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RESEARCH ARTICLE

Assessment of Effect of *Patol-katurohinyadi Kwatha* in Paracetamol Induced Hepato-toxicity in Albino Wistar Rats - An Experimental Study

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Abstract

Background: The present study was planning to assess the hepato-protective and hepato-curative property of *Patol-katurohinyadi (PK) kwath,* in paracetamol induced hepatotoxicity in albino rats. Hepatotoxicity due to paracetamol and dushi visha concept can be correlated with each other and hepatoprotective formulations mentioned in *Ayurveda* samhita can play major role in this concept. A classical *Ayurvedic* formulation *Patol-katurohinyadi* of *Ashtanga hridayam,* has been reported to be effective in management of liver related diseases. **Objective:** To study the hepato-protective and hepato-curative properties of *PK Kwatha* in paracetamol induced hepatotoxicity in albino rats. **Materials and Methods:** The experimental study was carried out in albino rats as per CPCSEA guidelines after getting approval from the Institutional Animal Ethics Committee (IAEC). 30 Albino rats, each weighing about 150 gm to 200 gm, were procured from APT Testing and Research Pvt. Ltd. Pune. The rats were housed in the institutional animal house in standard conditions. 30 albino rats were equally divided into five groups having six animals in each group as normal control, disease control, test group, preventive group and curative group. To assess hepato-toxicity, the blood investigations such as RBC, WBC etc. Haematological parameters, biochemical parameters, and histopathological findings were carried out by monitoring rats on 0, 7 and 14 days of study. The results were derived by applying appropriate test to the data. **Result:** The animals administered with PCM only shows increase in values of SGPT, SPOT, ALP, Albumin, total protein compared to animals of control group. Whereas animals administered with *PK Kwatha* prevented PCM-induced changes in biochemical parameters and histopathological features. **Conclusion:** The histopathological results of liver show the potent efficacy of *Patol-katurohinyadi kwatha* on paracetamol induced hepatotoxicity. Also, we found that *Patol-katurohinyadi kwatha* as a good hepatoprotective and hepato-curative drug.

Keywords: *Dushivisha,* Hepatotoxicity, Hepato-protection, Histopathology, *Patol-katurohinyadi Kwatha*, Paracetamol

1. Introduction

Liver is a vital organ for conducting many metabolic functions and a target for several toxic compounds. Liver is vulnerable to the toxicity of such compounds and plays an essential role in the transformation and removal of toxins. Hepatotoxicity means liver damage caused by chemicals or drugs[1].

Paracetamol (PCM) is easily available basic allopathic drug which is available without doctor's prescription over the counter. While in higher and repeated doses, it causes liver injury. One of the main metabolites in Paracetamol is N-Acetyl-P-Benzoquinonemine (NAPQI). It mainly depletes the liver natural antioxidants like glutathione which directly damages the liver cells leads to necrosis, phagocytic and fat degeneration in liver which may finally leads to hepatic failure[2]. Hypoglycaemia, coma, renal tubular necrosis, acute liver failure (ALF), and centrilobular hepatic necrosis are common adverse effects of paracetamol intoxication[3]. About 42% cases ALF are attributable to PCM overdoses. 63% paracetamol

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induced liver toxicities are caused by consuming unintentional harmful dosage which having PCM in combination[4]. Paracetamol consumption and related hepatotoxicity is a crucial issue which affecting many people across the world[5].

On the other hand, *Patol-katurihinyadi kwatha*, a classical *Ayurvedic* polyherbal formulation from *Sutrasthan* of *Ashtang hridyam* and *Sushrut samhita* has been evaluated for its anti-poisonous and hepatoprotective properties. *Patola Trichosanthes dioica, Katurohini (Picrorhiza kurroa), Chandana (Santalum album), Madhusrava (Leptadenia reticulate), Guduchi (Tinospora cordifolia)* and *Patha (Cissampelos pareira*) are the 6 herbal ingredients of *Patol-katurohiniyadi Kwatha*. Acoording to many *Ayurvedic* texts, the *Kwatha* and its individual contents are having anti-toxic (*Vishanasaka*) property[6-11].

