Biofunctional significance of multi-herbal combination against paracetamol-induced hepatotoxicity in Wistar rats

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Abstract

A multi-herbal combination (MHC) of five herbs, namely *Punica granatum* L., *Putranjiva roxburghii* Wall., *Swertia chirata* Buch.-Ham., *Tinospora cordifolia* (Willd.) Miers and *Trigonella corniculata* L., was assessed against the paracetamol-induced acute hepatotoxicity in female Wistar rats. The animals were randomly assorted into seven groups with six animals in each group. The rats were pre-treated with MHC (50, 100, and 200 mg/kg bw) and silymarin (50 mg/kg bw) once daily for seven consecutive days via oral route followed by administration of paracetamol (3 g/kg bw) on day 7, an hour after the last administration of MHC and silymarin. It was observed that MHC administration significantly ($p \leq 0.05$) overturned the paracetamol-induced increase in serum liver function biomarkers (serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, and total bilirubin), phase I reaction enzymes (NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase), and oxidant biomarkers (lactate dehydrogenase, lipid peroxidation, lipid hydroperoxides, and protein content). MHC administration also reinstated the paracetamol-induced significant decrease ($p \leq 0.05$) in haematological indices (haematocrit, haemoglobin, red and white blood cells, and platelets), phase II reaction enzymes (glutathione-S-transferase and DT-diaphorase), membrane-bound enzymes (Na^+/K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase), and antioxidant biomarkers (reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase). Overall, MHC at 200 mg/kg bw dose significantly ($p \leq 0.05$) sheltered the red blood cells from the assault of free radicals, stabilized the structural and functional integrity of hepatocytes, hindered APAP biotransformation to its toxic metabolites, and endorsed conjugating abilities to detoxify toxic entities. Further, MHC significantly ($p \leq 0.05$) activated enzymatic machinery to scavenge/inhibit the formation of reactive oxygen species, regulated nucleic acid metabolism, surface potential, and membrane fluidity, attenuated tissue breakdown, quenched peroxyl radicals, and provided protection against tissue injury. The necroinflammatory scores revealed strong evidence of MHC (200 mg/kg bw) effectiveness against the paracetamol-induced hepatotoxicity in rats at $p \leq 0.05$. The synergistic effect of major inherent phytoconstituents (kaempferol, ellagic acid, and gallic acid), detected by HPLC-PDA, in MHC might have overturned the paracetamol-induced biochemical toxic alterations in rat liver.

Introduction

At present time, a holistic therapeutic approach using botanicals is intensely required to underscore the complex cell biology coupled with multi-factorial pathogenesis. Concerning this, the notion of the pharmacological action of botanicals due to a single active principle or purified compound has now been substituted by the concept of the cumulative effect of several phytoconstituents (Carmona and Pereira 2013). This perspective engrosses the interaction between different plant-derived bioactive constituents and forms the basis of phytotherapy. Hence, phytotherapeutics protrudes as a multi-target strategy that encompasses combinatorial intervention of multiple bioactive constituents in the management of pathological conditions (Long et al. 2015). The core of phytotherapy is the diverse cluster of phytochemicals present in herbs, fruits, vegetables, green tea, cereals, legumes, and nuts which exhibit

therapeutic or prophylactic action through their complex intervention. For example, polyphenols (a group of secondary metabolites), as a segment of this cluster, contribute to the prevention of many diseases at the cellular level and exert potential health benefits in humans (Tresserra-Rimbau et al. 2017). From a long-term perspective, pharmaceutical practices involving single active compound extraction models often lead to the resistance and failure of drug response under definite treatments of various ailments (Gnanaraj et al. 2017). Consequently, based on the folklore uses and beneficial health effects five plants i.e. *Punica granatum* L., *Putranjiva roxburghii* Wall., *Swertia chirata* Buch.-Ham., *Tinospora cordifolia* (Willd.) Miers, and *Trigonella corniculata* L. were scrutinized for the development of a multi-herbal combination (MHC) with biofunctional importance in antioxidative and antihepatoma studies, as discussed in our previous study (Kaur et al. 2019). For the MHC development, peel, leaves, whole herb, stems, and seeds of *P. granatum*, *P. roxburghii*, *S. chirata*, *T. cordifolia*, and *T. corniculata* were used, respectively. The study revealed that synergistic intervention of inherent phytoconstituents in MHC might have a multi-target prophylactic effect with better bioavailability due to effectual solubility in the aqueous medium. The study provided the evidence for anti-hepatocarcinoma action of MHC in HepG2 cells. However, the conventional two-dimensional (2D) planar cultures of HepG2 cells could presage capricious results which may cause inconsistent outcomes in normal hepatocytes (Kaur et al. 2018). The present study is the auxiliary investigation of MHC for its *in vivo* hepatoprotective significance against acetaminophen (APAP)-induced acute toxicity or drug-induced liver injury (DILI) in Wistar rats.

DILI may predispose to intrinsic and extrinsic environmental risk factors. The intrinsic implications are linked to the endogenous circadian rhythm, intestinal microbiome, hepatic clock, inflammation, and infection. Besides, extrinsic risk factors are associated with the regional geographic variation, socioeconomic status, environmental pollution, smoking, and alcohol consumption (Stine and Chalasani 2017). Amid these risks, alcohol consumption is a prime factor for paracetamol or APAP-induced hepatoxicity. APAP, a medication listed in the World Health Organization's list of essential medicines, is the most frequently used drug for the management of pain and fever (WHO 2019). For adolescents, of 50 kg or above weight, the U.S. Food and Drug Administration (FDA) recommended dosage of APAP is 1 g (Q6H: every 6 hours) or 0.65 g (Q4H: every 4 hours) restraining to a limit of 4 g per day (FDA 2015). Cases of acute liver failure linked with APAP overdose often result in liver transplantation or death. Many cases of acute liver injury associated with this drug frequently involve its presence in one or more OTC (over-the-counter) combination medications and prescribed medications. Several studies report that plant-based extracts and compounds with antioxidant and anti-inflammatory actions have a potential hepatoprotective effect and inhibit the biotransformation of APAP to its toxic metabolites (Abdel-Daim et al. 2018). Apropos to this, our newly developed MHC is assessed for its viability as a prospective formulation in *in vivo* hepatoprotective studies in Wistar rats against APAP-provoked acute hepatic dysfunction.

Material And Methods

Chemicals and reagents

APAP or N-(4-hydroxyphenyl)acetamide powder was procured from Sigma-Aldrich, Bangalore (India). All standard polyphenols with ≥ 90% purity for HPLC were procured from Sigma-Aldrich, Bangalore (India). For chromatographic analysis, HPLC-grade methanol and water were used. All other reagents and chemicals were of analytical grade.

Preparation of MHC extract

The disintegrated plant samples of *P. granatum*, *P. roxburghii, S. chirata*, *T. cordifolia*, and *T. corniculata* with a proportion of 33, 27, 25, 10, and 5% respectively, were soaked mutually and decoction was prepared as described in our previous study (Kaur et al. 2019). The filtered decoction was dried on a water bath and the extract was used for further experiments. The acquaintances of five herbs in this multi-herbal combination (MHC) were based on three essential points. The first point was the identification of prominent or primary herb(s), the second was the identification of the nourisher herb(s) and the third point was the categorization of active stimulator herb(s) (Fig. 1).

High-performance liquid chromatography-photo diode array (HPLC-PDA) analysis

HPLC-PDA analysis of MHC extract was performed on Nexera UHPLC (Shimadzu, Kyoto, Japan) as per the standardized procedure in our previous publication (Kaur et al. 2019). The Enable C-18 G (150 × 4.6 mm, 5 µm particle size) column was used at 25°C column oven temperature. The photo diode array detector was used with a 280 nm detection wavelength. The elution system (low-pressure gradient) with 0.1% acetic acid in water (A) and methanol (B) mobile phase were used. The flow rate was 1 ml/min with an injection volume of 5 µl. The gradient program started at 30% B to 45% B in 12 min, 45–75% B in 13.5 min, maintaining 75% B until 15 min, 75–50% B in 16.6 min, 50–25% B in 18 min, upholding 25% B until 20 min, 25–30% B in 21 min and stopped at 22 min with sustained elution until 26 min.

Animal care and handling

Healthy female Wistar rats weighing 150–200 g (6–8 weeks old) used in the present study were procured from the National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab (India). The rats were housed in the Central Animal House of Guru Nanak Dev University, Amritsar under hygienic conditions in polypropylene cages employing paddy husk bedding (changed regularly) at a temperature of 25 ± 2°C with a 12-hour light / 12-hour dark cycle. The animals were fed on a standard pellet diet and had tap water *ad libitum* throughout treatment. The animals for the experimental purpose were allowed to acclimatize for two weeks prior to the commencement of the treatment schedule.

Experimental design

The animals were randomly assorted into seven groups (Group I-VII) with six animals in each group (n = 6). The rats were pre-treated with MHC and silymarin once daily for 7 consecutive days followed by administration of paracetamol on the 7th day an hour after the last dose of the respective drug was given. Hepatotoxicity was induced by a single toxic dose of paracetamol (3 g/kg bw) suspended in distilled water as reported previously by Madkour and Abdel-Daim (2013). The different concentrations of

MHC and standard silymarin were administered as per body weight (bw) and the doses were given to animals via oral route using a canula (Table 1).

