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PHARMACOLOGICAL EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF TURMESAC® ON PARACETAMOL-INDUCED LIVER TOXICITY IN RATS

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ABSTRACT
The present study were carried out to assess hepatoprotective potential for Turmesac® (formulated by using turmeric rhizome extract) against paracetamol (PCM) induced hepatotoxicity in rats. Oral administration of Turmesac® in two doses 250mg/kg and 500mg/kg body weight were subjected for the evaluation of hepatoprotective potential against PCM (2g/kg) induced liver injury. Silymarin (50mg/kg) was used as a standard drug. The biochemical parameters like serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), glutathione (GSH), super oxide dismutase (SOD), total bilirubin (TBR), total protein (TP) and catalase (CAT) were assayed in liver homogenates for all studied groups. In addition histopathological study was also carried out. The results revealed significant decrease in SGPT, SGOT and TBR and increase in GSH, TP, SOD and CAT levels when compared with toxic control. These data indicate that Turmesac® is a natural antioxidant hepatoprotective agent against hepatotoxicity induced by paracetamol model. Thus, Turmesac® may have a therapeutic value of drug-induced hepatotoxicity as well as in paracetamol therapy.

KEYWORDS: Turmesac®, Hepatoprotective activity, Paracetamol, Silymarin.

INTRODUCTION
The liver is the main organ responsible for the biosynthesis, uptake and degradation of proteins and enzymes. It is the second largest organ in the body, and is often considered as the most important one. The liver receives a dual blood supply with about 20% of blood coming from the hepatic artery and 80% from the portal circulation. The most common diseases includes infections such as hepatitis (A, B, C, E) alcohol damage, fatty liver, cirrhosis and cancer. More than 1000 drugs have been reported to have toxic effect on the liver and subsequently induce oxidative stress, steatosis and cell death. Most of the anti-cancer, anti-analgesic and anti-inflammatory drugs and antidepressants can be hepatotoxic. The main problem with these medications is the usage of high doses, which usually lead to hepatotoxicity in humans and experimental animals. Worldwide Paracetamol (acetaminophen), which is commonly used as an antipyretic, is the chief causative of drug-induced liver damage.[1] Natural products provide a repertory for the discovery of new leads drugs that can be used in treating different types of illnesses such as cancer, inflammation and liver diseases. More than half of all pharmaceutical products were discovered from natural compounds or their derivatives.[2] In the United States and Europe, approximately 65% of patients use herbal medicines against liver disease, due to their wide availability, low toxicity, pharmacological activity, chemical diversity and low side effects compared to synthetic drugs.[3–5] *Curcuma longa* commonly known as Turmeric has been used as a medicinal herb in India from ancient times. It played a great role in the day-to-day life of ancient Indians as a treatment for wounds, stomach ache, cough, poison *etc*. In addition, it is used for dyeing clothes and in the worship their god and goddesses. This plant has acquired great importance in the modern world with its anti-inflammatory, anticancer, antioxidant, and a variety of other medicinal properties.[6] The rhizomes of *C. longa* contain approximately 2% volatile oils, composed mainly of α and ß-turmerone, monoterpenes[7] (Leung AY et al., 1996), 5% curcuminoids, mainly curcumin[8] (Budavari S, 1996), demethoxycurcumin, bis-demethoxycurcumin and dihydrocurcumin, minerals, carotene and vitamin C[9] (Kapoor LD, 1990). Manal et al.(2014)[10] showed that curcumin supplementation at doses of 50 and 100 mg/kg/day to experimental rabbits with paracetamol-induced hepatotoxicity lowers the elevated aspartate, alkaline phosphatase and alanine transaminase levels and raises the total protein and albumin levels in plasma. In addition to these changes, curcumin increased the levels of red blood cells and platelets. In another study, the efficacy of curcumin to manipulate the protein content, Succinate dehydrogenase (SDH), Thiobarbituric acid

reactive substances (TBARS), Adenosine triphosphatase activity (ATPase), alkaline phosphatase activity (ALKase), acid phosphatase activity (ACPase), SOD and body weight of chloroquine phosphate (CQ)-induced hepatotoxicity was observed in a rat model.[11] Therefore, this study has been conducted to evaluate the hepatoprotective activity of Turmesac® on paracetamol induced hepatotoxic in Rats.