All individual contents of the *Kwatha* have shown hepatoprotective property against different experimental toxicity models[12-14]. The vitiated *Pitta* is the major cause of all liver disorders. *Patol-katurohinyadi Kwatha* is a *Tikta rasa* (Bitter taste) predominant formulation. *Tikta rasa* itself possesses *Vishaghna* property and it is *Pittashamaka* in nature. *Pitta* and *Rakta* have *Ashrayashrayi sambandha*. Hence treatment used to cure diseases of *Pitta*, ultimately treats the *Raktadushti*. As *Yakrita* is *Moolsthana* of *Raktavaha-strotasa*, the *Vishaghna dravyas* pacifying *Raktadushti* also helps to improve function of *Yakrita*. Hence *Pittashamaka* treatment is useful in paracetamol induced hepatotoxicity. In short, the *Patol-katurohniyadi Kwatha* has hepatoprotective, hepatocurative and antioxidants property but less studies are done to be elaborated their antitoxic properties in poisoning in modern era[15,16]. The formulation should be effectively established using the standard of animal experiments, just as it was before the clinical trial. Hence, this formulation is selected for animal experiment to evaluate the effect on hepato-toxicity induced by paracetamol.

2. Materials and Methods

2.1 Materials

The drugs and chemical used are shown in Table 1. Paracetamol (400 mg/kg) was given for 7 days to administered orally in disease control, preventive and curative groups except normal control and test (*Kwatha*) group[17]. *Patol-katurohinyadi kwatha* (8.7ml/kg/day) was given to test and preventive group for 7 days and to curative group for next 7 days after paracetamol administration for 7 days[18,19]. The duration of study was 14 days.

2.1.1 Collection of the Ingredients of the Patol-katurohinyadi Kwatha (PKK)

Contents of *PK Kwatha*-leaves of *Trichosanthes dioica,* rhizomes of *Picrorhiza kurroa*, heart wood of *Santalum album,* roots of *Leptadenia retuculate,* stem of *Tinospora cordifolia* and roots of *Cissampelos pareira* (Table 1) were collected from Wagh Brothers Herbal Pharmacy Nagpur.

2.1.2 Authentication of Sample

Authentication of the contents of *kwatha* were done in *Dravyaguna* Department, Govt. *Ayurvedic* College, Nagpur.

Table 1. Ingredients of *Patol-katurohinyadi Kwatha* with the part used and quantity

Sr. No.	*Dravya*	Latin Name	Family	Part used	quantity
1	Patola	*Trichosanthes dioica* Roxb.	Cucurbitaceae	Leaves	1 Part
2	*Katurohini*	*Picrorhiza kurroa* Royle ex Benth.	Plantaginaceae	Rhizome	1 Part
3	*Chandan*	*Santalum album* L.	Santalaceae	Heart Wood	1 Part
4	*Madhusrava*	*Marsdenia tenacissima* R. Br.	Asclepiadaceae	Root	1 Part
5	*Guduchi*	*Tinospora cordifolia* Thunb Miers	Menispermaceae	Stem	1 Part
6	*Patha*	*Cissamples pareira* L.	Menispermaceae	Root	1 Part

2.1.3 Preparation of Kwatha

Preparation of *Patol-katurohinyadi kwatha* was done in the Laboratory of Rasashastra Department, Govt. Ayurvedic College, Nagpur.

2.1.4 Procedure

20 gm *Bharad* of *Kwatha Dravyas* (i.e. *Patola, Katurohini, Chandana, Murva, Guduchi, Patha*) was soaked in a stainless steel vessel in 320 ml of water and kept overnight. Next day morning, heating was started to the vessel, with *Mandagni* (low flame). Continuous stirring was done to avoid the burning of *Kwatha Dravyas*. The heating was given to the contents till it reduce to 1/8th i.e. 40 ml. Then after it was filtred through a clean cotton cloth and stored in a container[15].

2.1.5 Standardization of Drugs

Standardization of *kwatha* contents and *kwatha* were done at Savitribai Phule Pune University as per API (*Ayurvedic* Pharmacopoeia of India) parameters.

2.1.6 Experimental Animals

A total number of thirty apparently healthy adult albino rats (weighing 150 gm to 200gm) were obtained from the Laboratory Animal House, APT Testing and Research Pvt. Ltd. Pune. Unique identification number was given to cage tag and by corresponding colour body markings. Per group 6 animals were tested. Before start of actual dosing the rats were housed in their cages for five days in experimental room after veterinary examination. They were fed on slandered diet and water during the investigation (Table 2).

2.2 Methods

2.2.1 Experimental Design

The approval for present study was taken from Institutional Animal Ethics Committee (IAEC), APT Testing and Research Pvt. Ltd., Pune, having protocol number 25/2021 and carried out according to the CPCSEA guidelines (Table 3).

2.2.2 Sampling

2.2.2.1 Blood Samples

On 8th day blood samples of animals of 1st four groups were collected through cardiac puncture for serum bio-chemical evaluations and on 15th day blood samples of animals of 5th group were collected through cardiac puncture for serum bio-chemical evaluations.