Table 1
The experimental design and animal grouping

Treatment groups: Female Wistar rats (*n* = 6)						
I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Dose administration via p.o. **route**						
Day 1–7	**Day 7**	**For seven consecutive days**				
Standard pellet diet and tap water *ad libitum* (Untreated control)	Paracetamol (3 g/kg bw)	MHC (200 mg/kg bw) per se	MHC (50 mg/kg bw)	MHC (100 mg/kg bw)	MHC (200 mg/kg bw)	Silymarin (50 mg/kg bw)
			Paracetamol (3 g/kg bw) on day 7, an hour after the last administration of MHC and silymarin			
Animals in above stated seven groups were sacrificed 48 h post-administration of paracetamol						

Group I: Normal control included the animals that were put on a normal pellet diet. **Group II**: Paracetamol (3 g/kg bw) in which animals received a single high dose of paracetamol on the 7th day to assess the severity of acute hepatic damage. **Group III**: MHC (200 mg/kg bw) per se included rats which were administered with the high dose of MHC once daily for seven days to ensure that it did not provoke any sort of toxicity itself. The animals of **Group IV**: MHC (50 mg/kg bw) + Paracetamol, **Group V**: MHC (100 mg/kg bw) + Paracetamol, and **Group VI**: MHC (200 mg/kg bw) + Paracetamol received low, mid, and high doses of MHC respectively for seven days and were intoxicated with paracetamol an hour after the administration of the last dose of combination on the 7th day. **Group VII**: Silymarin (50 mg/kg bw) + Paracetamol in which animals were treated with the aqueous suspension of silymarin once daily for seven days followed by a single oral dose of paracetamol on the 7th day. The choice of dose of silymarin was based on a previous study in the literature (Paul et al. 2016).

Estimation of haematological indices

Blood samples were withdrawn from the rats in the above stated seven groups 48 h post-administration of paracetamol. The samples were collected by puncturing the retro-orbital venous plexus with sterilized capillary under light diethyl ether anaesthesia into EDTA coated vacutainers for determination of haematological parameters (haematocrit, haemoglobin concentration, red blood cells (RBCs), white blood cells (WBCs), and platelet count) using Automated Haematology Cell Counter.

Estimation of serum liver function biomarkers

For serum biochemical assessment blood samples were collected in clot activator vacutainers and samples were kept for 20 min at room temperature followed by centrifugation of the coagulated blood at 3000 rpm for 10 min. The clear supernatant designated as the serum was kept at -80°C until analysed (Kale et al. 2012). The biomarkers of hepatic injury *viz*., serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (ALP), and total bilirubin were estimated by means of Erba Mannheim XL-640 Autoanalyzer using Erba Mannheim XL SYSPACK kits.

Preparation of liver homogenate

Following blood collection, the rats were sacrificed by cervical dislocation and the livers of animals were perfused instantly with ice-cold sodium chloride (0.9%), isolated carefully, trimmed free of any adhering fat and other extraneous tissues. The organs were then suspended and rinsed in chilled 0.15 M Tris-KCl buffer (pH 7.4) consisting of potassium chloride (0.15 M) and tris-hydrochloride (10 mM). Thereafter, liver samples were blotted dry, weighed immediately, and homogenized by means of a glass Teflon homogenizer in a buffer to yield 10% (w/v) liver homogenate. To 500 µL of this homogenate trichloroacetic acid (5%) was added following centrifugation at 2000 rpm to allow sedimentation of tissue debris and the supernatant was utilized to determine the content of reduced glutathione. The remaining homogenate was centrifuged for 20 min at 10,000 rpm and supernatant thus obtained, after disposing of unbroken cells, any floating lipid layer, cell debris, was used for the assessment of different enzymes such as phase-I, phase-II, antioxidant and membrane-bound enzymes (Kaur and Arora 2013).

Estimation of phase-I reaction enzymes

NADPH-cytochrome P450 reductase (CPR)

The activity of CPR was determined based on the rate of oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) and reduction of potassium ferricyanide as reported by Omura and Takesue (1970). The specific activity was expressed in terms of IU/mg protein. Here, IU signifies µM NADPH oxidised/min/mg protein content.

$$Specificactivity = \frac{Changeinabsorbance \times Totalreactionvolume \times dilution}{Extinctioncoefficient \times Proteincontent}$$

NADH-cytochrome b5 reductase (b5R)

The specific activity of b5R based on the rate of oxidation of nicotinamide adenine dinucleotide hydride (NADH) was estimated as per the method outlined by Mihara and Sato (1972). The specific activity of b5R was expressed in terms of IU/mg protein. Here, IU signifies µM ferricyanide reduced/min/mg protein content.

$$Specificactivity = \frac{Changeinabsorbance \times Totalreactionvolume \times dilution}{Extinctioncoefficient \times Proteincontent}$$

Estimation of phase-II reaction enzymes

Glutathione-S-transferase (GST)

The enzymatic activity of GST, to detoxify the xenobiotics, was determined as described by Habig et al. (1974) and expressed in terms of IU/mg protein. Here, IU signifies μM glutathione-CDNB (1-Chloro-2,4-dinitrobenzene) conjugate formed/min/mg protein content.

$$Unitactivity = \frac{Changeinabsorbance/minute \times Totalreactionvolume}{Extinctioncoefficient \times Volumeofhomogenateused}$$

$$Specificactivity = \frac{Unitactivity}{Proteincontent}$$

DT-diaphorase (DTD)

NAD(P)H quinone oxidoreductase 1 (DTD) was assayed adopting the methodology of Ernster (1967) in which NADH is used as an electron donor, 2,6-dichlorophenolindophenol (DCPIP) as an electron acceptor, and bovine serum albumin (BSA) as an activator. The values were expressed as IU/mg protein. This assay engrosses oxidation of NADH and reduction of DCPIP catalysed by DTD. Thus, here IU signifies μM DCPIP reduced/min/mg protein content.

$$Unitactivity = \frac{Changeinabsorbance/minute \times Totalreactionvolume}{Extinctioncoefficient \times Volumeofhomogenateused}$$

$$Specificactivity = \frac{Unitactivity}{Proteincontent}$$

Estimation of membrane-bound enzymes

Na^+/K^+-ATPase

The Na^+/K^+-ATPase enzyme pumps out Na^+ and K^+ into cells against the concentration gradient using energy from adenosine triphosphate (ATP) and is measured by the release of inorganic phosphorous. The assay was performed as per the methodology given by Bonting (1970). The assessment of phosphorous content was carried out as per the procedure described by Fiske and Subbarow (1925). The enzymatic activity was expressed as μM phosphorous liberated/min/mg protein.

Ca^{2+}-ATPase

The methodology of Hjerten and Pan (1983) was followed for the determination of Ca^{2+}-ATPase activity. The amount of phosphorous liberated from the substrate was assessed as per the method described by Fiske and Subbarow (1925). The enzyme activity was expressed in terms of μM phosphorous liberated/min/mg protein.

Mg^{2+}-ATPase

The Mg^{2+}-ATPase enzyme activity was assayed by the method prescribed by Ohnishi et al. (1982) and phosphorous content was estimated by following the protocol of Fiske and Subbarow (1925). The results of enzyme activity were expressed as µM phosphorous liberated/min/mg protein.

Estimation of oxidant/antioxidant biomarkers

Oxidant biomarkers

Lactate dehydrogenase **(LDH) -** The methodology of Kuznetsov and Gnaiger (2010) was followed to evaluate the rate of oxidation of pyruvate by NADH, determining the enzymatic activity of LDH. The results of specific activity were expressed as IU/mg protein where IU represents µM NADH reduced/min/mg protein content.

$$Unitactivity = \frac{Changeinabsorbance/minute \times Totalreactionvolume}{Extinctioncoefficient \times Volumeofhomogenateused}$$

$$Specificactivity = \frac{Unitactivity}{Proteincontent}$$

Lipid peroxidation (LPO)

LPO was determined as per the method described by Devasagayam et al. (2003) in terms of the formation of characteristic pink-coloured thiobarbituric acid reactive substances (TBARS) and the absorbance was measured at 532 nm using an ELISA microplate reader (Synergy HT, BioTek). The results were expressed as µM malondialdehyde (MDA) equivalent/g tissue calculated from the calibration curve obtained using MDA as a standard.

Lipid hydroperoxide

The content of lipid peroxides was calculated from the regression equation obtained by using hydrogen peroxide as standard and values were expressed as mM H_2O_2 equivalent/g tissue. This method involves the oxidation of ferrous ions to ferric ions in an acidic environment. Ferric ions then form a complex with xylenol orange which is measured at 560 nm (Jiang et al. 1992).

Protein content

The protein content in tissue homogenate was estimated using the procedure given by Smith et al. (1985) in which sodium salt of bicinchoninic acid forms a purple complex with cuprous ions under alkaline conditions. BSA was used as a standard to draw the regression equation and to determine the protein content in terms of mg/g tissue.

Antioxidant biomarkers

Reduced glutathione **(GSH)**

The content of GSH in liver tissues was assessed by its reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) following the methodology of Anderson (1985). The glutathione content was calculated in terms of mM SH content/g tissue from the regression equation obtained by using GSH as a standard.

Superoxide dismutase **(SOD) -** The activity of SOD was assayed following the method proposed by Kono (1978) based on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) accompanying autoxidation of hydroxylamine. Specific activity of the enzyme was expressed as IU/mg protein. IU indicates units of SOD enzyme/min/mg protein.

