhibitory effect on the cytokine release shown in figure 4.

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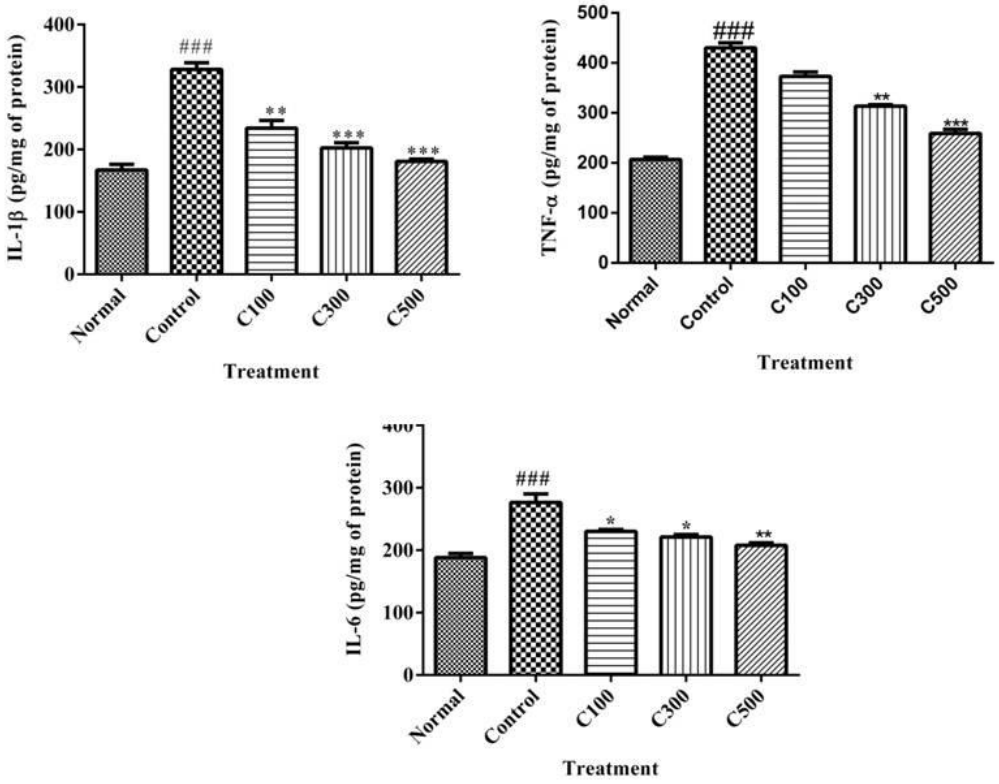

Figure 4- Effect of DCPT on serum cytokine levels

Histopathological finding

The microscopic study of the liver tissue sections from the control group showed large polygonal cells with eosinophilic cytoplasm and round natural core arranged around the central vein and several hepatic sinuses. In contrast, the second group received only cisplatin, which revealed obvious histological abnormalities including severe fatty change, central venous sinus congestion, and necrosis mainly around the central vein. While groups received 300 and 500 mg/ kg b.w. of DCPT along with cisplatin, it showed a regression of the fatty changes in the liver cell cytoplasm. A greater improvement and the scattering of the apoptotic cells were seen at doses of 500 mg/ kg b.w. of DCPT.

