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Efficacy Study of Livartho against Paracetamol Induced Hepatotoxicity in Adult Sprague Dawley Rats

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Abstract

Liver disease is a worldwide problem. It is an exposed to many kinds of xenobiotics and therapeutic agents. The purpose of this study was to evaluate therapeutic effect of Livartho drug in paracetamol induced hepatic damage Sprague dawley rats. Paracetamol at the dose level of 900mg/kg body weight administered through intraperitoneal route once daily for one week to produced liver damage in rats as manifested by the significantly decrease body weight and feed consumption along with significantly increase liver organ weight and serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin, cholesterol and triglyceride and decrease the total protein and albumin levels along with significantly increase myloperoxidase enzyme activity, lipid peroxidation and protein carbonyl levels as compared to control group. The antioxidant parameters such as reduced glutathione (GSH), oxidized glutathione (GSSG), total thiol and GSH/ GSSG ratio were also found lowered in paracetamol treated group as compared with control. After treatment with Livartho drug (206.6 mg/kg body weight) once daily for 16 days to paracetamol treated rats shows significantly lowered the afore mentioned biochemical parameter whereas protein and albumin levels were increased along with significant increased above mentioned antioxidant enzymatic parameters. The results of this study strongly indicate that Livartho drug is most effective medicine which has potent hepatoprotective action and free radical scavenger against paracetamol induced hepatic damage in Sprague dawley rats.

Keywords: Livartho; Paracetamol; Hepatic damage; Hematological; Biochemical; Antioxidant parameters

Abbreviations: PCM- Paracetamol; MDA- malonaldialdehyde or lipid per-oxidation; GSH-reduced glutathione; GSSG- oxidized glutathione; MPO- myeloperoxidase; TSH- total thiol; PC- protein carbonyl; SGOT- Serum glutamic oxaloacetic transaminase; SGPT-serum glutamic pyruvic transaminase; LDH-lactate dehydrogenase; TGA-triglyceride

Introduction

Liver disease has become one of the serious health problems [1]. It is an exposed to many kinds of xenobiotics and therapeutic agents. Moreover the rapidly growing morbidity and mortality from liver disease are attributable to the increasing number of chemical compounds and environmental pollution. Liver plays many essential roles in maintaining the normal physiology process. It is also a vulnerable target of many drugs or other chemicals because the liver is involved in complex metabolism. A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage [2]. Therefore the liver is one of the primary organs tested in drug safety evaluations. Most of hepatotoxic chemicals cause damage liver cells mainly by inducing lipid peroxidation and oxidative stress in liver. Paracetamol (Acetaminophen) is an analgesic, antipyretic drug and metabolized by cytochrome P450 system, which leads to the formation of N-acetyl-p-benzoquinoneimine (NAPQI) [3,4,5]. Paracetomol is a powerful inducer of cytochrome P450. The action of the P450 system on paracetomol produces a highly reactive quinoneimine that combines to the sulfhydryl groups of proteins. The toxicity occurs because of its reactive metabolite, NAPQI. NAPQI exerts its toxicity primarily *via* its oxidative effect on cellular proteins. The inactivation of proteins leads to death of liver cells. Over dose of paracetamol in both animals and man has been shown to produce hepatic necrosis, ascribed to a toxic metabolite of the parent drug [6].

Therefore it is necessary to search alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful safety and efficacy [7]. Traditional systems of medicine like Ayurveda, Siddha, Unani have a major role in the treatment of liver ailments [8]. In the recent years, importance is being given to Ayurvedic polyherbal formulations due to their effective therapeutic action and lack of side effects. Now a days, there is various marketed formulation available for the treatment of liver disorders such as Liv-52, Liv -42 and Livercure [9,10,11].

Livartho is a poly herbal formulations which consist of 10 active constituents of medicinal plants *viz*, *Andrographis paniculata*, *Cichorium intybus, Tephrosia purpurea, Solanum nigrum, Phyllanthus amarus, Tinospora cordifolia, Eclipta alba, Berberis aristata, Piper longum* and *Emblica officinalis*. These ingredients have been reported to possess hepatoprotective effect and antioxidant properties [12,13]. So the aim of present investigation was to determine the therapeutic efficacy of new poly herbal medicine Livartho in paracetamol induced hepatic damage SD rats.