Percent inhibition:

$$\frac{Changeinabsorbance/minute(Blank) - Changeinabsorbance/minute(Sample)}{Changeinabsorbance/minute(Blank)} \times 100 = x$$

Where,

x = % inhibition of NBT reduction by 70 µL of homogenate

$$SODenzymein70\mu Lofhomogenate = \frac{1}{50} \times x = yunit$$

$$Unitactivity = \frac{y}{70} \times 1000$$

$$Specificactivity = \frac{Unitactivity}{Proteincontent}$$

Catalase **(CAT)** - CAT was analyzed on the basis of the rate of disappearance of hydrogen peroxide at 240 nm as per the method given by Aebi (1984). The specific activity of CAT was expressed as IU/mg protein. Here, IU indicates the µM hydrogen peroxide decomposed/min/mg protein content.

$$Unitactivity = \frac{Changeinabsorbance/minute \times Totalreactionvolume}{Extinctioncoefficient \times Volumeofhomogenateused}$$

$$Specificactivity = \frac{Unitactivity}{Proteincontent}$$

Glutathione peroxidase **(GPx) -** GPx was assayed according to the procedure described by Paglia and Valentine (1967) which involves indirect measurement of its activity. The specific activity was expressed in terms of IU/mg protein. IU signifies µM NADPH oxidized/min/mg protein content.

$$Unitactivity = \frac{Changeinabsorbance/minute \times Totalreactionvolume}{Extinctioncoefficient \times Volumeofhomogenateused}$$

$$Specificactivity = \frac{Unitactivity}{Proteincontent}$$

Glutathione reductase **(GR) -** The protocol of Carlberg and Mannervik (1985) was followed to determine the activity of GR based on the amount of NADPH consumed in the conversion of oxidized glutathione to reduced glutathione. The results were expressed as IU/mg protein. Here, IU represents μM NADPH oxidised/min/mg protein content.

$$Unitactivity = \frac{Changeinabsorbance/minute \times Totalreactionvolume}{Extinctioncoefficient \times Volumeofhomogenateused}$$

$$Specificactivity = \frac{Unitactivity}{Proteincontent}$$

Histopathological examination of liver

The livers from each of the six animals of all experimental groups were excised and preserved in formalin (10%) solution. Then liver specimens were processed, embedded in paraffin blocks, and 3–4 μm thick sections were prepared followed by staining with haematoxylin and eosin in compliance with the standard techniques. Liver sections were coded and then observed microscopically for pathological findings such as coagulative necrosis, apoptosis, and portal triad inflammation by the pathologist. Ishak modified histological index grading (HAI) was used to score and evaluate liver damage (Ishak et al. 1995).

Statistical analysis

The resultant data of six animals were presented as mean ± standard error (SE). The statistical differences among mean data were assessed by one-way analysis of variance (ANOVA) at a 5% level of significance i.e. $p \leq 0.05$. Post hoc Tukey's HSD was performed at $p \leq 0.05$ and statistical pairwise comparisons were presented by lowercase letters displayed on bar graphs. The Kruskal-Wallis H test with Dunn-Bonferroni post hoc was performed at $p \leq 0.05$ to test the significant differences between seven treatment groups (Group I-VII) of independent variables on ordinal dependent variables i.e. scores of Ishak modified HAI necroinflammatory grading.

Results

HPLC-PDA analysis

The HPLC chromatogram revealed the presence of major polyphenolic compounds as shown in Fig. 2. It was observed that the highest amount of kaempferol (40.68 μg/mg) was detected in MHC followed by ellagic acid, gallic acid, quercetin, and epicatechin.

In vivo **hepatoprotective studies**

Percentage change in body weight

The percentage change in body weight of Wistar rats was observed at the termination of the experiment as shown in Fig. 3. It was found that the highest body weight change (8.718%) was observed in rats pre-treated with 200 mg/kg bw dose of MHC while the minimum body weight change (5.481%) was seen in paracetamol intoxicated animals. No statistical difference was observed at a 5% level of significance on comparing the normal and paracetamol intoxicated group. Furthermore, pre-treatment with MHC at all the three tested doses (50, 100, and 200 mg/kg bw), silymarin (50 mg/kg bw), and MHC (200 mg/kg bw) per se also revealed no significant difference in comparison to normal control at $p \leq 0.05$. Supplementary data are given in Table S1.

Haematological indices

Haematocrit

Hepatic injury incited by paracetamol caused a significant decrease ($p \leq 0.05$) in haematocrit (35.70%) as compared to untreated control (44.52%). However, administration of MHC resulted in a significant increase in the percentage of haematocrit in a dose-responsive manner, i.e. 39.23, 42.65, and 45.03% at doses of 50, 100, and 200 mg/kg bw, respectively (Fig. 4a). It was also noticed that the highest dose of MHC restored the parameter to the normal range with no statistically significant difference at $p \leq 0.05$. Silymarin (50 mg/kg bw) used as a standard in the present study showed a value of 42.63% while MHC per se displayed 41.42%. The feeding of animals with MHC (200 mg/kg bw) per se did not reveal any significant sign of toxicity. Supplementary data are given in Table S2.

Haemoglobin

The protective effect of pre-treatment of MHC and silymarin on paracetamol-induced alterations in haemoglobin content is presented in Fig. 4b. Intoxication of hepatotoxin at 3 g/kg bw dose to the rats caused a significant reduction ($p \leq 0.05$) in haemoglobin (8.567 g/dL) when compared with normal control (12.70 g/dL). Regarding MHC, regulation in haemoglobin was observed with values of 10.63, 11.20, and 12.33 g/dL at the doses of 50, 100, and 200 mg/kg bw respectively. Silymarin pre-administration showed a value of 12.40 g/dL. Both the highest dose of MHC and silymarin reflected statistical similarity at a 5% level of significance. Also, 200 mg/kg bw dose of MHC per se demonstrated the value of 11.55 g/dL and had no significant toxic effect on haemoglobin content. Supplementary data are given in Table S2.

Total RBCs count

The effect of paracetamol intoxication as well as pre-treatment of MHC and silymarin on total RBCs count is presented in Fig. 4c. Group II animals that were given a toxic dose (3 g/kg bw) of paracetamol showed a marked decrease ($p \leq 0.05$) in total RBCs count (3.225×10^{12}/L) in comparison to normal group animals (4.898×10^{12}/L). Pre-administration of MHC at 50, 100, and 200 mg/kg bw showed dose-dependent increase in values i.e. 4.670×10^{12}/L, 4.812×10^{12}/L, and 4.983×10^{12}/L respectively. Silymarin administration (50 mg/kg bw) displayed the total RBCs count of 4.767×10^{12}/L. However,

group comparison revealed no significant difference among the groups pre-treated with the three doses of MHC and silymarin. In addition, the total RBCs count was observed to be 4.952 x 10^{12}/L in rats administered with MHC (200 mg/kg bw) per se reflecting no significant difference at $p \leq 0.05$ in comparison to untreated control. Supplementary data are given in Table S2.

Total WBCs count

The protective effect of MHC and silymarin pre-treatment on total WBCs count against paracetamol intoxication is presented in Fig. 4d. Group II animals that were challenged with a toxic dose of paracetamol (3 g/kg bw) showed a marked decrease ($p \leq 0.05$) in total WBCs count (3.430 x 10^9/L) in comparison to normal group animals (6.627 x 10^9/L). Pre-treatment with MHC at 50, 100, and 200 mg/kg bw doses showed the values 6.478 x 10^9/L, 6.522 x 10^9/L, and 6.688 x 10^9/L respectively. Also, silymarin pre-administration displayed the total WBCs count of 6.828 x 10^9/L. However, group comparison among the three tested doses of MHC and silymarin revealed no significant difference at $p \leq 0.05$. In addition, the total WBCs count was observed to be 6.432 x 10^9/L in animals administered with MHC at 200 mg/kg bw dose per se indicating no apparent sign of toxicity. Supplementary data are given in Table S2.

Platelet count

The toxic effect of paracetamol intoxication as well protective response of MHC and silymarin pre-treatment on platelet count is presented in Fig. 4e. Animals that were intoxicated with paracetamol showed a significant decrease ($p \leq 0.05$) in platelet count (134.5 x 10^9/L) in comparison to the normal group (327 x 10^9/L). However, MHC at 50, 100, and 200 mg/kg bw dose administration showed the values 332.9 x 10^9/L, 335.2 x 10^9/L, and 352.3 x 10^9/L respectively. Pre-treatment with silymarin displayed a platelet count of 335.8 x 10^9/L. Group comparison revealed no significant difference among the groups that were given 50, 100, and 200 mg/kg bw dose of MHC and 50 mg/kg bw dose of silymarin. In addition, platelet count was observed to be 359 x 10^9/L in rats administered with MHC (200 mg/kg bw) per se indicating no apparent sign of toxicity. Supplementary data are given in Table S2.

Serum liver function biomarkers

Serum glutamic oxaloacetic transaminase (SGOT)

The level of SGOT was seen to be 785 IU/L in paracetamol intoxicated animals, which was observed to be significantly higher ($p \leq 0.05$) than that of control (106.3 IU/L). This induction in serum level was diminished dose-dependently by oral pre-treatment of MHC. As seen from Fig. 5a, values were 447.2, 267.8, and 175.4 IU/L in groups administered with 50, 100, and 200 mg/kg bw doses of MHC respectively. The value of 98.27 IU/L in animals that were given 200 mg/kg bw dose of MHC indicated that the combination by itself did not show any toxicity and no significant difference in comparison to the control group was observed. Similarly, silymarin pre-treatment also reduced the elevated level of SGOT to 147.8 IU/L. Further, the highest dose of MHC (200 mg/kg bw) and silymarin (50 mg/kg bw) reflected the

potential to counteract the toxic effects of paracetamol with no statistical difference at a 5% level of significance. Supplementary data are given in Table S3.