Table 2. Protocol for animal experiment

Animal Species	Albino Rats
Source of animals	Research centre and Animal care shelter
Average weight of animals	150-200 grams
Number of groups	5
Number of Rats	6 rats per group
Age of Animals	6 to 8 weeks
Sex of Animals	Males- 50% and Females- 50%
Route of Dose administration	Paracetamol in glucose water was given orally and *Patol-katurohinyadi kwatha* was given orally in once a day (O.D) dose

Table 3. Showing groups of animals

Group 1: Control group	only distilled water for 7 days
Group 2: Paracetamol Test group	only Paracetamol 400 mg/kg/ day for 7 days
Group 3: Test group	*Kwatha* of *Patol-katurohinyadi* for 7 days
Group 4: Prophylactic group	Paracetamol 400 mg/kg/ day for with simultaneous administration of *Patol-katurohinyadi kwatha* 8.7ml/kg/ day for 7 days
Group 5: Curative group	For 7 days- Paracetamol 400 mg/kg/day mg/kg/days fallowed by administration of *Patol-katurohinyadi kwatha* 8.7ml/kg/day for next 7 days

2.2.2.2 Tissue Specimens

Liver from all the animals were dissected weighed. Half of the liver was preserved in formalin for histopathology study and half was used to determine an antioxidant enzyme. A homogenate was prepared with 0.5 g of the liver tissue with 2.5ml of 5% TCA. The precipitated

protein was centrifuged at 1000 rpm for 10 minutes. The supernatant (0.1ml) was used for the estimation of GSH. The supernatant (0.1ml) was made up to 1.0 ml with 0.2 M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 µmoles were also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer at 412 nm after 10 minutes. The values were expressed as µmoles GSH/g sample.

2.2.2.2.1 Hematological Studies

WBS, Lymphocyte, MID, Neutrophil, RBS, HCB, HCT, MCV, MCH, MCHC, and Platelets were measured as per standard methods.

2.2.2.2.2 Serum Biochemical Parameters

SGPT, SPOT, ALP, Albumin, Bilirubin and Total Protein were measured as per standard methods.

2.2.2.2.3 Anti-oxidant Assays

Lipid Peroxidation (LPO), Glutathione (GSH) were measured as per standard methods.

2.2.2.2.4 Reduced Glutathione Assay

A homogenate was prepared with 0.5g of the liver tissue with 2.5ml of 5% TCA. The precipitated protein was centrifuged at 1000rpm for 10 minutes. The supernatant (0.1ml) was used for the estimation of GSH. The supernatant (0.1ml) was made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 µmoles were also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer at 412nm after 10 mins and their values were denoted in µmoles GSH/gram of the sample.

2.2.2.2.5 Lipid Peroxidation Assay

A 10% weight/volume of liver homogenate in phosphate buffer with a pH of 7.4 of test sample was made. OD was measured at 535 nm against blank. (OD X 1.56 $M^{-1}cm^{-1}$ is equal to n moles of MDA/gm of tissue).

2.2.2.2.6 Histopathological Examination of Liver

All the rats were sacrificed after treatment protocol. Liver was isolated from animals and then stored in 10% formalin solution for fixation. Tissue sample was processed to make paraffin block which was then sectioned with microtome. Section was then stained with haematoxylin and eosin. Histology was analysed then under microscope (Nikon) at magnification of 100x-400x.

2.3.2 Statistical Analysis

A microsoft excel spreadsheet was used to record the collected data. Microsoft was used to create the charts and tables. Comparison of body weight and food consumption before (day-0) and after treatment (day-7) by performing paired t-test. Weight of organs, haematological parameters, biochemical parameters, absorbance, absorbance blank and n moles of MDA/gm of tissue and Reduced Glutathione (GSH) were compared across 5 groups by performing one-way test of ANOVA. Bonferroni t-test was performed for comparing difference between 2 groups. Histopathological findings (severity of toxicity) were compared among 5 groups by chi2-square test method. Samples, Fisher exact test was utilized for small sizes. Statistics were considered significant if $p<0.05$. For data analysis statistical software STATA version 14.0 was used.

3. Result

3.1 Heamatological Results

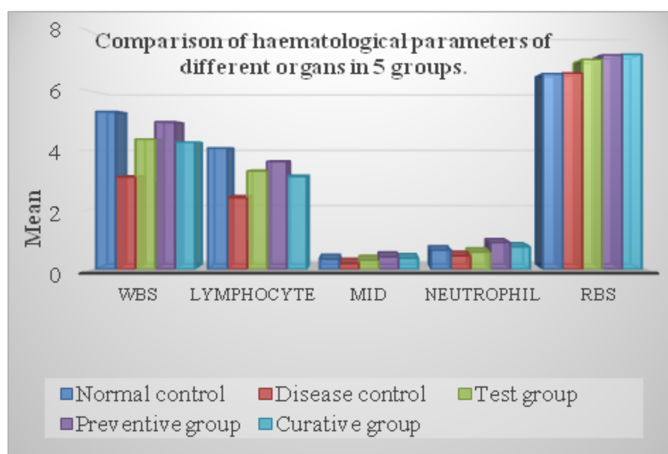
The Haematological parameters (WBS, lymphocyte, MID, neutrophil, RBS, HCB, HCT, MCV, MCH, MCHC, platelet) data has shown non-significant changes in animals across 5 groups. No multiple comparison test was performed (Table 4, Figures 1A and 1B).