Materials and Methods

Chemicals

All biochemicals such as reduced glutathione, oxidized glutathione, 5,5' dithionitrobis 2-nitrobenzoic acid (DTNB) used in the present study were procured from Sigma, St. Louis, MO, USA. Other biochemicals

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& chemicals purchased locally were of Himedia and analytical grade. Ketamine hydrochloride was purchased from Samarth Life Science Pvt. Ltd. Mumbai. Other biochemical kits were procured from Erba Diagnostics Mannheim Gmb, Germanny.

Drug

Livartho drug was obtained from sponsor Lavanaya Pharmacy Pvt. Ltd. Lucknow for the efficacy study. The drug has 10 major active constituents *Andrographis paniculata*, *Cichorium intybus, Tephrosia purpurea, Solanum nigrum, Phyllanthus amarus, Tinospora cordifolia, Eclipta alba, Berberis aristata, Piper longum* and *Emblica officinalis*. The purity and chemical analysis is not part of this study and it is responsibility of sponsor. The drug concentration was 500 mg/capsules.

Animals

The study was performed under the GLP condition. Animals were obtained from the animal house facility of Central Drug Research Institute Lucknow U.P India. The experiment was carried out after getting approval from the Institutional Animal Ethics Committee (IAEC). The IAEC number for this study was IAEC/L/2014-05. The study was performed on female SD rats weighing 210±5 g, housed in polypropylene cages in an air-conditioned room with temperature maintained at 25 ± 2 °C and 12 h alternating day and night cycles. The animals were allowed standard rat chow diet and sterile distilled water.

Experimental procedure

Total thirty female SD rats (weighting 210±5 g) were selected and randomized on the basis of individual body weight. These animals were divided into 3 groups and each group contain 10 animals

- **G I** (n=10) control and treated with distilled water
- **G II** (n=10) treated with paracetamol (PCM) at dose level 900 mg/kg bodyweight
- **G III** (n=10) treated with paracetamol (PCM)+ Livartho at dose 206.6 mg/kg body weight

Hepatic damage was induced *via* intraperitoneal route administration of PCM for one week. Hepatic damage was confirmed by increase the levels SGOT, SGPT, LDH and total bilirubin parameters, After increased above parameters, treatment was started with livartho drug for 16 days. Body weight and food intake were recorded daily throughout the experimental periods.

At the end study, overnight fasted animals were scarified by cervical dislocation and blood samples were collected from cardiac puncture into heparinized and non-heparinized vials for measurement of hematological and biochemical examinations. Liver, kidney and spleen organs of each animals were separated, get dried and immediately weight these organs and further used for histopathological examination.

Hematological & biochemical estimation

1.5 ml blood samples were collected into heparinized vials. 300 µl blood samples were used for measurement of hematological parameters; hemoglobin (Hb), White blood cell (WBC), platelet count, Red blood cell (RBC), Pack cell volume (PCV),Mean corpuscular hemoglobin (MCH) and Mean corpuscular volume (MCV) through fully automatic cell counter (Mindray Vector, Model No.BC-2300). Rest part of blood samples were used for preparation of plasma and these samples were used for determination of oxidant and antioxidant enzymatic levels.

The blood samples were collected without any anticoagulant and were allowed to clot for 10-20 minutes at room temperature. The blood was centrifuged at 2500 rpm for 20 minutes at 30°C. The obtained serum was stored at 4°C for the estimation of SGOT, SGPT, LDH, Total billirubin, albumin and total protein parameters. These estimations were done according to the standard procedures given along with the kits purchased.

Anti-oxidant parameters

Reduced glutathione (GSH) measurement: Reduced glutathione was assayed by the method of Ellman [14]. 0.5 ml plasma sample was mixed with equal amount of 5% (w/v) TCA reagent and kept for 10 min at room temperature, proteins were precipitated and the filtrate was removed carefully after centrifugation at 3500 rpm for 15 minutes. Filtrate (0.25 ml) was taken and added to 2.0 ml of Na_2HPO_4 (4.25%) and 0.04 ml of DTNB (0.04%). A blank sample was prepared in similar manner using double distilled water instead of the filtrate. A pale yellow color was developed and optical density was measured at 412 nm by a spectrophotometer.