Serum glutamic pyruvic transaminase (SGPT)

The biochemical investigation of serum revealed a prominent elevation ($p \leq 0.05$) in SGPT level in animals administered with a 3 g/kg bw dose of paracetamol as compared to the normal group. The values were observed to be 128.6 IU/L and 32.39 IU/L in the paracetamol and normal group respectively. The results presented in Fig. 5b indicated that rats administered with MHC for seven consecutive days reduced the elevated level to 84.43, 55.03, and 37.46 IU/L in a dose-responsive manner while in the silymarin treated group, the value was observed to be 30.43 IU/L. It was elucidated that values exhibited by 200 mg/kg bw dose of MHC and 50 mg/kg bw dose of silymarin were statistically similar ($p \leq 0.05$) to the basal value as seen in the control group. In addition, MHC per se showed the value 37.61 IU/L and did not produce any significant change in the parameter studied in comparison to control. Supplementary data are given in Table S3.

Alkaline phosphatase (ALP): In the present study, it was observed that level of ALP was significantly augmented ($p \leq 0.05$) in animals receiving paracetamol (838.0 IU/L) than the control group (276.2 IU/L). However, pre-treatment of MHC at 50, 100, and 200 mg/kg bw for seven consecutive days significantly influenced the toxic effects by reducing the values in a dose-dependent way (Fig. 5c). The values were observed to be 551.6, 364.4, and 274.7 IU/L at 50, 100, and 200 mg/kg bw dose respectively. A significant suppression (184.3 IU/L) at a 5% level was also seen in the silymarin-treated group in comparison to the paracetamol intoxicated group. Also, the value exhibited by MHC per se (208.4 IU/L) was not statistically different from the silymarin administered group. Supplementary data are given in Table S3.

Total bilirubin

As given in Fig. 5d, it was observed that paracetamol intoxication significantly raised ($p \leq 0.05$) the level of total bilirubin (1.055 mg/dL) in comparison to the control group (0.157 mg/dL). However, pre-treatment of animals with 50, 100, and 200 mg/kg bw dose of MHC revealed a significant reduction in total bilirubin with values of 0.560, 0.348, and 0.313 mg/dL respectively. The degree of protection was observed to be increased in a dose-responsive manner. Silymarin treatment afforded protection against injurious effects of paracetamol by displaying the value of 0.213 mg/dL. Further, MHC at the mid and highest dose (100 and 200 mg/kg bw) and the silymarin group displayed no significant variation ($p \leq 0.05$) when compared with normal control. This also indicated that MHC revealed a similar protective effect to that of silymarin. Also, it was noted that MHC per se did not display any adverse outcome as there was no significant difference in the parameter studied in comparison to the normal group. Supplementary data are given in Table S3.

Phase-I reaction enzymes

NADPH-cytochrome P450 reductase (CPR)

A significant augmentation (41.62 IU/mg protein) in enzyme level of CPR was seen in the paracetamol treated group as compared to the untreated group (16.89 IU/mg protein) at $p \leq 0.05$. While pre-administration of MHC witnessed a reduction in the enzyme level with values 33.43, 23.74, and 11.46 IU/mg protein in a dose-dependent way i.e. at 50, 100, and 200 mg/kg bw respectively (Fig. 6a). In the present findings, it was seen that the mid and highest dose of MHC (100 and 200 mg/kg bw) reflected the better effect in bringing down the elevated level to near basal value as no statistical significance at $p \leq 0.05$. Similarly, silymarin rendered protection against the toxic effects of paracetamol and showed an enzymatic value of 21.93 IU/mg protein which was comparable to the normal group. In MHC per se group, the value was found to be 14.35 IU/mg protein and did not reach any statistical significance when compared with normal control indicating no sign of toxicity by the highest dose itself. Supplementary data are given in Table S4.

NADH-cytochrome b5 reductase (b5R)

In the present findings, the level of b5R was markedly increased (27.11 IU/mg protein) in the paracetamol-treated group as compared to the untreated group (11.82 IU/mg protein). As observed from Fig. 6b, statistical analysis between the normal and paracetamol groups revealed a significant difference at $p \leq 0.05$. MHC pre-treatment attenuated the paracetamol-induced hepatic dysfunction by reducing the values to 21.73, 11.50, and 6.027 IU/mg protein in a dose-dependent manner at the doses of 50, 100, and 200 mg/kg bw respectively. However, the mid and highest dose of MHC (100 and 200 mg/kg bw) and silymarin pre-administration exhibited a statistical similarity with the normal group. Meanwhile, MHC alone when administered to rats showed the value of 11.86 IU/mg protein and revealed no significant effect ($p \leq 0.05$) with the parameter being comparable to normal control animals. Supplementary data are given in Table S4.

Phase-II reaction enzymes

Glutathione-S-transferase (GST): Fig. 6c represents the specific activity of the GST enzyme in different experimental groups. It was found that after a single high dose of paracetamol (3 g/kg bw), the activity of GST was markedly decreased (1.840 IU/mg protein) in comparison to the control group (7.390 IU/mg protein). A significant difference ($p \leq 0.05$) in the level of GST of the normal and paracetamol group was noticed. Pre-treatment of animals with three different doses of MHC (50, 100, and 200 mg/kg bw) and silymarin (50 mg/kg bw) elevated the expression of enzymes to 3.705, 4.767, 5.958, and 6.766 IU/mg protein respectively. Further, it was observed that 200 mg/kg bw dose of MHC and silymarin significantly increased the level of enzyme and restored the activity to the values that were statistically similar to the normal group at a 5% level of significance. While in MHC per se group, the enzymatic value was seen to be 4.513 IU/mg protein. Supplementary data are given in Table S4.

DT-diaphorase (DTD)

In the present design of the experiment, paracetamol administration to Group II animals resulted in the depletion of specific activity of DTD enzyme (2.768 IU/mg protein) as compared to the control group

(6.944 IU/mg protein). Group comparison between the two revealed a significant difference at a 5% level of significance. Pre-administration of animals with MHC offered protection against the depleted enzymatic activity. At all the tested doses of combination i.e. 50, 100, and 200 mg/kg bw, the enzymatic activity was recovered displaying values 3.599, 5.326, and 5.679 mg/kg bw respectively (Fig. 6d). However, the mid and highest dose (100 and 200 mg/kg bw) represented statistical similarity at $p \leq 0.05$ with the normal group. Similarly, there was no significant difference in the activity of the enzyme under study in silymarin pre-treated rats (7.181 IU/mg protein) and normal rats. Also, the value was observed to be 4.434 IU/mg protein on the administration of animals with MHC (200 mg/kg bw) alone which was not seen to be apparent with the normal control. Supplementary data are given in Table S4.

Membrane-bound enzymes

Na^+/K^+-ATPase

The activity of Na^+/K^+-ATPase in the liver of different treatment groups is presented in Fig. 7a. The level of this enzyme was significantly decreased ($p \leq 0.05$) in paracetamol-challenged animals (0.129 µM phosphorous liberated/min/mg protein) when compared with normal rats (0.377 µM phosphorous liberated/min/mg protein). On the contrary, the level of Na^+/K^+-ATPase was seen to be increased in a dose-responsive manner when animals were pre-administered with MHC i.e. the values were 0.155, 0.184, and 0.211 µM phosphorous liberated/min/mg protein at 50, 100, and 200 mg/kg bw dose respectively. However, as revealed by statistical analysis, the highest dose of MHC exhibited a value similar to the silymarin treated group (0.288 µM phosphorous liberated/min/mg protein) with no statistical difference at a 5% level of significance. Also, the activity of this enzyme was seen to be 0.262 µM phosphorous liberated/min/mg protein when animals were given MHC at 200 mg/kg bw dose per se, showing statistical similarity with the untreated control. Supplementary data are given in Table S5.

Ca^{2+}-ATPase: Fig. 7b shows the modulatory effect of MHC pre-treatment on paracetamol-induced altered Ca^{2+}-ATPase activity in the liver tissue of different treatment groups. A significant decrease ($p \leq 0.05$) in the level (0.114 µM phosphorous liberated/min/mg protein) was observed in the paracetamol group in comparison to the basal value in control animals (0.633 µM phosphorous liberated/min/mg protein). While, the activity of the enzyme was found to be increased in MHC at a dose of 50, 100, and 200 mg/kg bw (0.180, 0.287, and 0.499 µM phosphorous liberated/min/mg protein respectively) in a dose-responsive way counteracting the adverse effects of paracetamol intoxication. Similarly, the level of Ca^{2+}-ATPase in the silymarin pre-treated group was seen to be 0.526 µM phosphorous liberated/min/mg protein. Based on the statistical comparison, it was revealed that pre-treatment with the highest dose of MHC (200 mg/kg bw) and silymarin were equally effective in normalizing the diminished enzyme level. In addition to this, it was also found that exposure of animals to MHC alone did not significantly alter the parameter under study when compared with normal animals in group I. Supplementary data are given in Table S5.

Mg^{2+}-ATPase

Intoxication of animals with paracetamol resulted in a significant decrease ($p \leq 0.05$) in Mg^{2+}-ATPase activity (0.544 µM phosphorous liberated/min/mg protein) in comparison to control group (1.563 µM phosphorous liberated/min/mg protein) (Fig. 7c). However, pre-treatment of animals with MHC increased the level of this membrane-bound enzyme in a dose-responsive manner with values 0.886, 1.048, and 1.359 µM phosphorous liberated/min/mg protein at 50, 100, and 200 mg/kg bw dose respectively and protected the animals against paracetamol provoked membrane damage. Likewise, a decline in the specific activity of the enzyme was counteracted by silymarin pre-treatment (1.786 µM phosphorous liberated/min/mg protein). It was seen that both the highest dose of MHC (200 mg/kg bw) and silymarin (50 mg/kg bw) demonstrated statistically similar values as that of the normal group. Also, MHC alone exhibited the value of 1.396 µM phosphorous liberated/min/mg protein with no significant difference at $p \leq 0.05$ as compared to untreated control. Supplementary data are given in Table S5.