MATERIALS AND METHODS

Turmeric rhizome (Sangli Turmeric) was collected from Maharashtra, March 2019, India and then authenticated by a botanist, R&D division, Star Hi Herbs Pvt. Ltd, Jigani, Bangalore, and Karnataka, India. The turmeric rhizomes were carefully cleaned, dried, powdered and stored in an airtight container until the extraction procedure.

Preparation of the Turmesac®

Turmesac® is manufactured and registered by Star Hi Herbs Pvt. Ltd, Jigani, Bangalore, Karnataka, India. Turmeric rhizome powders were subjected to steam distillation and the turmeric oil was separated and collected. The rhizomes were further extracted by refluxing with water in a commercial extraction facility. The water extract was concentrated by distillation under vacuum and the resultant concentrated liquid was spray dried to obtain a free flowing powder. Turmesac® was prepared by blending 95% of spray dried water extract of turmeric rhizome, 3% of the ethanolic extract curcuminoids and 2% of the turmeric oil.

Hepatoprotective activity of Turmesac®

The studies were carried out using male wistar albino rats (180-200g). They were obtained from the animal house, Bharathi College of pharmacy Mandya, Karnataka, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25°c) with dark and light cycle (12/12 h). All the animals were acclimatized to laboratory condition for a week before commencement of experiment. The ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before the experiment (1135/PO/Re/S/07/CPCSEA).

Study design

30 Wistar albino rats of either sex were used for the study. They were randomly divided into 5 groups with six (6) rats in each group.

Group I: Normal control rats (distilled water given p.o)
Group II: Distilled water+Paracetamol (2 g/kg bw, orally) (Toxic control)
Group III: Silymarin (50 mg/kg/day p.o) +Paracetamol (2 g/kg bw, orally) (positive control)
Group IV: Turmesac® (250 mg/kg/day respectively) +Paracetamol (2 g/kg bw, orally)
Group V: Turmesac® (500 mg/kg/day respectively) +Paracetamol (2 g/kg bw, orally)

Biochemical Studies

The blood samples were drawn from all the animals by puncturing retro-orbital plexus on 10th day of the treatment. Serum was separated by centrifuging blood at 2500 rpm for 15 min and the levels of SGOT, SGPT, Total bilirubin, and total protein were analyzed by using a commercially available enzymatic kit (AGAPPE, India) and an Autoanalyser (Chemistry Analyser (CA 2005), B4B Diagnostic Division, China).

In tissue samples, a part of liver tissue was minced and homogenized in ice-cold 1.15% w/v KCl in a Potter Elvehjem Teflon glass homogenizer for 1 min to make a 10% w/v liver homogenate. The quantitative estimation of hepatic antioxidant enzymes such as superoxide dismutase (SOD)[12], catalase (CAT)[13], reduced glutathione (GSH)[14] were performed in liver homogenate respectively.

Histopathology

A section of the liver was collected and immediately fixed in 10% formalin, and then dehydration in ascending grades of alcohol (ethanol) of 70, 80 and 95% and absolute alcohol for 2 changes each. The tissues were cleared in xylene and embedded in paraffin wax. Serial section of 5-6 microns in thickness were obtained using rotary microtome and stained with hematoxylin and eosin. The stained sections were examined under microscope for analyzing any changes in the architecture of the liver tissue due to paracetamol challenge and improved liver architecture due to pre-treatment with test extract and standard drug.[15]

Statistical analysis

A result of biochemical estimation has been expressed as mean ± Standard Error of Mean (SEM). The values were subjected to One Way Analysis of Variance (ANOVA) using Graph Prism version 3.0. The variance in a set of data has been estimated by Tukey multiple compare test. The values of $P < 0.05$ were considered statistically significant.