Total thiol determination: Total thiol content was analyzed by the method of Hu [15]. Plasma samples (0.2 ml) were taken in test tubes and 0.6 ml of Tris EDTA buffer (Tris 0.25 M; EDTA 20mM; pH 8.2) was added, followed by 40 µl of 10 mM of 5,5'dithionitrobis 2-nitrobenzoic acid (DTNB in methanol) and the total reaction volume of 4.0 ml was obtained by adding 3.16 ml of methanol. All test tubes were sealed and the color was developed for 15–20 min, followed by centrifugation at 3000 g for 10–15 min at room temperature. The absorbance of the supernatant was measured at 412 nm wavelength.

Oxidative stress parameters

Measurement of myeloperoxidase (MPO; EC 1.11.2.2): Myeloperoxidase was determined by O-dianisidine method with slight modification Kurutas **et al.,** [16]. The assay mixture consisted of 0.3 ml of sodium phosphate buffer (0.1 M; pH 6.0), 0.3 mL of H_2O_2 (0.01 M), 0.2 ml of O-dianisidine (0.02 M) (freshly prepared in distilled water) and final volume of 3.0 ml was obtained with distilled water. The reaction was started by addition of 0.1 ml samples. The change in absorbance was recorded at 460 nm wavelength. All measurements were carried out in duplicate. One unit of enzyme activity was defined as the increase in absorbance of 0.001 $min^{-}1$.

Determination of protein carbonyl content: The protein carbonyl content was followed the method described by Levine *et al.* [17] with slight modifications. Briefly, two tubes of 0.5 ml plasma sample were taken; one was marked as "test" and the other as "control". 4.0 ml of 10 mM 2,4-dinitrophenylhydrazine (DNPH) prepared in 2.5 M HCl was added to the test sample and 4.0 ml of 2.5 M HCl alone was added to the control sample. The contents were mixed thoroughly and incubated in the dark (room temperature) for 1 hour. The tubes were shaken intermittently every 15 minutes. Then 5 ml of 20% TCA (w/v) was added to both tubes and the mixture left in ice for 10 minutes. The tubes were then centrifuged at 3,500 rpm for 20 min to obtain the protein pellet. The supernatant was carefully aspirated and discarded. This was followed by a second wash with 10 % TCA as described above. Finally the precipitates were washed three times with 4 ml of ethanol: ethyl acetate (1:1, v/v) to remove unreacted DNPH and lipid remnants. The final protein pellet was dissolved in 2 ml of 6 M guanidine hydrochloride and incubated at 37°C for 10 min and absorbance was recorded at 362nm spectrophotometer (Beckman DU 640, Fullerton, CA, U.S.A). Each sample was read against the control sample (treated with 2.5 M HCl). The carbonyl content was calculated from peak absorption

at 370nm using an absorption coefficient (e) of 22,000 $M^{-1}Cm^{-1}$. The protein carbonyl content was expressed as µmole/mg protein.

Measurement of lipid peroxidation level: Free radical mediated damage was assessed by the measurement of lipid peroxidation in the term of malondialdehyde (MDA) formed, essentially according to method of Ohkawa *et al.*, [18]. It was determined by thiobarbituric reaction. The reaction mixture consisted of 0.2 ml samples, 0.20 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of (20%, pH 3.5) acetic acid, 1.5 ml of 0.8% thio barbituric acid (TBA) and 0.6 ml distilled water to see find volume of 4.0 ml. The tubes were kept in boiled water at 95°C for one hr and cooled immediately under running tap water. The amount of 1.0 ml of water was added to and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) and vortexed. The tubes were centrifuged at 3500 rpm for 15–20 minutes. The upper layer was aspirated and optical density was measured at 532 nm. The molar extension coefficient 1.56x 105 was used for calculation.

Histopathology

The liver and renal tissues were dissected out and fixed in the 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin wax. The sections, which were 5-6 mm thick, were then prepared using rotary microtome (Leica RM 2125 RTS, Singapore) and stained with hematoxylin and eosin dye for microscopic observation of histopathological changes in the liver and renal organs.