Oxidant/antioxidant biomarkers

Oxidant biomarkers

Lactate dehydrogenase (LDH)

The animals treated with a single toxic dose (3 g/kg bw) of paracetamol developed significant hepatic injury as observed from elevated level (49.55 IU/mg protein) of LDH enzyme in comparison to the control group (10.10 IU/mg protein). Statistical comparison between both these groups displayed a significant difference at $p \leq 0.05$. Pre-feeding of animals with MHC afforded protection against paracetamol-induced elevation in a dose-responsive way, i.e. the values were found to be 38.22, 26.68, and 16.54 IU/mg protein at 50, 100, and 200 mg/kg bw dose respectively (Fig. 8a). The LDH level was seen to be 7.901 IU/mg protein in the silymarin administered group. Meanwhile, the highest dose (200 mg/kg bw) of MHC and silymarin represented no statistical significance ($p \leq 0.05$) with the normal group. However, a comparison between these two treatments illustrated a significant difference. Supplementary data are given in Table S6.

Lipid peroxidation (LPO)

In the present investigation, the normal basal value of TBARS content was seen to be 48.54 µM MDA equivalent/g tissue while paracetamol treatment raised the value to 157.9 µM MDA equivalent/g tissue which was significantly highest ($p \leq 0.05$) among all treatment groups. However, MHC at 50, 100, and 200 mg/kg bw declined the formation of TBARS to 117.0, 92.4, and 63.16 µM MDA equivalent/g tissue respectively in a dose-responsive way (Fig. 8b). MHC per se and silymarin group animals displayed TBARS content of 52.63 and 56.73 µM MDA equivalent/g tissue. Further, it was elucidated that the highest dose of MHC and silymarin displayed no statistical difference at $p \leq 0.05$ when compared with the normal group. Thus, the effect rendered by the highest dose was equivalent to that of standard silymarin. Also, the statistical similarity between MHC alone and the normal group represented no significant toxicity of combination by itself. Supplementary data are given in Table S6.

Lipid hydroperoxides

The amount of lipid hydroperoxides in group I rats was observed to 0.913 mM H_2O_2 equivalent/g tissue. This value was considered a normal basal value. Paracetamol intoxicated animals showed the maximum amount of lipid hydroperoxides among all treatment groups (4.807 mM H_2O_2 equivalent/g tissue) and represented a significant difference at $p \leq 0.05$. Animals pre-treated with MHC at the dose of 50, 100, and 200 mg/kg bw afforded the protection in a dose-dependent manner and exhibited lipid hydroperoxide content of 3.766, 2.218, and 1.149 mM H_2O_2 equivalent/g tissue respectively (Fig. 8c). MHC at 200 mg/kg bw dose per se displayed 1.920 mM H_2O_2 equivalent/g tissue and animals pre-treated with silymarin showed the lipid hydroperoxide content close to the basal value (0.960 mM H_2O_2 equivalent/g tissue). From the results, it was noticed that 100 mg/kg bw and 200 mg/kg dose of MHC showed no significant difference in lipid hydroperoxide content as compared to the untreated control group. Also, the statistical similarity between MHC per se and the normal group presented no appreciable toxicity of combination. Supplementary data are given in Table S6.

Protein content

The variations in the protein content displayed by animals in different experimental groups are given in Fig. 8d. Group I animals that served as normal control exhibited the highest amount of protein content (91.57 mg/g tissue) while Group II animals that were given a highly toxic dose (3 g/kg bw) of paracetamol showed the minimum amount of protein content (29.45 mg/g tissue) among all treatment groups. This variation was observed to be statistically different at $p \leq 0.05$. The animals pre-treated with MHC at three doses (50, 100, and 200 mg/kg bw) showed the dose-dependent increase in protein content i.e. 41.28, 60.53, and 76.40 mg/g tissue respectively. The protein content displayed by the highest dose of MHC and silymarin exhibited no significant difference ($p \leq 0.05$) when compared with the normal group. In addition, MHC at 200 mg/kg bw dose per se exhibited 83.86 mg/g protein content and presented statistical similarity with normal rats at a 5% level of significance indicating no toxic effect. Supplementary data are given in Table S6.

Antioxidant biomarkers

Reduced glutathione (GSH)

In the present findings, it was seen from Fig. 9a that rats fed on a normal diet exhibited 410.5 µM SH content/g tissue while paracetamol challenged rats showed significant reduction ($p \leq 0.05$) in the level i.e. 151.4 µM SH content/g tissue. Pre-treatment of rats with MHC at doses 50, 100, and 200 mg/kg bw significantly restored the level of glutathione content in a dose-responsive manner exhibiting values 199.5, 261.1, and 307.7 µM SH content/g tissue respectively. Silymarin also reversed the adverse effect elicited by hepatotoxin and showed the value 366.1 µM SH content/g tissue. Animals that were given MHC alone exhibited 348.3 µM SH content/g tissue. In addition, MHC per se and silymarin group revealed

no significant differences at $p \leq 0.05$. Overall, the experimental groups I to VI showed statistical difference at a 5% level of significance. Supplementary data are given in Table S7.

Superoxide dismutase (SOD)

The specific enzymatic activity of SOD was evaluated in different treatment groups and results are presented in Fig. 9b. It was observed that the toxicity promoted by paracetamol markedly reduced ($p \leq 0.05$) the enzymatic activity (84.42 IU/mg protein) as compared to basal control value 179.0 IU/mg protein. The administration of MHC raised the level of SOD in a dose-dependent manner i.e. 100.2, 121.7, and 155.2 IU/mg protein at 50, 100, and 200 mg/kg bw respectively. Meanwhile, the highest dose of MHC (200 mg/kg bw) restored the paracetamol mediated altered enzymatic level with statistical similarity to that of the normal group. Similarly, the silymarin-treated group also raised the level of hepatic antioxidant enzyme effectively (203.8 IU/mg protein) and indicated no statistical variation when compared with normal control. MHC alone exhibited enzymatic activity with a value of 124.2 IU/mg protein. Supplementary data are given in Table S7.

Catalase (CAT)

The activity of CAT was significantly depleted ($p \leq 0.05$) in the liver of rats intoxicated with paracetamol. The enzymatic value was calculated to be 4.901 IU/mg protein as compared to the control value, i.e. 15.23 IU/mg protein as observed in the normal group. In contrast, pre-treatment of MHC was observed to be effective in boosting the CAT activity in a dose-dependent manner and reflected 6.145, 10.01, and 12.60 IU/mg protein at 50, 100, and 200 mg/kg bw dose respectively (Fig. 9c). The treatment of the highest dose of MHC (200 mg/kg bw) prior to paracetamol intoxication represented no significant deviation at $p \leq 0.05$ as compared to the normal group. Similarly, silymarin pre-administration raised the enzymatic level to 13.71 IU/mg protein and manifested statistical similarity with Group I normal animals ($p \leq 0.05$). In addition, the enzymatic value was observed to be 8.552 IU/mg protein in animals that were given 200 mg/kg bw dose of MHC alone. However, this value was not observed to be apparent with the basal value. Supplementary data are given in Table S7.

Glutathione peroxidase (GPx)

The level of hepatic GPx was measured in all treatment groups and results are shown in Fig. 9d. The activity of the enzyme was significantly diminished in the paracetamol exposed group (3.172 IU/mg protein) in comparison to the normal control (11.56 IU/mg protein) at a 5% level of significance. Pre-treatment with 50, 100, and 200 mg/kg bw dose of MHC significantly augmented the antioxidant enzyme level (4.586, 7.358, and 10.52 IU/mg protein) in a dose-dependent way. It was observed from the results that the highest dose of MHC was effective in restoring the reduced enzyme level to near normal as it did not represent a significant difference at $p \leq 0.05$. In the same way, the value was seen to be 11.11 IU/mg protein in the silymarin administered group which also did not reach any statistical difference in comparison to the control. Simultaneously, it was also seen that MHC (200 mg/kg bw) and silymarin (50 mg/kg bw) showed statistical similarity, raising the possibility that the highest dose of MHC is effective

in restoring the altered enzyme activity as that of standard silymarin. Supplementary data are given in Table S7.

Glutathione reductase (GR)

The changes in specific activity of GR among different treatment groups are shown in Fig. 9e. Hepatic damage provoked by paracetamol resulted in a significant diminution (5.357 IU/mg protein) when compared with animals of the control group (17.23 IU/mg protein) at $p \leq 0.05$. While, the pre-administration of MHC indicated a dose-dependent rise in the level of GR i.e. 7.222, 10.28, and 16.37 IU/mg protein at 50, 100, and 200 mg/kg bw respectively. It was found that the highest dose caused a successive revival towards normalization as the statistical comparison did not reveal a significant difference ($p \leq 0.05$) when compared with the normal group. Further, silymarin treatment and MHC per se at the dose of 200 mg/kg bw illustrated no significant difference and showed the values of 12.10 and 12.71 IU/mg protein respectively. Supplementary data are given in Table S7.