3.2 Serum Biochemical Results

The ALP, total protein, albumin, SGPT, SGOT of animals was observed in each group. Test applied was one-way ANOVA to see the difference in mean of biochemical parameters of animals. It was observed that there is high statistically significant difference was noted except bilirubin in means difference and p value obtained was <0.05, here p value is <0.001, which shows significant difference between the means (Table 5; Figures 2A and 2B).

Table 4. Showing comparison of haematological parameters between 5 study groups

	Normal control		Disease control		Test group		Preventive group		Curative group		F -value	p-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
WBC	5.31	0.71	3.1	1.35	4.38	1.66	4.96	2.33	4.28	2.23	138	0.2693 NS
Lymphocyte	4.08	0.63	2.41	1.09	3.31	1.57	3.63	1.75	3.13	1.48	1.22	0.3261 NS
MID [Type of WBC]	0.35	0.05	0.21	0.09	0.31	0.16	0.43	0.23	0.38	0.21	1.43	0.2532, NS
Neutrophil	0.65	0.12	0.46	0.19	0.58	0.31	0.90	0.38	0.76	0.58	1.29	0.2993, NS
RBC	6.58	1.03	6.6	0.75	7.06	0.21	7.21	0.29	7.23	0.36	1.66	0.1908, NS
HGB	12.66	2.20	12.43	1.88	13.31	0.76	14.2	0.47	13.88	0.88	1.72	0.1760, NS
HCT	53.03	8.83	52.33	6.13	56.03	2.18	58.41	2.90	56.3	3.43	1.34	0.2825, NS
MCV	80.61	1.97	79.38	1.58	79.66	1.30	80.98	1.75	77.86	3.49	1.91	0.1408, NS
MCH	19.16	0.85	18.73	1.06	18.86	0.71	19.66	0.90	19.13	0.87	0.97	0.4406, NS
MCHC	23.8	0.59	23.6	1.06	23.71	0.59	24.3	1.06	24.58	0.11	1.79	0.1632, NS
Platelets	484.6	106.5	412.6	165.6	515.8	94.3	584.1	128.3	526.1	165.3	1.29	0.2997, NS



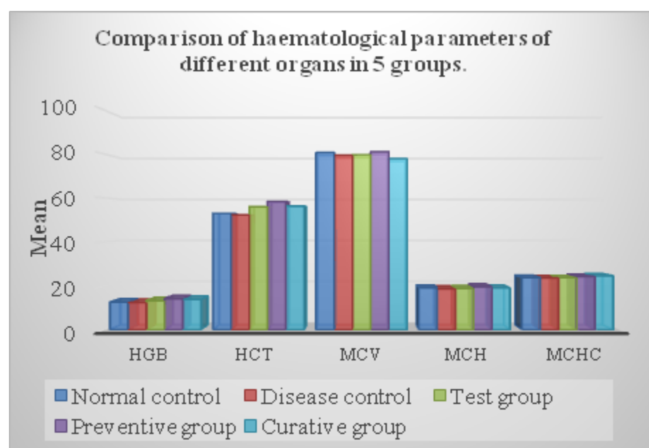
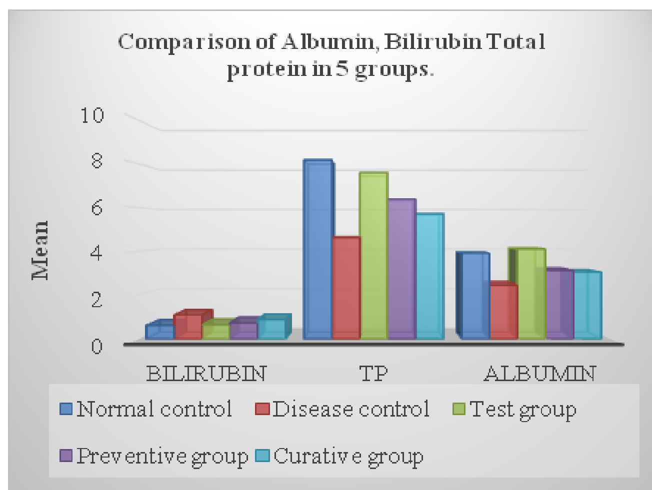


Figure 1A and 1B. Graphical presentation of haematological parameters between 5 groups.