Parameters	Group I	Group II	Group III
Body weight (g)	224.75 ± 29.40	159.6 ±13.11^{a}	202.5 ± 31.81^{b}
Feed weight (g)	60.71 ± 7.77	49.2 ± 5.76^{b}	63.86 ± 8.10^{a}
Organs weight (g)			
Liver	5.81 ± 0.50	8.50 ± 0.56^{a}	6.15 ± 0.21^{a}
Kidney	1.32 ± 0.09	1.07 ± 0.04^{a}	1.25 ± 0.15^{b}
Spleen	0.591 ± 0.03	0.724 ± 0.14^{b}	0.600 ± 0.08^{c}
Relative organ weight (%)			
Liver	2.63 ± 0.52	5.54 ± 0.42^{a}	3.05 ± 0.35^{a}
Kidney	0.73 ± 0.15	0.67 ± 0.05^{ns}	0.72 ± 0.11^{ns}
Spleen	0.27 ± 0.05	0.45 ± 0.07^{a}	0.3 ± 0.10^{b}

All data are mean ± SD of each group of 10 animals. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test are used to determine statistical difference between control (group I) *vs* paracetamol (PCM) induced hepatotoxicity group(group II) and paracetamol (PCM) induced hepatotoxicity group (group II) *vs* livartho treated group (group III). Where a; p<0.0001(highlysignificant) b; p<0.001(significant) c; p <0.01 (significant). d; p<0.05 (significant). The p-values >0.05 were considered statistically non-significant.

Table1: Status of body weight (g), feed intake (g), organ weight (g) and relative organ weight (%) in PCM induced hepatic damage and Livartho treated groups.

Parameters	Group I	Group II	Group III
Hb (g/dL)	14.15 ± 3.0	11.68 ± 2.1^{a}	14.88 ± 0.699^{b}
RBC (10^{12}/L)	6.4 ± 0.61	5.30 ± 1.05^{c}	6.31 ± 0.98^{c}
WBC(10^{9}/L)	18.87 ± 1.67	12.53 ± 2.05^{a}	17.0 ± 2.87^{a}
PCV (%)	37.25 ± 2.33	30.2 ± 5.43^{b}	36.75 ± 2.61^{b}
MCH (pg)	23.175 ± 1.32	22.12 ± 0.85^{d}	24.20 ± 1.42^{a}
MCV(fL)	58.45 ± 2.83	57.14 ± 3.86^{Ns}	61.75 ± 1.70^{a}
Platelet (10^{9}/L)	755 ± 53	499.6 ± 159^{a}	736.5 ± 108.4^{a}

All data are mean ± SD of each group of 10 animals. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test are used to determine statistical difference between control (group I) *vs* paracetamol (PCM) induced hepatotoxicity group(group II) and paracetamol (PCM) induced hepatotoxicity group (group II) *vs* livartho treated group (group III). Where a; p<0.0001(highly significant) b; p <0.001(significant) c; p <0.01(significant). d; p<0.05 (significant). The p-values >0.05 were considered statistically non-significant.

Table 2: Status of hematological parameters in PCM induced hepatotoxicity and Livartho treated groups.

Statistical Analysis

All values were expressed as Mean ± SD. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test was used to determine statistical difference between control group I *vs* paracetamol induced hepatic damage group II and paracetamol induced hepatic damage group II *vs* Paracetamol plus livartho treated group III. The **p**-values <0.05 were considered statistically significant.

Results

Effect on physiological parameters

The results shows that a statistically significant (p>0.0001, p>0.001) decreased body weight and feed consumption in paracetamol treated group II as compared to control group I. After treatment with livartho drug for 16 days, these parameters were found to be significant (p>0.001, p>0.0001) increased in paracetamol plus livartho treated group III when compared with paracetamol treated group II. The body weight and feed intake were enhancing almost near to control level (Table 1).

Effect on organ weight and relative organ weight

Liver and spleen organs weight and its percentage of relative organ weights were found significant (p>0.0001, p>0.001, p>0.0001) higher along with significant (p>0.0001) lower the kidney weight and insignificant (p<0.05) changed the percentage of relative organ weight in paracetamol induced group II as compared to control group I. These organs weight and its percentage of relative organ weight were further significant (p>0.0001, p>0.001, p>0.01) recovered by administration of livartho drug after 16 day treatment in livartho treated group III, when compared with paracetamol treated group II and come back almost near to base line level (control) (Table1). In case of kidney, the percentage of relative organ weight was insignificant (p<0.05) changes observed in group III after treatment with livartho drug for 16 days.