Histopathological findings

Gross necropsy of the liver of all the treatment groups was conducted to observe any abnormal or irregular changes in the appearance of liver lobes. It was seen that the liver of Group I control animals was dark maroon with a smooth surface. While the liver of paracetamol intoxicated rats showed changes in colour and appearance. The liver lobes were more or less brown with irregular white spots as depicted in Fig. 10. These gross abnormalities were remarkably reduced when animals were pre-treated with MHC and silymarin.

Further, the histopathological examination of liver tissues was done using Ishak modified HAI grading to determine the necroinflammatory scores in different treatment groups. The various features of hepatic injury are displayed in Table 2 and **Fig. 11**. The sections of liver tissues of animals treated with paracetamol (3 g/kg bw) showed marked hepatic damage with extensive panacinar/multiacinar necrosis, coagulative type necrosis of hepatocytes, loss of cellular boundaries, apoptosis, portal triad inflammation with the highest score (10/18) as seen from Table 2. On the other hand, liver sections of the control group depicted healthy normal hepatocytes with well-defined sinusoidal spaces and a central vein scoring 0/18. Similarly, in MHC (200 mg/kg bw) per se group, the hepatocytes did not show any significant sign of necrosis or degeneration and well preserved hepatic cells with cytoplasm and nucleus were observed. Paracetamol provoked massive necrosis and inflammation was significantly improved in MHC pre-treated rats in a dose-responsive manner. Group pre-treated with the lowest dose (50 mg/kg bw) had mild to moderate piecemeal necrosis, focal confluent necrosis in some areas with mild focal and portal inflammation (score 4/18). The medium dose (100 mg/kg bw) demonstrated mild focal inflammation with no sign of confluent necrotic region (2/18) indicating partial protection of hepatocytes. The highest dose (200 mg/kg bw) afforded the marked protection and represented the normal architecture of hepatocytes with no necrotic zones. However, mild portal inflammation was seen and the grading score was calculated to be 1/18. Also, in the silymarin treated group, no major abnormality or irregularity in the

structure of hepatic cells was observed. Overall, the Kruskal-Wallis H test revealed a statistically significant difference in necroinflammatory scores between different treatment groups (I-VII). The necroinflammatory scores for "*periportal or periseptal interface hepatitis (piecemeal necrosis)*" were significantly different between treatment groups (I-VII) with $H = 28.24$, $p = 0.000085$ and $df = 6$. Similarly, the necroinflammatory scores for "*confluent necrosis*" ($H = 35.82$, $p = 0.000003$ and $df = 6$), "*focal (spotty) lytic necrosis, apoptosis, and focal inflammation*" ($H = 31.89$, $p = 0.000017$ and $df = 6$) and "*portal inflammation*" ($H = 30.06$, $p = 0.000038$ and $df = 6$) were significantly different between treatment groups (I-VII). The corresponding average rank scores of groups (I-VII) with pairwise comparisons for twenty-one pairs of groups by Dunn-Bonferroni post hoc test are shown in **Fig. 12a, 12b, 12c and 12d**. There was strong evidence of difference at $p \leq 0.05$ (adjusted by the Bonferroni correction) between treatment group II with group I, group III, group VI, and group VII. Moreover, there was statistically no significant difference among groups I, III, VI, and VII revealing the effectiveness of MHC by overturning the paracetamol-induced hepatotoxicity in rats.

Table 2
Summary of the hepatic necroinflammatory score in Ishak modified HAI scoring of all treatment groups

Group (n = 6)	Treatment type	Perportal or periseptal interface hepatitis (piecemeal necrosis)	Confluent necrosis	Focal (spotty) lytic necrosis, apoptosis and focal inflammation	Portal inflammation	Grading score
I	Normal control	0	0	0	0	0/18
II	Paracetamol (3 g/kg bw)	1	6	2	1	10/18
III	MHC (200 mg/kg bw) per se	0	0	0	0	0/18
IV	MHC (50 mg/kg bw) + Paracetamol	1	1	1	1	4/18
V	MHC (100 mg/kg bw) + Paracetamol	1	0	1	0	2/18
VI	MHC (200 mg/kg bw) + Paracetamol	0	0	0	1	1/18
VII	Silymarin (50 mg/kg bw) + Paracetamol	0	0	0	0	0/18

Discussion

Antioxidant phytoconstituents have the ability to overturn the toxic alterations in the liver by reducing oxidative stress and inflammatory responses (Jeyadevi et al. 2019). The hepatoprotective effect of MHC could be correlated to the presence of high amounts of kaempferol, ellagic acid, and gallic acid. Structurally, high planarity due to the existence of three OH groups on the chroman ring in kaempferol contributes to the efficacious radical scavenging capacity (Kim and Lee 2004). The antioxidant action of ellagic acid is supported by high 5-O radical stability via maintaining overall spin density delocalization due to dual orthodiphenolic functionalities of this polyphenol (Chen et al. 2015). The benzene ring of gallic acid has three vicinal hydroxyl groups which enhance the antioxidant ability of this compound (Kim and Lee 2004). Overall, the effective hepatoprotective ability of MHC can correlate to the vicinal substitution of OH functional groups on catechol moiety, 4-oxo functionality with 2,3- double bonds

conjugation, and presence of 3- and 5-OH functional groups on chroman ring (Kim and Lee 2004). The possible synergistic effect of polyphenols in MHC may be attributed to the high synchrony within polyphenolic structures and efficacious electron/hydrogen donating capability. Bioavailability is a crucial endpoint for documentation of *in vivo* effective action of nutritional commodities (de Pascual-Teresa et al. 2010). As reported in our previous study, the protective ability of MHC in liver cells might be attributed to the higher bioavailability of antioxidants at cellular levels due to the water-soluble characteristics of MHC (Kaur et al. 2019).

Paracetamol (acetaminophen; N-acetyl-p-aminophenol) is a commonly utilized antipyretic and analgesic drug worldwide. It is considered safe when taken at a therapeutic dose. However, its overdose has been documented to cause acute liver poisoning and is one of the most frequently reported products promoting DILI (Yoon et al. 2016; Tittarelli et al. 2017). Phenolic phytochemicals can impact APAP metabolism, effectiveness, and toxicity. These compounds can affect its bioavailability via a variety of mechanisms, including competition with cytochrome P450 (CYP) enzymes, esterases, uridine diphosphate glucuronosyltransferases, and transporters including P-glycoprotein, organic anion transporting polypeptides, breast cancer resistance protein, multidrug resistance-associated proteins, and monocarboxylate transporters (Abdel-Daim et al. 2018).

In the present study, the statistically insignificant differences in average body weight did not reveal any signs of paracetamol-induced hepatotoxicity in rats as compared to the normal control group. Similar results were observed in a previous study on paracetamol-induced toxicity in rats (Mahmood et al. 2014). The study revealed that the ratio of liver weight to body weight is a better approach for observing the toxic effects. The aggregation of extracellular matrix protein and collagen in liver tissue can be linked to the toxic enlargement of the liver caused by paracetamol. However, our study did not reveal significant enlargement of the liver as compared to the normal control group. The haematopoietic system is considered a unique target for haematotoxins due to its high susceptibility to the intoxication of xenobiotics or their secondary toxic effects. This may affect the supply of iron and nutrients, production of erythropoietin, clearance of urea, and other essential functions which in turn alter the normal range of different blood cells like erythrocytes, leucocytes, and platelets (Adeneye et al. 2008). Results of the present study showed that haematological changes induced by paracetamol had the haematotoxic effect and MHC showed the potential protective activity against this effect. The parameters like haematocrit, haemoglobin, total RBCs, total WBCs, and platelet count were significantly decreased relative to normal control due to interference in the rate of erythropoiesis associated with overdose of paracetamol. However, oral pre-treatment with MHC at 200 mg/kg bw dose reduced the deleterious effect of haematotoxic injury with subsequent augmentation of these haematological indices indicating synthesis of blood cells. This might be attributed to the possibility that interaction among constituents (phenolic acids, flavonoids, and other bioactive constituents) in MHC could have stimulated the formation or secretion of erythropoietin, haematopoietic growth factors, and stem cells (Uboh et al. 2010). Previous studies have also demonstrated that under the conditions of hypoxia, herbal extracts activate the hypoxia response element signalling pathway to promote the mRNA and protein expression of erythropoietin (Lam et al. 2016). This might be the probable response of MHC in the present case to regulate the

haematological profile from the adverse scenarios. In addition to this, MHC could have sheltered the blood cells from the assault of free radicals which can be related to its antioxidant efficacy as seen in CAP-e assay (Kaur et al. 2019). The protective effect of MHC in the present study is supported by results of the previous findings which reported that an aqueous ethanolic polyherbal mixture consisting of leaves of *Gongronema latifolia, Vernonia amygdalina*, and *Ocimum gratissimum* raised levels of most of the haematological parameters and annulled the haematopoietic injury promoted by paracetamol (Iroanya et al. 2014).