Table 5. Comparison of biochemical parameters between 5 groups

	Normal control		Disease control		Test group		Preventive group		Curative group		F value	P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
ALP	132.0	42.59	279.4	65.84	129.6	18.2	170.4	41.1	223.4	48.5	11.72	<0.001, HS
Bilirubin	0.62	0.29	1.10	0.80	0.64	0.11	0.73	0.12	0.89	0.27	1.46	0.2450, NS
TP	8.16	1.05	4.63	0.91	7.58	0.69	6.38	1.11	5.71	0.51	15.34	<0.001, HS
Albumin	3.91	0.74	2.46	0.33	4.1	0.63	3.13	0.66	3.05	0.49	7.69	0.0003, HS
SGPT	44.01	10.63	144.0	23.64	56.5	21.8	67.53	17.63	109.9	32.8	20.36	<0.001, HS
SGOT	146.6	25.63	289.0	65.08	154.4	31.3	154.5	19.47	201.3	51.8	12.02	<0.001, HS



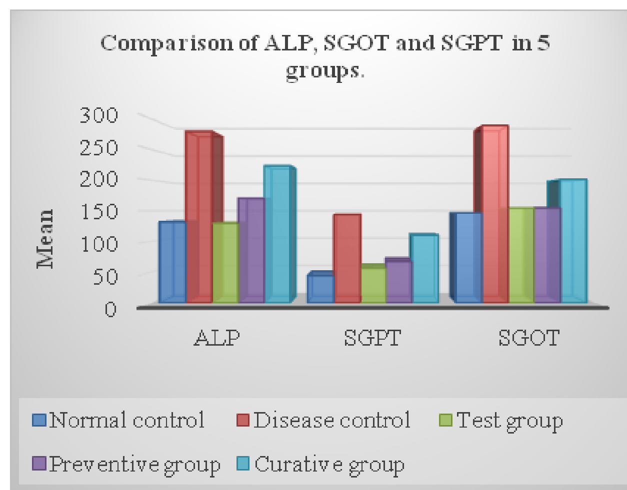


Figure 2A and 2B. Graphical presentation of biochemical parameters between 5 groups.

3.3 Lipid Peroxide Assay (LPO)

The absorbance, absorbance blank and n moles of MDA/gm of tissue of animals was observed in each group. Test applied was one-way ANOVA to see the difference in mean of animals. As p value obtained was <0.05 here, p value = 0.0002, which shows significant difference between the means. (Figure 3).

3.4 Reduced Glutathione (GSH)

The glutathione-absorbance of animals was observed in each group. Test applied was one-way ANOVA to see the difference between the mean of glutathione-absorbance value of animals. A significant statistical difference was noted across the mean value of glutathione-absorbance as p value obtained was obtained was <0.05 here F value=8.69 and p value = 0.0002, which shows significant difference between the means (Figure 4).

3.5 Histopathological Finding of Toxicity of Liver

Histopathological analysis shows Mild (+2) degenerative changes in the liver tissues in the disease control