RESULTS

The effect of Turmesac® on histopathological and various biochemical parameters are shown in Table No.1 and Fig 1-3. It was observed that, the activities of serum SGOT, SGPT and TBR were increased GSH, SOD, CAT and TB decreased markedly in paracetamol fed animals as compared to normal control group. The administration of Turmesac® 250mg/kg and 500mg/kg lowered the paracetamol induced elevation of serum parameters. The Standard Silymarin 50mg/kgbw treatment showed extremely significant ($P<0.001$) reduction in SGPT, SGOT, TBR and increased GSH, SOD and TB. Turmesac® (250mg/kg bw and 500mg/kgbw) treated animals showed moderately significant ($P<0.05$) reduction in SGPT, SGOT & TBR and increased GSH, SOD and TB levels as compared to toxic control group. Histopathological examination of liver sections of normal rats showed normal hepatic cells with cytoplasm

and nucleus whereas paracetamol treated group showed various degrees of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of Turmesac® at a dose of 500 mg/kg significantly normalized these defects in the histological architecture of the liver (Fig-3A-E).

Effect of Turmesac® on serum SGPT (AST) level in paracetamol induced hepatotoxicity in albino wistar rats

The effect of Turmesac® on SGPT level in serum of paracetamol induced hepatotoxicity in male albino wistar rats [Fig-1A]. The control had shown the SGPT level in serum of 371±3.4IU/L but after paracetamol treatment, it increased to 829±2.1IU/L. whereas after administration of Turmesac® at the doses of 250 mg/kg, and 500 mg/kg, bw po in paracetamol in toxicated rats, the SGPT level reduced to 731±1IU/L and 546±0.9 IU/L respectively. These data suggested that Turmesac® might protect the liver against paracetamol induced injury by attenuating oxidative stress.

Effect of Turmesac® on serum SGOT level in paracetamol induced hepatotoxicity in albino wistar rats

The effect of Turmesac® on SGOT level in serum of paracetamol induced hepatotoxicity in male albino wistar rats (Fig-1B). The control had shown the SGOT level in serum of 374±0.56 IU/L but after paracetamol treatment, it increased to 929±2.10 IU/L. Whereas after administration of Turmesac® at the doses of 250 mg/kg and 500 mg/kg, bw po in paracetamol intoxicated rats, the SGOT level reduced to 707± 1.20 IU/L and 568±1.4 IU/L respectively.

Effect of Turmesac® on serum Total bilirubin level in paracetamol induced hepatotoxicity in albino wistar rats

The effect of Turmesac® on Total bilirubin level in serum of paracetamol intoxicated male albino rats (Fig-1C). The control has showed to bilirubin level in serum of (0.05±0.0003) mg/dL, but after paracetamol, it increased to (0.25±0.008) mg/dL. Whereas after administration of Turmesac® at a dose of 250mg/ kg bw and 500 mg/kg bw in paracetamol intoxicated rats, the bilirubin level reduced to (0.17±0.01) mg/dL and 0.13±0.004 mg/dL. The reduced level bilirubin was also observed 0.05±0.0003 mg/dL control group.

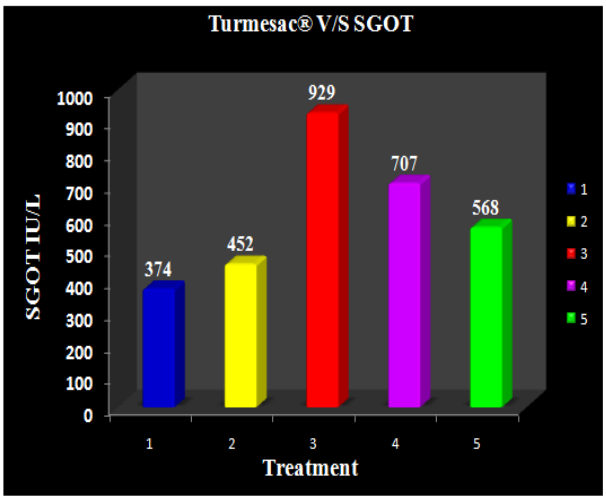
Effects of Turmesac® treatments on liver enzyme activities