Effect on hematological parameters

There were statistically significant (p>0.0001) decreased hemoglobin (Hb), white blood cell (WBC) and platelet count levels in paracetamol induced hepatotoxicity group II as compared with control group after one week administration of paracetamol. These levels were found to be statistically significant (p>0.001, p>0.0001) increased in paracetamol plus livartho treated group III after treatment with drug for 16 days as compared with PCM exposed group II. These levels were almost come near to control group (Table 2). Red blood cells (RBC) and pack cell volume (PCV) were also significant decreased (p>0.01, p>0.001) in group II as compared with control group I after one week induction of hepatic injury by PCM administration. After treatment with livartho drug for 16 days, these above parameters were found to be statistically significant increased (p>0.01, p>0.001) in group III as compared with paracetamol induced hepatotoxicity group II and come back almost near to control group (Table 2). Mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were also found (p>0.05, p<0.05) lowered in group II as compared with control group I. After treatment with livartho drug for 16 days, the MCH and MCV parameters were found to be improved (p>0.0001) in group III as compared to PCM exposed group II and come back near to control (Table 2).

Effect on biochemical parameters

The results of liver function test and some other biochemical parameters of the rats studied are shown in (Table 3) and results

Parameters	Group I	Group II	Group III
SGOT (IU/L)	140 ± 10.61	217.3 ± 55.2[a]	155.8 ± 7.77[b]
SGPT (IU/L)	48.5 ± 3.0	67.5 ± 7.5[a]	57.75 ± 9.9[c]
LDH (IU/L)	403.5 ± 85.9	755.5 ± 156.8[a]	261.75 ± 56.6[a]
Total bilirubin (mg/dL)	0.813 ± .212	1.647 ± 0.2[a]	0.433 ± 0.03[a]
Cholestrol (mg/dL)	61.33 ± .212	78.0 ± 5.58[a]	63.25 ± 8.89[a]
TGA (mg/dL)	66.5 ± 3.59	143.7 ± 50.2[a]	107.75 ± 12.3[c]
Protein (mg/dL)	7.85 ± 0.778	4.5 ± 0.212[a]	7.25 ± 0.778[a]
Albumin (mg/dL)	3.475 ± 0.299	2.02 ± 0.636[a]	3.3 ± 0.212[a]

All data are mean ± SD of each group of 10 animals. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test are used to determine statistical difference between control (group I) *vs* paracetamol (PCM) induced hepatotoxicity group(group II) and paracetamol (PCM) induced hepatotoxicity group (group II) *vs* livartho treated group (group III). Where a; p<0.0001(highly significant) b; p <0.001(significant) c; p <0.01(significant). d; p<0.05 (significant). The p-values >0.05 were considered statistically non-significant.

Table 3: Status of biochemical parameters in PCM induced hepatotoxicity and Livartho treated groups.

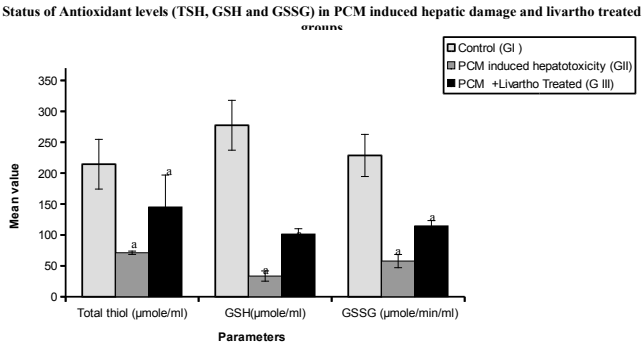

Figure 1: All data are mean ± SD of each group of 10 animals. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test are used to determine statistical difference between control (group I) *vs* paracetamol (PCM) induced hepatotoxicity group(group II) and paracetamol (PCM) induced hepatotoxicity group (group II) *vs* livartho treated group (group III). Where a; p<0.0001 (highly significant) b; p <0.001(significant) c; p <0.01(significant). d; p<0.05 (significant). The p-values >0.05 were considered statistically non-significant.

showed that serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH) levels of rats treated with paracetamol (group II) were found to be significantly (p>0.0001) higher than control group. In contrast, the rats treated with livartho drug for 16 days, the SGOT, SGPT and LDH levels had significantly (p>0.001, p>0.0001, p>0.0001) lowered in group III when compared with the group II and these levels were come back near to control group. Total protein and albumin levels were also found significantly (p>0.0001) lowered along with significant (p>0.0001) increased total bilirubin level in group II as compared with control group I. After treatment with livartho drug for 16 days, these parameters were significant (p>0.0001) improved in livartho treated group when compared with group II and the levels were come back near to control group.