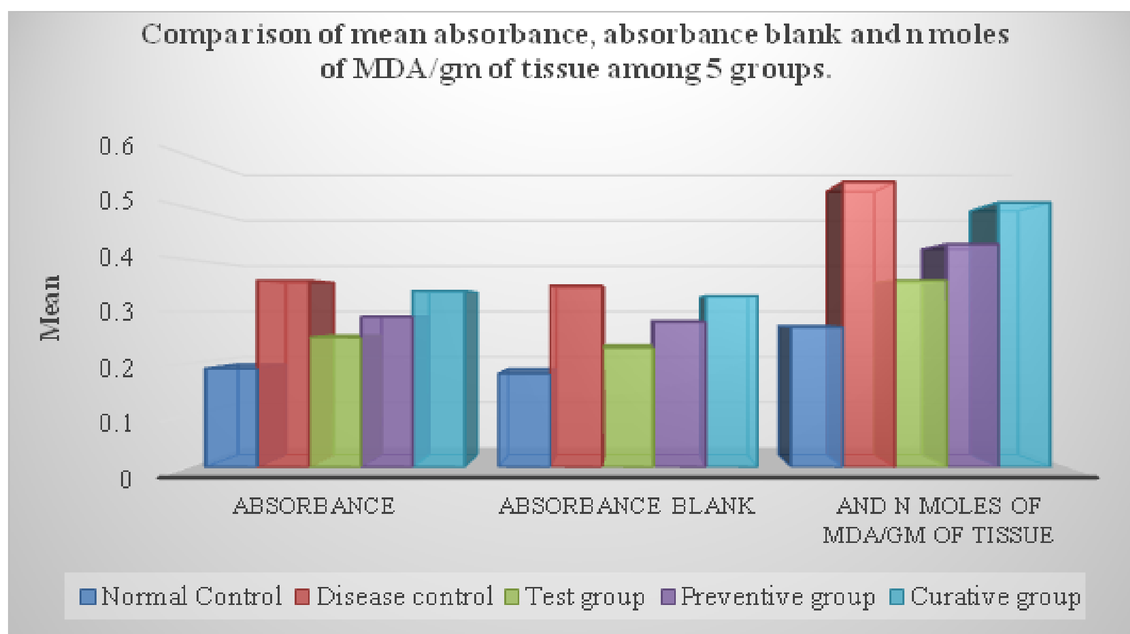


Figure 3. Graphical presentation of LPO between 5 groups.

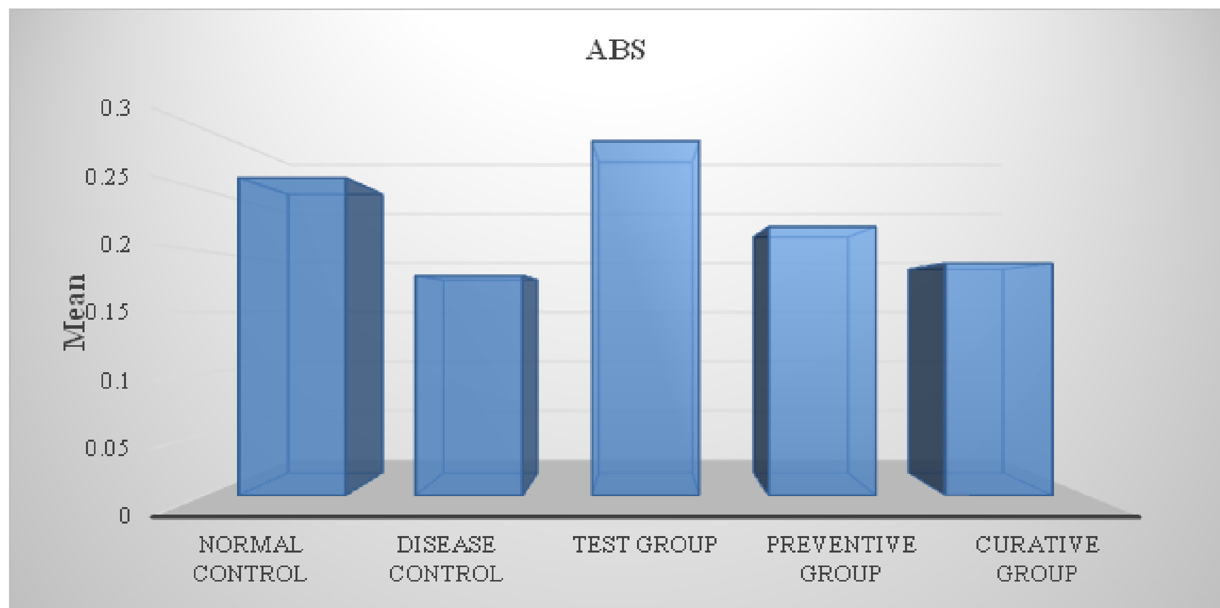


Figure 4. Graphical presentation of Reduced Glutathione (GSH) between 5 groups.

group where hepatic parenchyma were shown cellular swelling and cytoplasmic vacuolar changes. Multiple foci of centrilobular necrosis were observed in the hepatic parenchyma. While the other groups showed Minimal (+1) focal degenerative changes in hepatocytes (Figure 5).

4. Discussion

Paracetamol for hepatotoxicity was selected as per standard methods of induced hepatotoxicity OECD guidelines no 423. Research work on *Patol-katurohinyadi Kwatha* shows, it has *Yakritgamitva* i.e., an affinity towards the *Yakrita* and it is efficacious in treatment of liver disorders. The ingredients of *Kwatha* i.e., *Patol, katurohini, Chandana, Murva, Guduchi, Patha* are mentioned as having anti-poisonous property in many classics. All the contents of the *Patol-katurohinyadi kwatha* are known to have hepatoprotective properties against many experimental hepatotoxicity models. Research work on *PK Kwatha* shows, it's all contents are having hepatocurative, antioxidant and hepatoprotective properties.