The Effects of treatments for antioxidant enzyme activities Fig-1,D,E,F and G show changes of TP, GSH, SOD and CAT activities in the liver tissue indicating liver oxidative damage. Exposure to rats to paracetamol produced significant drops in TP (3.83±0.27), GSH (16±1.1), SOD (25±0.4) and CAT (46±1.1) ($P<0.05$) enzyme activities compared to other groups respectively. The rats received 250mg/kg.bw Turmesac®+PCM exhibited significantly elevated levels of TP (4.51±0.07), GSH (21±1.19), SOD (46±1.1) CAT (58.5±0.8) respectively. The administration of 500mg/kg.bw Turmesac®+ PCM exhibited significantly increased levels of TP (5.13±0.25), GSH (24±0.95) and SOD (60±1.6) CAT (62.6±0.88) in comparison with a paracetamol group ($P < 0.05$) but not with the control. In contrast, treatment of Turmesac® resulted in a significant amelioration of the enzyme (TP, GSH, SOD and CAT) activities.

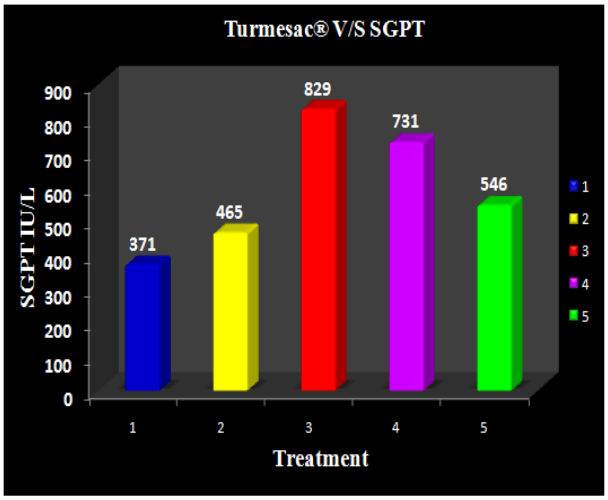
Histopathological studies of rat liver

Microscopic examination on normal liver section shows intact parenchymal cells. Mucosal glands are seen compactly arranged, consisting of cells with vesicular nuclei with nucleoli and abundant eosinophilic cytoplasm. Basement membrane is thick and intact (Fig-2 Group-1). The degeneration and necrosis of liver cells, presence of pycnotic nuclei, granular cytoplasm and increase in intercellular spaces with inflammatory collections and loss of cellular boundaries indicated with yellow circle (Fig-2 Group II). Group III treated with silymarin 50 mg/kg, body weight as reference drug shows intact parenchymal cells. Mucosal glands are seen compactly arranged without any abnormality or any degenerative changes of hepatocytes. In rats group treated with Turmesac® in two different doses 250mg/kg.bw and 500mg/kg.bw (Fig-2 Group IV and V), shows marked changes at the periphery, granular cytoplasm and decrease in intercellular spaces (indicated by circle) as compared to hepatotoxic control rats. Liver sections show minimal degenerative changes of hepatocytes with minimal swelling. The treatment with extracts showed that there is a significant reduction in tissue damage along with minimal evidence of inflammation.

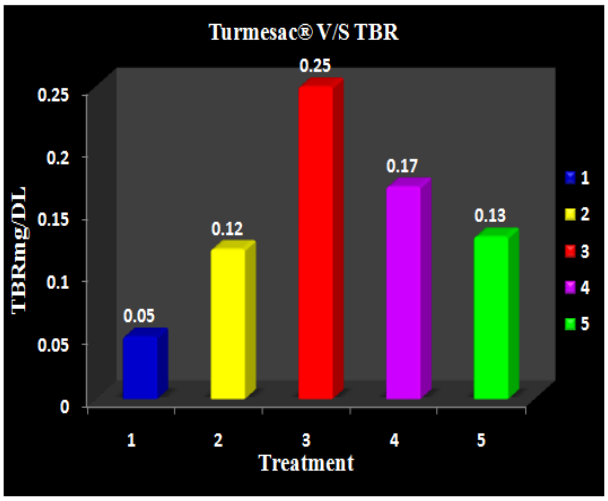
[A]. Effect of Turmesac® on serum SGOT level in paracetamol induced hepatotoxicity in albino wistar rats