Total cholesterol and triglyceride (TGA) levels were also found statistically (p>0.0001) significant higher in paracetamol exposed group in comparison to control group.When paracetamol plus livartho treated animals (group III) treated with livartho drug for 16 days treatment, these parameters were found significantly (p>0.0001, p>0.01) lowered in group III in comparison to group II and these parameters were almost come near to control group.

Effect on antioxidant levels

The results showed that there were a significantly (p>0.0001) decreased total thiol (TSH) , reduced glutathione (GSH) and oxidized glutathione (GSSG) antioxidant levels in paracetamol hepatic damage induced group II in comparison to control group I. When livartho drug treated for 16 days treatment, these anti-oxidant levels were found significantly (p>0.0001) increased in paracetamol plus livartho treated group III as compared to paracetamol induced group II and these parameters were further come back to control group (Figure 1). A highly significant (p>0.0001) decreased the GSH/GSSG ratio in group II as compared with control group after one week induction of hepatic damage by administration of paracetamol. After treatment with livartho drug for 16 days, the ratio was significant (p>0.01) enhanced in paracetamol plus livartho treated group when compared with paracetamol induced group and ratio was reached almost near to control group (Figure 3).

Effect on oxidative stress levels

Malondialdehyde (MDA) level is widely used as a marker of free radical generation in the term of lipid peroxidation injury of the liver when treated with paracetamol. The oxidant enzymes are also enhances along with protein oxidation during liver tissue injury caused by PCM. So in the study, the results showed that a significant (p>0.0001) increased malonaldialdehyde and protein carbonyl levels along with increased (p>0.0001) myloperoxidase (MPO) enzyme activity in paracetamol exposed group in comparison to control group. When livartho drug treated for 16 days treatment, the MDA, PC levels and MPO enzyme activity were found significant (p>0.001, p>0.0001) lowered in paracetamol plus livartho treated group as compared with paracetamol induced heptatotoxicity group and these levels were come back near to control group (Figure 2).

Histopathological examination

Histopathological study of liver tissue revealed that normal control group (GI) showed normal hepatic cells appear with intact cytoplasm and well defined nucleus and inflammatory cells were restricted (Figure 4 A). Whereas paracetamol treated group (GII), clear showed central vein dilated (CVD) along with hemorrhage and presence of inflammatory cells in hepatic cells, cytoplasmic membrane destruct and scattered lymphocyte and plasma cells was seen in around portal triad (Figure

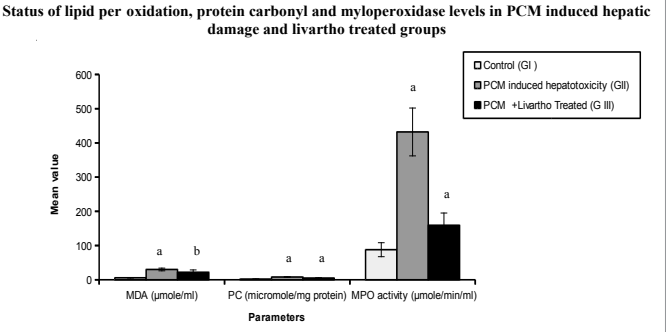

Figure 2: All data are mean ± SD of each group of 10 animals. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test are used to determine statistical difference between control (group I) *vs* paracetamol (PCM) induced hepatotoxicity group(group II) and paracetamol (PCM) induced hepatotoxicity group (group II) *vs* livartho treated group (group III). Where a; p<0.0001(highly significant) b; p <0.001(significant) c; p <0.01(significant). d; p<0.05 (significant). The p-values >0.05 were considered statistically non-significant.

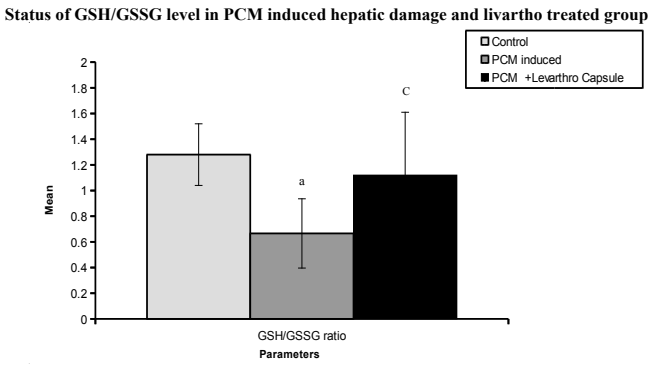

Figure 3: All data are mean ± SD of each group of 10 animals. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test are used to determine statistical difference between control (group I) *vs* paracetamol (PCM) induced hepatotoxicity group(group II) and paracetamol (PCM) induced hepatotoxicity group (group II) *vs* livartho treated group (group III). Where a; p<0.0001(highly significant) b; p <0.001(significant) c; p <0.01(significant). d; p<0.05 (significant). The p-values >0.05 were considered statistically non-significant.