The hepato-specific markers i.e. SGOT, SGPT, ALP, and total bilirubin are deemed sensitive indicators of hepatocellular dysfunction (Kullak-Ublick et al. 2017). Hepatocytes participate in various metabolic functions and their destruction during hepatic injury substantiates efflux of their cytosolic content along with serum transaminases into extracellular spaces from where these enzymes enter into the circulatory stream (Ozer et al. 2008). ALP enzyme hydrolyzes monophosphatases at an alkaline pH and is associated with the cell membranes of hepatocytes. Generally, its elevation in serum represents biochemical abnormality indicating disruption of the plasma membrane (Ozer et al. 2008). The high concentration of bilirubin (haemoglobin degradation product) in serum is a marker of hepatobiliary injury which signifies its excessive excretion during damage to the hepatic parenchyma (Ozer et al. 2008; Nithianantham et al. 2011). In this study, paracetamol intoxication to rats significantly augmented liver function indices reflecting membrane damage and leakage of enzymes into the bloodstream. However, the amplified levels were ameliorated in a dose-dependent manner by pre-treatment with MHC. The normalisation of these serum markers with the administration of the highest dose was seen to be comparable with standard silymarin indicating the metabolic regulatory potential of MHC. The active phyto-ingredients in the combination might have facilitated the stabilization of plasma membrane affording protection to hepatic cells against paracetamol and ultimately preserved the structural and functional integrity of hepatocytes. Earlier studies have also reported that pre-treatment of animals with the herbal formulation comprising a spray-dried extract of *Andrographis paniculata*, *Phyllanthus niruri*, and *Phyllanthus emblica* for 5 days at 100 and 200 mg/kg bw dose significantly reduced the level of serum biomarkers provoked by paracetamol overdose (Tatiya et al. 2012). Another hepatoprotective study also presented similar results with pre-treatment of Livshis formulation regularizing transport function of liver cells (Bera et al. 2011; Bera et al. 2017).

In the phase-I system, the metabolism of paracetamol primarily involves its oxidation in the liver via CYP enzymes to make it more water-soluble and facilitates its easy removal from the body (Williams 2013). In this system, CYP-catalyzed metabolism of paracetamol forms intermediate hepatotoxic products like N-acetyl-p-benzoquinone imine (NAPQI) and 3-hydroxy-acetaminophen (3-OH-APAP) (Gu et al. 2005, Abdeen et al. 2018). P450 enzymes (especially CYP1A2 and CYP2E1) play an important role in the metabolic activation of paracetamol to its toxic biotransformation. CPR is an obligate redox companion for all P450 enzymes and thus its elevated expression may signify the toxic biotransformation of paracetamol in the liver (Gu et al. 2005). In the present study, the significant augmentation of CPR indicated the hepatic biotransformation of paracetamol to its toxic byproducts in the paracetamol-treated group as compared to its expression in the untreated group. While it was seen that the highest dose of MHC brought down the

elevated level of enzymes to the near basal value. The activity of MHC was observed to be dose-dependent rendering protection against the toxic effects of paracetamol.

In catalytic biotransformation of paracetamol, the P450 cycle requires the sequential translocation of two electrons via CPR and b5R (Finn et al. 2008). Thus, more abundance of cytochrome b5 reductase along with CPR reveals the toxic biotransformation of paracetamol. In the present investigation, the level of b5R was markedly increased in the paracetamol-treated group. This analysis highlights the toxic biotransformation of paracetamol in rat liver as compared to the untreated group. It was observed that pre-treatment of MHC inhibited the excess augmentation of b5R at the highest dose facilitating diminution of toxic biotransformation of paracetamol. MHC pre-treatment attenuated the paracetamol-induced hepatic dysfunction by reducing the level of b5R in a dose-dependent way.

GST is an important enzyme for the detoxification of many xenobiotics from the liver. The enzyme catalyzes the conjugation of N-acetyl-p-benzoquinone imine (NAPQI) with a reduced form of intracellular glutathione, ultimately assisting in the excretion of NAPQI from the liver as conjugates of cysteine and mercapturic acid (McCrae et al. 2018). Thus, excess NAPQI causes antioxidant glutathione depletion which leads to oxidative stress. In the present study, the paracetamol-treated group revealed a reduction in the level of GST as compared to the control group. The highest dose of MHC (200 mg/kg bw) augmented the level of this enzyme to near the level of the control group. The augmentation in enzymatic level was found to be in a dose-dependent manner.

DTD is an important enzyme that can scavenge superoxide anions formed as a consequence of oxidative stress and help in the reestablishment of endogenous reduced forms of defensive antioxidants (Aleksunes et al. 2006). DTD facilitates the metabolization of toxic byproduct (NAPQI) of paracetamol into harmless hydroquinone (Lancaster et al. 2015). The excess of paracetamol can reduce the level of enzyme in the liver, thereby causing abatement of enzymatic expression (Liu et al. 2016). The decreased expression may lead to aggravated toxicity in the liver. In the current study, it has been revealed that the specific activity of the DTD enzyme was significantly reduced in the paracetamol intoxicated group as compared to the control group. However, MHC treatment recovered the enzymatic level in a dose-dependent manner with greater activity at the highest dose of 200 mg/kg bw comparable to the normal group.

ATPases are a class of energy-linked master enzymes in all organisms accountable for membrane permeability, transport of ions, and regulation of osmotic pressure (Al-Numair et al. 2015). The membrane-bound enzymes such as Na^+/K^+, Ca^{2+}, and Mg^{2+}-ATPases transport ions across the cell membrane at the expense of adenosine triphosphate. Na^+/K^+ and Ca^{2+}-ATPase are actively associated with the transport of sodium, potassium, and calcium ions across the plasma membrane while Mg^{2+}-ATPase directs intracellular magnesium ion concentration and regulates protein synthesis and cellular growth (Ji et al. 2009). In the present investigation, decreased activities of ATPases in the paracetamol administered group was observed. The inactivation of these enzymes in hepatotoxin administered group might be possibly due to their vulnerability to LPO induced as a result of over generation of free radicals

(Bhagavathy and Sumathi 2012, Madkour and Abdel-daim 2013). Consistent with the previous studies (Bafna and Balaraman 2013; Padma et al. 2016), the present research revealed that following MHC pre-treatment the activities of membrane-bound enzymes were raised. The polyphenolic compounds in the combination might have protected the membrane phospholipids from deleterious effects of peroxyl radicals by modulating the alteration in surface potential and membrane fluidity which in turn would have regularized enzymatic activities and cellular functions.

Another important enzyme linked with cellular integrity is LDH. During the absence of sufficient oxygen, it regulates the constant generation of ATP to sustain the glycolytic cycle. It regenerates NAD^+ by catalyzing the conversion of pyruvate to lactate in a reversible reaction (Augoff et al. 2015). However, the extensive-expression of this enzyme is related to its release into the blood or other body fluids during tissue breakdown. The intoxication of paracetamol elevated the level of LDH due to the damage exerted by free radicals or electrophiles generated as a consequence of induction of phase-I and reduction of phase-II enzymes. On the other hand, the activity of the enzyme was found to be lowered by the administration of herbal combination in a dose-responsive manner highlighting the potential of MHC in attenuating the toxic impact of paracetamol. The findings of Mayuren et al. (2010) also reported that Livactine containing extracts of nine medicinal plants at a dose of 2 mL/kg significantly reduced the elevation of LDH along with other marker enzymes in serum. The authors correlated the resultant protective efficacy of formulation against paracetamol to the free radical scavenging property of phytoconstituents like flavonoids, alkaloids, and sterols.

Free radicals hit the lipid bilayer of cell membranes, stimulate the formation of lipid hydroperoxides and malondialdehydes, inactivate cellular proteins, membrane-bound enzymes and disrupt ionic homeostasis (Birben et al. 2012). The current study showed high lipid hydroperoxide and malondialdehyde content in the paracetamol group while pre-treatment with MHC demonstrated its reduction in a dose-dependent fashion. It was also noticed in *in vitro* experimental results that the MHC demonstrated the potential of scavenging peroxyl radicals in the LPO assay (Kaur et al. 2019). Hence extrapolating these results in liver homogenates, it might be feasible that the prophylactic effect of the combination is due to scavenging of free radicals and reactive oxygen species. Comparable with the findings in earlier studies reported by Olaleye et al. (2010), Parmar et al. (2010), Sabir et al. (2012) for various plant extracts, our results also indicated that bioactive constituents present in MHC might have been accountable for its capability to lessen the paracetamol exaggerated LPO.

The protein content in paracetamol intoxicated rats was observed to be the lowest among the studied animal groups. The reactive oxygen species in rats generated as a result of paracetamol metabolism might have targeted amino acids and promoted oxidative scission of DNA with altered activities of repair enzymes (Bkhairia et al. 2018). However, pre-treatment with MHC repressed proteolytic degradation possibly by regulating nucleic acid metabolism, preventing oxidative damage mediated structural modifications of proteins and hence promoting DNA and RNA formation. The results obtained as a consequence of administration of MHC are similar to findings where leaf extracts of *Citrus hystrix* and *C. maxima* increased the protein content (Abirami et al. 2015).