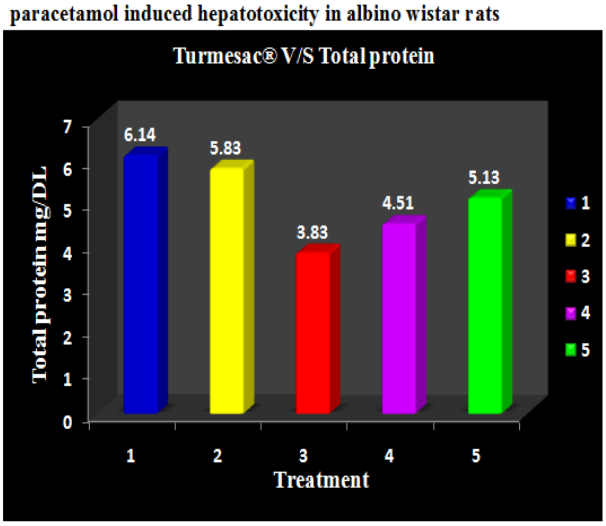
[B]. Effect of Turmesac® on serum SGPT level in paracetamol induced hepatotoxicity in albino wistar rats



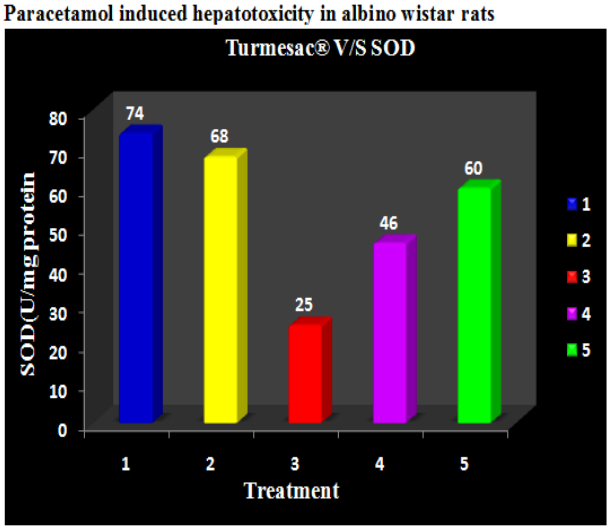
[C]. Effect of Turmesac® on serum Total bilirubin level in paracetamol induced hepatotoxicity in albino wistar rats



[D]. Effect Turmesac® on serum Total protein level in paracetamol induced hepatotoxicity in albino wistar rats



[E]. Effect of Turmesac® on serum SOD level in Paracetamol induced hepatotoxicity in albino wistar rats



[F]. Effect of Turmesac® on GSH level in paracetamol induced hepatotoxicity in albino wistar rats