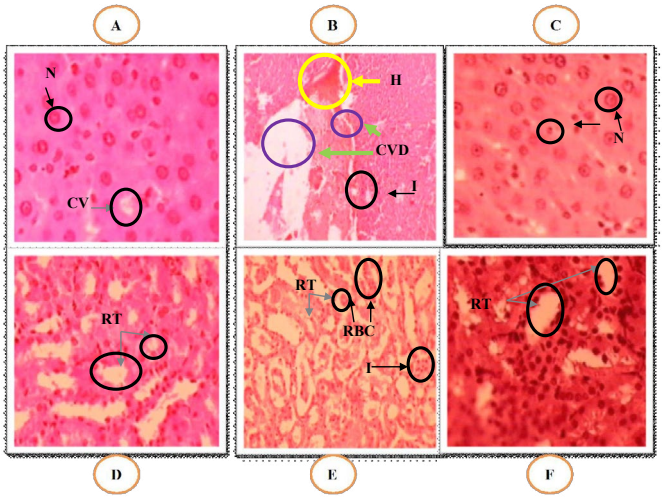

Figure 4: Histological changes in liver and renal organs of Control group, PCM induced group and livartho treated groups. Slide A, B and C shows liver organ of different groups whereas D, E and F shows renal organs of control, PCM and PCM +livartho treated groups . **CVD**; central vein dilated , **I**; inflammation , **RT**; renal tubules, **H**; hemorrhage.

4 B). The livartho treated group (GIII) showed less inflammatory cells with intact cytoplasm and nucleus and appear normal hepatic cells (Figure 4C). Renal histopathological showed that control group appear normal renal tubules (RT) and renal parenchyma (Figure 4D) whereas paracetamol treated group shows a collection of chronic inflammatory cells (plasma cells and lymphocyte) in peri-renal alveolar tissues. RBCs and protein materials were also seen in the lumen of several renal tubules (Figure 4E). After treatment with livartho drug in group III, renal tubules appear normal and free from RBC and protein cast.

Discussion

Paracetamol is considered to be the safest non-steroidal antipyretic drug. It is commonly available over the counter if it is used in recommended doses; it is also capable of producing hepatic damage on consuming single overdoses or chronic low dose [19-21]. Hence liver toxicity induction model was developed using paracetamol drug to see the therapeutic and hepatoprotective activity of new ayurvedic drug livartho in female SD rats. The mode of action of paracetamol on the liver is by covalent binding of its toxic metabolite, n-acetyl-p-benzoquinone-amine to the sulfhydryl group of protein resulting in cell necrosis and lipid peroxidation [22]. The result of present study reveals that paracetamol induced significant increase in organs weight, implying impaired animal growth and organs function, which is occur due to blocking of secretion of hepatic triglyceride into plasma [23]. However after treatment with livartho drug, ameliorated the increased organs weight and its percentage of relative organ weight in PCM group, restore the altered organs weight and its percentage to near normal.

The liver plays a central role in hematopoiesis and synthesis of coagulation proteins and liver disease is associated with a broad range of hematological abnormalities [24]. So in present study, it was found that a significant reduction of hematological parameters (Hb, RBC, Platelet count, PCV, MCH and MCV levels) in paracetamol induced group as compared with control group. The results clear show that the paracetamol causes destruction of matured RBC, reduction in the rate of erythropoiesis and inhibit the erythropoietin enzyme which release from the kidney. Hb concentration decreased due to reduction in the oxygen-carrying capacity of blood and less amount of oxygen delivered to the tissues. Due to less supply of oxygen carrying capacity of blood, PCV value was also decreased in paracetamol treated group and it clear indicate that an induction of anemia. The reduction of platelet count was found in paracetamol treated group as compared with control group. The result clear showed that high dose of paracetamol metabolites on platelets which leads to thermbocytopenia. The reduction of these hematological parameters may be attributed to the hyperactivity of bone marrow, which leads to the production of red blood cells with impaired integrity that are easily destroyed in the circulation, as well as marked leucopenia [25,26]. It has reported that high dose paracetamol causes alteration in the hematological parameters in rats [27]. After treatment with livartho drug for 16 days, these following parameters were significant improved in paracetamol plus livartho treated group and come back to control base line. The reversal of the hematological levels in treatment group may be due to increased erythropoiesis induced by drug or by prevention of the hemolysis of the acanthocytes (spur cells) which results from abnormal lipid composition of the red blood cell membrane following hepatic dysfunction [28].