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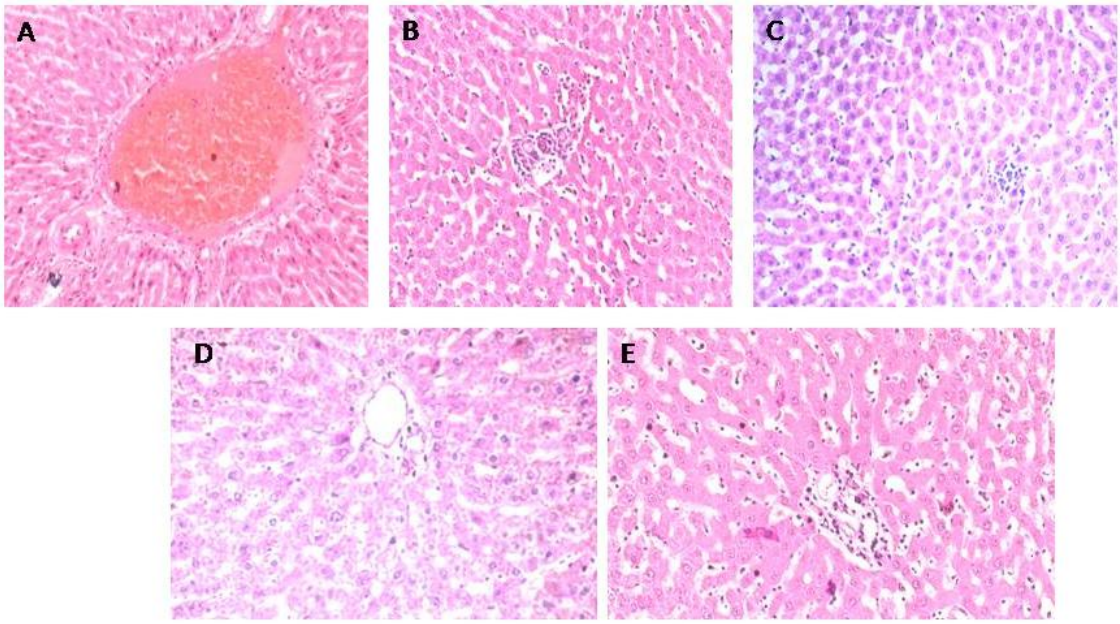

Figure 5: Effect of DCPT on histological finding of liver (A- Normal, B- Control group, C-C100, D-C300, C-500)

Discussion

Cisplatin was widely used as an antineoplastic agent but its therapeutic potential is limited due to hepatotoxicity effects. Near about 40% of patients suffering from renal dysfunction after a single dose of Cisplatin treatment[21]. The protective effect of DCPT on Cisplatin-induced nephrotoxicity and its molecular mechanism has not been proved yet.

In this study, we investigated the potential protective effect of polyherbal tablet against cisplatin-induced hepatotoxicity through experimental models.

AST, ALP, ALT, TB and HP constitute hepatic injury markers and hence, serve as diagnostic markers for cell injury. These markers are likely to be released into the blood from damaged tissue when the cell membrane becomes more permeable or get ruptured. Damaged hepatic tissue is the predominant source of elevated levels of these hepatic injury markers[22]. Cisplatin injected rats showed an increased level of AST, ALP, ALT, TB and HP. Treatment with DCPT inhibited the increase in the level of liver injury markers. Several types of research have confirmed that ROS-caused oxidative damage is involved in the pathogenesis of hepatotoxicity. In this study, we recognized the importance of oxidative stress in the improvement of cisplatin-induced hepatotoxicity by DCPT. Those phenomena were effectively reversed with the help of DCPT treatment for fseven days. Similarly, to oxidative stress, inflammation performs an essential component within the pathogenesis of cisplatin-prompted hepatotoxicity.

TNF-α is a prototypical inflammatory mediator, which promotes cytokines generation and irritation reaction via stimulating neutrophils and macrophages, in the long run ensuing in cell toxicity. Further, IL-1β promotes inflammation, reasons fever, as well as stimulates the development and differentiation of the immune system. The inhibitory effect exhibited by DCPT may contribute to the suppression of the inflammation associated with excessive secretion of TNF-alpha, IL-6, and IL-1β induced by Cisplatin due to loss of liver function.

Conclusion

In conclusion, DCPT at 100, 300, and 500 mg/kg doses exert significant and dose-dependent protective effects against Cisplatin induced hepatotoxicity. From this study we conclude that prepared polyherbal tablet shows protective effect in hepatotoxicity.

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