Body weight, biochemical parameters etc. were presented as Mean ± SD. Body weight, biochemical parameters, haematological parameters, organ parameters were compared across 5 different groups by performing one-way ANOVA test for data and all the tests were 2 sided. P value < 0.05 was considered as statistically significant. Body weight and body organ weight parameters showed non-significant difference across 5 groups, no multiple comparison test was performed.

4.1 Haematological Parameters

WBS, lymphocyte, MID, neutrophil, RBS, HCB, HCT, MCV, MCH, MCHC, platelet shown non-significant changes in animals across 5 groups.

4.2 Biochemical Parameters

The animals administered with PCM only shows increase in values of SGPT, SPOT, ALP, albumin, total proteinas compared to animals of control group. Whereas animals administered with *PK Kwatha* prevented PCM-induced changes in biochemical parameters.

4.3 LPO

The values of absorbance, absorbance blank and n moles of MDA/gm of tissue of animals are increased in disease control group and reduced in normal control group whereas these values are decreased in preventive and curative group.

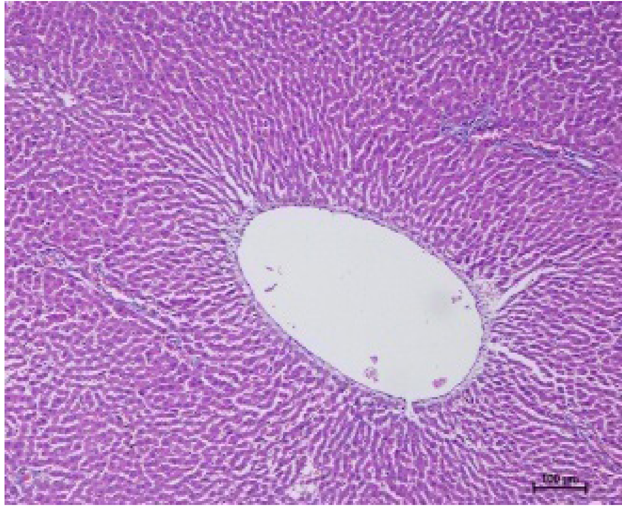


Figure 5A - Group-1

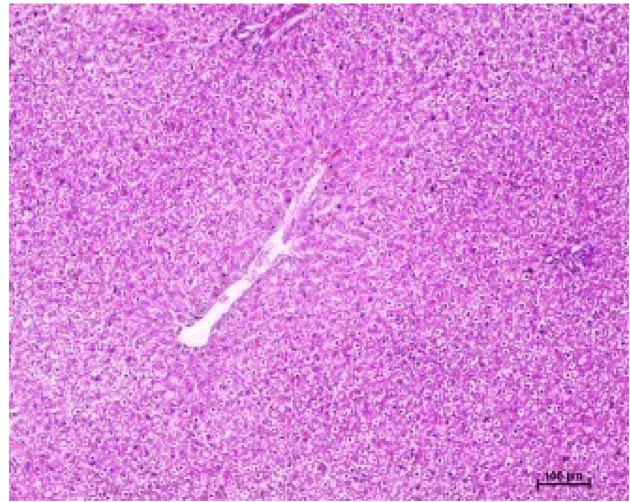


Figure 5B – Group - 2

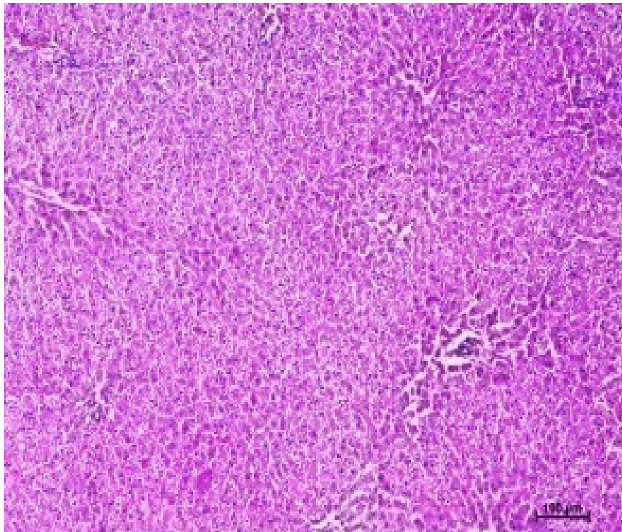


Figure 5C – Group – 3

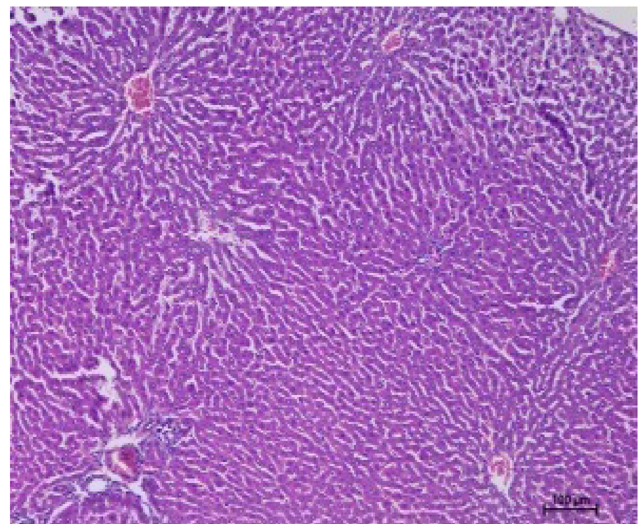


Figure 5D – Group - 4

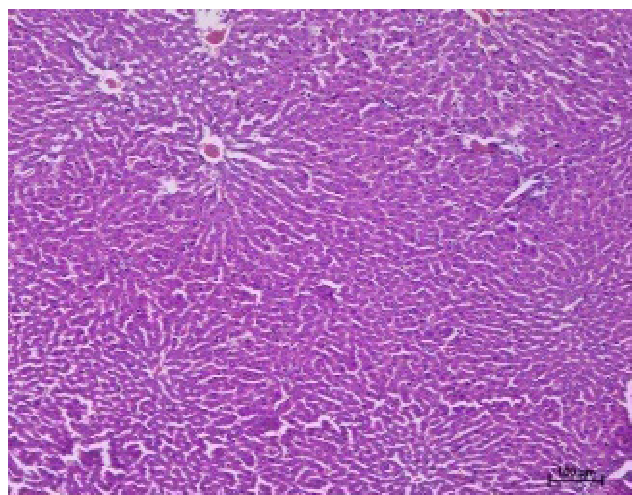


Figure 5E - Group - 5

Figure 5. Histopathological findings of liver.

4.4 Histopathological Findings of Liver

Histopathological analysis shows mild (+2) degenerative changes in the liver tissues in the disease control group. Multiple foci of centrilobular necrosis were observed in the hepatic parenchyma. While the other groups showed minimal (+1) focal degenerative changes in hepatocytes.

4.5 Mode of Action of *Patol-katurohinyadi Kwatha*