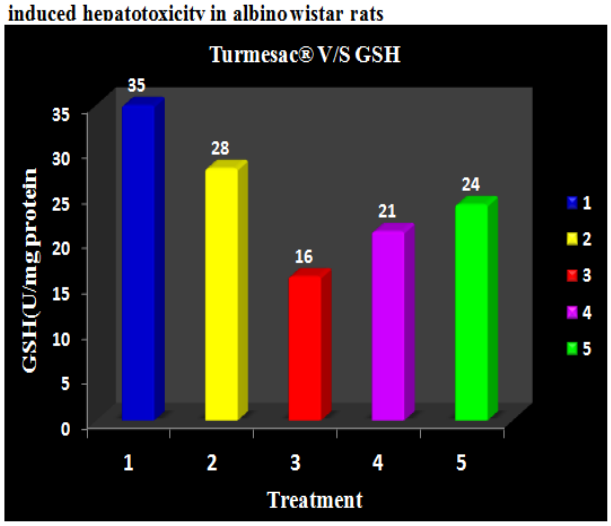
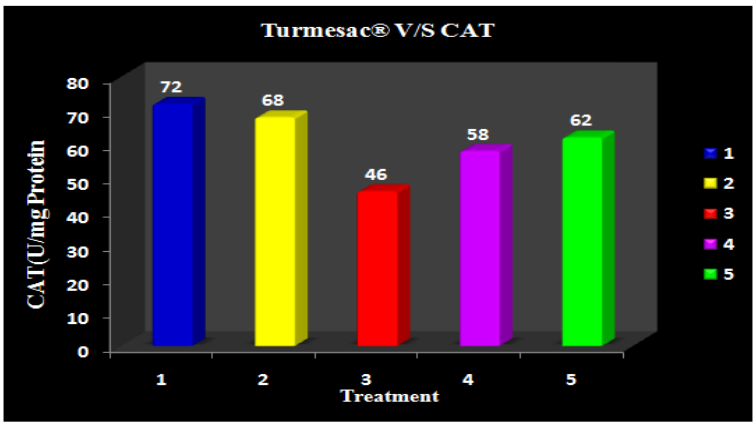
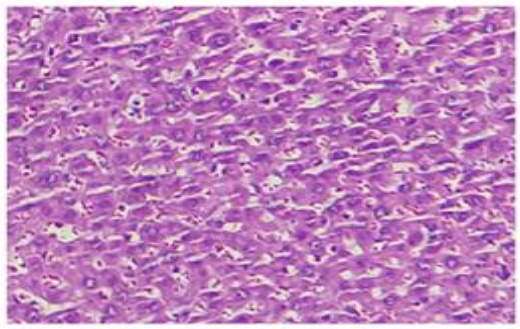


Figure 1: Biochemical evaluation of Turmesac® on liver different enzymes.

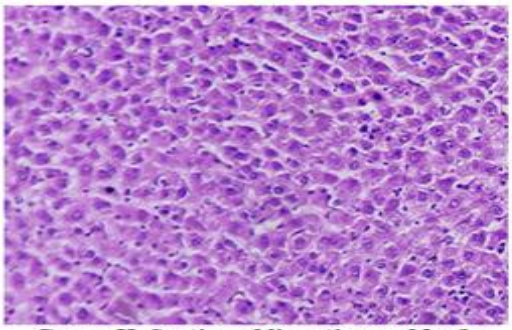
[G]. Effect of Turmesac® on CAT level in paracetamol induced hepatotoxicity in albino wistar rats



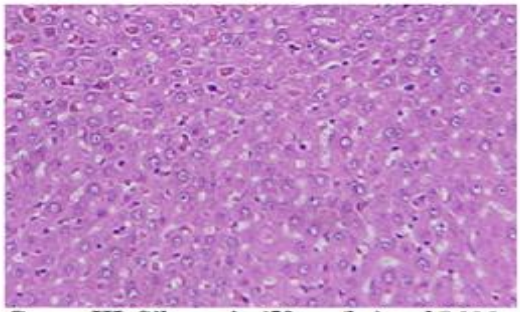
(1) Normal (D/W) (2) Silymarin (50 mg/kg) + Paracetamol (3) Paracetamol toxic Control (2g/kg) (4) Turmesac® (250 mg/kg) + Paracetamol (5) Turmesac® (500 mg/kg) + Paracetamol



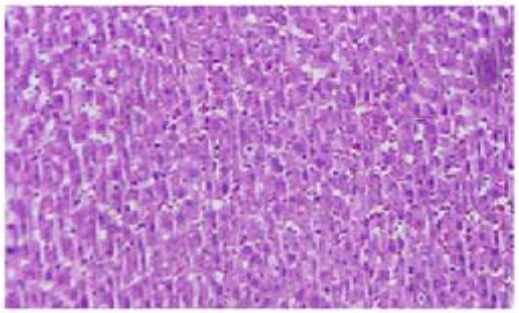
Group I. Normal control
Normal histological architecture of rat liver with central vein and radiating chords of hepatocytes.