During liver injury caused *via* overdose of paracetamol, the transport function of hepatocytes gets disturbed and resulting in the leakage of the plasma membrane [29], thus causing an increase serum hepatic enzyme levels. It is well known that many toxic compounds accumulate in the liver where they are detoxified [30]. Liver transaminases such as AST (aspartate transaminase) or SGOT (serum glutamic oxaloacetic transaminase), and ALT (alanine transaminase) or SGPT (serum glutamic pyruvic transaminase) have still remained the gold standards for the assessment of liver injury [31]. So in the present investigation, there were significant (p>0.0001) increased levels of SGOT, SGPT and total bilirubin along with increased LDH enzyme activity in paracetamol hepatic damage group in comparison to control group. The results clear indicate that these enzymes are cytoplasmic in location and are released into circulation after liver damage indicating hepatotoxicity. After treatment with livartho drug for sixteen days, a significant decreased hepatic enzyme levels SGOT, SGPT, total protein , albumin and LDH enzyme activity in paracetamol plus livartho treated group as compared with paracetamol treated group and come back near to control group. These results hypothesized that livartho drug inhibit the cytochrome P450 enzymes activity in hepatic microsomes and prevent stabilization of plasma membrane thereby preserving the structural integrity of cell

membrane which then prevents the leakage of enzymes into the blood stream. Various findings reported that usefulness of medicinal plants are effective for liver disorders [1,23]. In the present study, there were significant increased triglyceride and cholesterol levels in PCM induced group as compared with control group. The results clear shown that paracetamol affects in the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis and damage of hepatic parenchymal cells that lead to disturbance of lipid metabolism in liver. After treatment with drug, these levels were significantly decreased in paracetamol plus livartho treated group as compared to paracetamol induced group and come back to control group. These finding showed that mechanism of lipid lowering effects of drug might be attributed to an inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferease enzyme. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver. Protein and albumin level were decreased in paracetamol induced hepatic damage group in comparison to control group and restore further after treatment with livartho drug for 16 days in group III as compared to paracetamol treated group. The results indicate that poor liver functions or impaired synthesis, either primary as in liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndromes or malnutrition, or loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis. The increased protein and albumin levels after treatment with drug may attribute to decreased lipid peroxidation process and increase in the activities of plasma protein thiol. Liver damage is always associated with cellular necrosis which leads to increase in tissue lipid per-oxidation and depletion in antioxidant reduced glutathione (GSH) level. In this study, a significant reduction in antioxidant enzymatic levels (GSH, TSH, GSSG) and GSH/GSSG ratio along with significant increased lipid per-oxidation and protein carbonyl content levels and myloperoxidase enzyme activity in paracetamol treated group when compared with control group. The results shown that oxidant and antioxidant ratio are disturbed due to overdose of paracetamol which causes liver injury and increase excess free radical generation. Various studies have been reported that paracetamol causes oxidative stress and alteration in endogenous antioxidant enzyme activities in rat [32]. The levels of lipid per-oxidation, protein carbonyl content and myloperoxidase enzyme activity were also significantly ($p>0.001$, $p>0.0001$) decreased along with significant ($p>0.0001$) increased the antioxidant levels in livartho treated group and come back to normal level. This results clear indicate that livartho drug scavenge excess free radical generation, prevent the integrity of hepatic cells and protect the leakage of intracellular enzymes by its membrane stabilizing activity. The improvement in hematological, biochemical parameters and decrease free radical generation along with increased extra and intracellular antioxidant levels of drug treated group during due to presence of active constituent's flavonoids, alkoloids and polyphenolic in drug which possess hepatoprotective, antioxidant properties that are responsible for restoration of hepatic damage.

Conclusion

On the basis of above finding it is concluded that livartho is good efficacy and most effective drug for prevention of hepatic injury along with improved hematological biochemical parameters and decreased free radical generation and maintained antioxidants level during hepatic injury.

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Competing interests

The authors declare that they have no competing interests.

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