The Paracetamol toxicity can also be considered with concept of *Garavisha* which is explained in *Agadtantra*. The signs and symptoms of *Garavisha* i.e., *Mandagni, Udar-rog, Yakrit-pleeha vikara, Gulma, pandu* are also found in liver toxicity. In hepatotoxicity, liver is mainly affected by *Pitta* and *Kapha dosha*. Due to this, there is increase in *Aam Dosha* and this excessive quantity of *Aam dosha can* causes *Shrotaovarodha* which further leads to *Vimarg gamana* of *Vayu*. The properties of *Patol-katurohinyadi Kwatha* like *Tikta rasa* and *Deepan pachana gunas* are helpful in *Aam pachan* and releasing *Shrotavarodha*. The vitiation of *Pitta* is the major cause of all the liver related diseases and *Patol-katurohinyadi Kwatha*is having *Tikta rasa* predominance which is *Pittashamaka* in nature and *Tikta rasa* is also attributes to the *Vishanasaka* (anti-toxic) property, so it can be used for liver detoxification. *PK-kwatha* has a balancing effect on *Pitta* and *Kapha* vitiation. The formulation is effective in purifying blood, removing the blocks in Liver and remove the toxicity in cells also. *Patol, Katurohini, Chandana, Murva, Guduchi, Patha* are 6 main ingredients of *PK-Kwatha* are having hepatoprotective, antioxidant and hepato-curative. They also reduce the symptoms of *Panduta, Yakrit-pleeha Vikara, Agnimandya, Aruchi, Hrillas, Vishamjwara* etc. which perfectly resembles with the hepatotoxicity of paracetamol. All ingredients and also the *Kwatha* itself possess the property of *vishanashaka* (ant poisonous). The result obtained from Biochemical and histopathological examination shows the curative and preventive activity on paracetamol toxicity. So, both the criteria successfully achieved the aim of our subject, *Patol-katurohinyadi Kwatha* proved effective against paracetamol induced hepatotoxicity in albino rats.

5. Conclusion

From above results it can be concluded that paracetamol causes hepatic damage at the given concentration and based on results of biochemical parameters and histopathological observations this damage can be prevented by prophylaxis of *PK Kwatha*. In the present study, the curative efficacy of *PK Kwatha* was more promising as compared to preventive efficacy against paracetamol induced hepatotoxicity in experimental rats at the given dose. However detailed research is needed to find out the specific mechanism for effect.

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