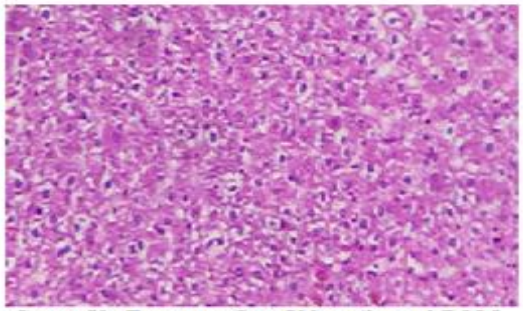
Group II. Section of liver tissue of 2 g/kg
PCM-treated group (p.o) showing massive coagulative necrosis, hemorrhage and inflammation.



Group III. Silymarin (50 mg/kg) and PCM treated group showing partial centrizonal protection.



Group IV. Turmesac® at 250 mg/kg and PCM showing almost complete protection of hepatocytes against paracetamol induced hepatotoxicity



Group V. Turmesac® at 500 mg/kg and PCM showing almost normal architecture of liver with few fatty vacuoles

Figure 2: Histopathological evaluation of the effect of various doses of Turmesac® against PCM-induced hepatic injury in rats.

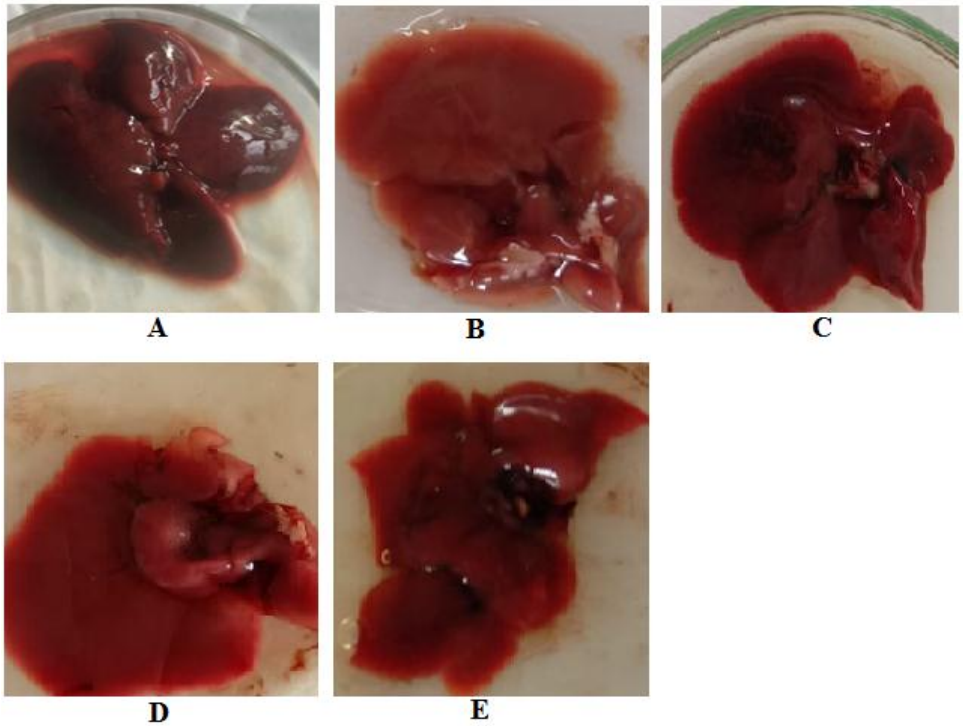


Figure 3: (A) Normal liver, (B) liver intoxicated with 2g/kg PCM: gross image shows major color changes of liver lobes (arrow), (C) liver pretreated with 50mg/kg silymarin and induced with PCM: spot of color changes was noted (arrow), (D) liver pretreated with 250 mg/kg Turmesac® and induced by PCM, (E) liver pretreated with 500 mg/kg Turmesac® and induced by PCM.

Table 1: Effect of Turmesac® on biochemical parameters.

Treatment Groups	SGOT (IU/L)	SGPT (IU/L)	GSH (/L)	TBR(mg/dL)	SOD U/mg protein	CAT	TP
Normal (D/W)	374±0.56	371±3.4	35±1.3	0.05±0.0003	74±0.6	72±0.95	6.14±0.24
Silymarin (50 mg/kg) + Paracetamol	452±3.16***	465±2.6***	28±0.6**	0.12±0.003***	68±0.6***	68±0.6*	5.83±.0.19^{ns}
Paracetamol toxic Control (2g/kg)	929±2.10***	829±2.1***	16±1.1***	0.25±0.008*	25±0.4***	46±1.1***	3.83±0.27***
Turmesac® (250 mg/kg) + Paracetamol	707± 1.20***	731±1.00***	21±1.19***	0.17±0.01***	46±1.1***	58.5±0.8***	4.51±0.07***
Turmesac® (500 mg/kg) + Paracetamol	568±1.4***	546±0.9***	24±0.95***	0.13±0.004***	60±1.6 ***	62.6±0.88***	5.13±0.25*

Values are given as mean±SEM (n=6rats)
*** Significant at p<0.001 when all treated compared with control group
* Significant at p<0.05

DISCUSSION

The liver is profoundly affected primarily by toxic agents. Hence, the liver marker enzymes are very sensitive markers for toxicity and are of great importance of the assessment of hepatic damage.[16] Activities of SGPT, SGOT and the level of serum bilirubin are mostly utilized as the most common SGPT, SGOT and TBR to examine the liver damage. Paracetamol-induced liver damaged is considered and also used for the toxic agent of liver toxicity.[17] This study, the important elevation of SGPT, SGOT and bilirubin level in rats treated with paracetamol, are suggestive of cellular leakage and defeat of functional integrity of the liver cell membrane.[18] Pretreatment group of rats with Turmesac® at doses levels of 250 mg/kg and 500 mg/kg showed significant (P < 0.001) different from control group, which proves at this doses the extract has hepatoprotective activity by restoring at the levels of SGOT (707± 1.20, 568±1.4), SGPT (731±1.00, 546±0.9) and TBR (0.17±0.01, 0.13±0.004) respectively.

Glutathione (GSH), extensively found in cells, protects cells against electrophilic attacks provided by xenobiotics such as free radicals and peroxides GSH deficiency leads to cellular damage in kidney, muscle, lung, jejunum, colon, liver, lymphocytes and brain (Orhan *et al*., 2007). The elevation of MDA level, which is one of the end products of lipid peroxidation in the

liver tissue, and the reduction in hepatic GSH levels are important indicators in paracetamol intoxicated rats.[19]

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found to the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Chance *et al*., 1952). Therefore the reduction in the activity of these enzymes may result in the number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of Turmesac® increased the activities of CAT in paracetamol induced liver damage in rats to prevent the accumulation of excessive free radical and protected the liver from paracetamol intoxication.[20]

Furthermore, Histopathological evaluation of the liver of paracetamol-treated rats showed necrosis and fatty degeneration with infiltration of inflammatory mediators. The animals treated with Turmesac® (250 mg/kg and 500 mg/kg) showed a significant improvement of paracetamol-induced liver injury evident from the presence of normal hepatic cords, the absence of necrosis, and a lesser degree of fatty infiltration. At the dose of 500 mg/kg, the liver architecture was near normal with only mild fatty changes (Fig-2 and 3). These observations were well correlated with the biochemical findings and gives clear evidence that there was not only improvement in the liver functions with the treatment of Turmesac®, but also in the hepatic architecture.

CONCLUSION

It can be concluded from the present study that the Turmesac® possesses hepatoprotective activity against paracetamol induced hepatotoxicity in a rat model. Turmesac® has demonstrated hepatoprotective activity based on biochemical parameters SGPT, SGOT, total bilirubin, GSH, SOD, total protein and CAT levels, and also by histopathological studies by preserving the normal architecture of liver tissue to a large extent. Further investigation into these promising protective effects of Turmesac® against paracetamol drug induced liver injury may have a considerable impact on developing clinically feasible strategies to treat patients with hepatotixicity.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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