GSH is an essential non-enzymatic antioxidant abundantly present in nuclei, mitochondria, and cytosol. It acts as a co-factor for GST and GR. The reduction in its content is coupled to excessive generation and amassing of reactive oxygen species that ultimately perturb the structural and functional homogeneity of cell organelles and their membranes (Sharma et al. 2010). In the present study, paracetamol exercised its detrimental effects by dropping the content of GSH. Moreover, the specific activity of GR was also diminished in paracetamol intoxicated animals, which was observed to be analogous with depletion of GSH content in liver homogenate of animals. MHC significantly elevated the level of GSH in a dose-dependent manner rendering it to endorse the conjugating abilities in order to detoxify toxic entities. The results of the present study seem to be conceivable with that obtained by Singh et al. (2016) who reported that combined administration of *Solanum xanthocarpum* and *Juniperus communis* fruit extracts significantly potentiated their protective effect in comparison to their effect per se. The biological system is equipped with a set of antioxidative defence grids offering a network of protective strategies to avert and deactivate the free radical aggravated oxidation of DNA, proteins, lipids, and other essential biomolecules (Ighodaro and Akinloye 2017). In the present research, the response of animals that were given MHC was studied against paracetamol-induced oxidative damage by monitoring antioxidant enzymes like SOD, CAT, GPx, and GR. SOD catalyses the disproportionation of highly reactive superoxide anion radicals to hydrogen peroxide while CAT and GPx function to neutralize hydrogen peroxide to oxygen and water employing different substrates (Lobo et al. 2010; Kaur and Arora 2013). GPx also catalyses the reduction of peroxide radicals to oxygen and corresponding alcohols protecting organisms against oxidative damage. GR maintains a pool of GSH during cellular metabolism via catalyzing NADPH-dependent reduction of glutathione disulfide i.e. oxidised glutathione is recycled back to its reduced form (Couto et al. 2016). In the present study, decreased level of these enzymes in the paracetamol group might be related to their consumption in detoxification of excessive reactive oxygen species produced as a result of its toxicity. Another possibility of decreased specific activity of enzymes might be their suppression by the insult of reactive oxygen species. The pre-treatment with MHC at 50, 100, and 200 mg/kg bw dose augmented the level of enzymes dose-dependently indicating the activation of enzymatic machinery to render protection to animals either by scavenging or inhibiting the formation of reactive oxygen species. An elevation in the specific activities of antioxidant enzymes has also been observed in a previous study following pre-treatment of animals with the herbal formulation comprising seven plants (*Eclipta prostrata*, *Wedelia calendulaceae*, *Indigofera tinctoria*, *Sphaeranthus indicus*, *Centella asiatica*, *Acalypha indica*, and *Coldenia procumbens*) against paracetamol-induced liver injury (Sen et al. 2015).

The protective action of MHC was further established by histopathological evaluation of the liver. The observations of paracetamol injured liver revealed confluent necrosis of hepatocytes but MHC pre-treated rats displayed normal architecture of hepatic cells. These findings were seen to be concurrent with liver function indices, antioxidant enzymes, and other oxidative stress parameters which showed values near to basal control against the toxic insult with paracetamol overdose. MHC was observed to be highly effective at 200 mg/kg bw dose revealing its possible protective action against paracetamol-induced hepatic dysfunction in rats. The effect of MHC was observed to be promising as it protected the hepatic

tissues from histopathological alterations invoked due to paracetamol intoxication probably by scavenging the surplus free radicals, triggering antioxidant defense system, and stabilizing cellular membranes through inhibition of LPO. The results were comparable to earlier studies presented by Devaraj et al. (2011) and Tatiya et al. (2012) who also reported that polyherbal formulations present high potential in abrogating the pathological damage against paracetamol-induced hepatotoxicity and sustaining normal liver morphology. Finally, **Fig. 13** depicts the possible protective action of MHC with mechanistic pathways against paracetamol-induced acute hepatotoxicity in female Wistar rats. The study revealed that MHC pre-administration overturned the APAP-mediated toxic alterations.

Herbs are natural resources of conventional, traditional, and complementary medicines, offering imperative therapeutic properties against many ailments. The present study gives the reflection that MHC treatment overturned the biochemical toxic alterations in rat liver against paracetamol-induced hepatic damage. Our previous study revealed that the hydrogen or electron-donating capability of bioactive phytoconstituents in MHC rendered the potential antioxidative action against *in vitro* oxidative stress. Further, the antiproliferative potential of MHC against HepG2 cells reflected the probable apoptotic mode of hepatoma cell death (Kaur et al. 2019). The biofunctional significance of herbal drugs emerges from the cumulative effect of synergistic interactions between several phytoconstituents and their specific affinity for many biological receptors in phytotherapeutic research (Carmona and Pereira 2013). The physiology of pathogenesis and disease progression encompasses multiple factors necessitating the transition of therapeutic approaches from conventional “single target-one drug” to “multiple target-multidrug” model (Efferth and Koch 2011; Long et al. 2015). The multi-component herbal therapy endows the holistic action originating from multifaceted positive/negative interactions between components (Rather et al. 2013). The mechanistic basis of these interactions consisting of better bioavailability, modulation in cellular transport functions, pro-drug activation, and synergistic effect at different targets of the same cell signalling cascade is referred to as multi-target effect (Efferth and Koch 2011). Decisively, the present study apprehends the synergistic intervention of inherent phytoconstituents (kaempferol, ellagic acid, and gallic acid) in MHC which might have a multi-target prophylactic effect with better bioavailability due to effectual solubility in the aqueous medium. The study necessitates the investigation of the mechanistic basis of cumulative interaction among various phytoconstituents in MHC for its viable therapeutic action in complementary and alternative healthcare practices.

Conclusion

A multi-herbal combination of *P. granatum*, *P. roxburghii, S. chirata*, *T. cordifolia*, and *T. corniculata* with a proportion of 33, 27, 25, 10, and 5% respectively was evaluated against the acute drug-induced liver injury by paracetamol in female Wistar rats. It was discovered that MHC intercepted the paracetamol mediated toxic alterations in liver function indices and xenobiotic-metabolizing enzymes affording protection against hepatic dysfunction. The effect of MHC was observed to be promising as it protected the hepatic tissues from paracetamol-mediated alterations by scavenging the surplus free radicals, triggering antioxidant defence system, and stabilizing cellular membranes through inhibition of LPO which might be correlated to the synergistic effect of inherent phytoconstituents like kaempferol, ellagic acid, and

gallic acid. These major findings advocate the enormous scope of MHC as a multiple target-multidrug model, after affirmative pharmacological investigations in the future. Thus, herbal formulations with a natural combination of active phytoconstituents may provide a viable alternative to modern medicines.

Declarations

Ethics approval and consent to participate

The *in vivo* experimentations were carried out in accordance with the norms and guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. The necessary humane care was provided and all efforts were undertaken to reduce the animal sufferings. The study was reviewed and duly approved by Institutional Animal Ethics Committee, Guru Nanak Dev University, Amritsar (approved protocol no. 226/CPCSEA/2014/19).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PK and RS performed the experiments, wrote the manuscript and contributed substantially to the design and execution of the study. RGM interpreted the data and contributed significantly to the writing of the manuscript. BS and SA provided the laboratory facility and helped in the designing of experimental procedures and data interpretations. All authors read and approved the final manuscript.

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Figures

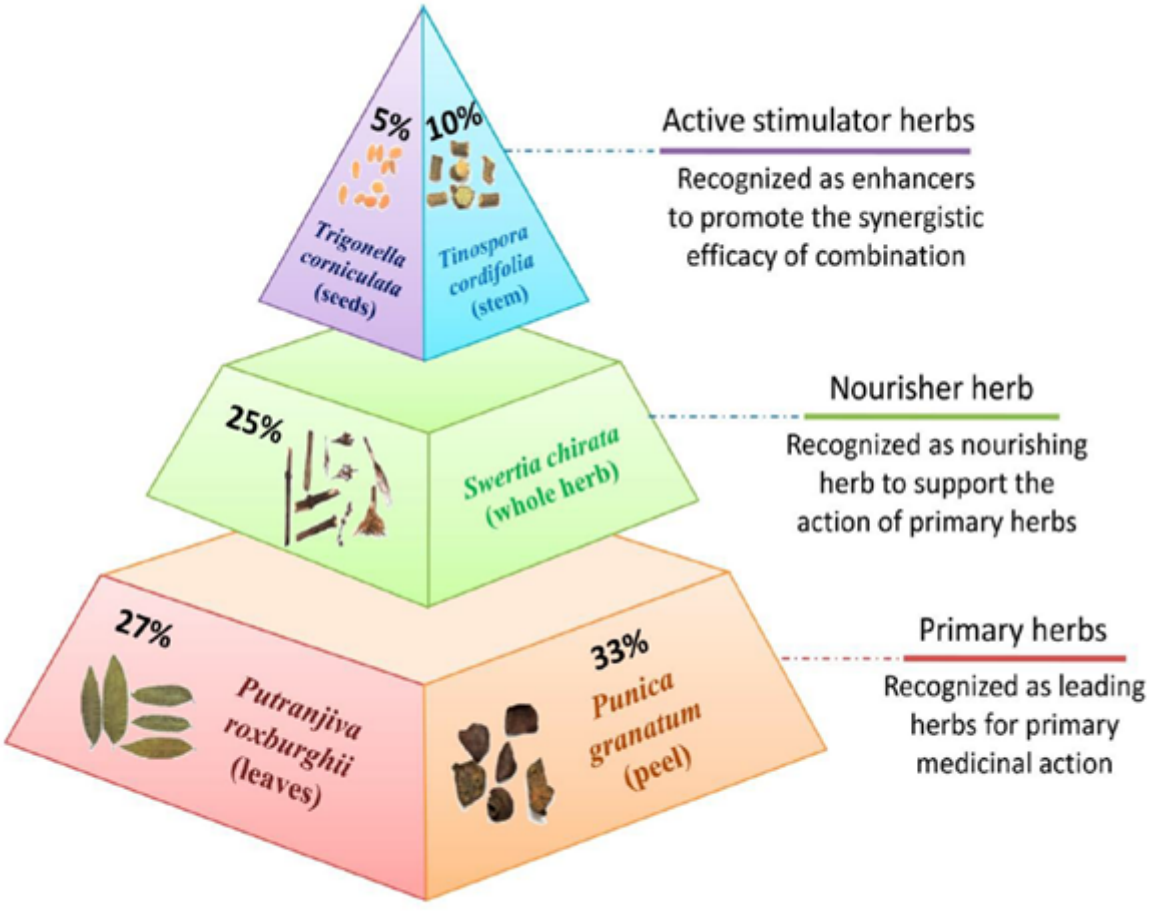
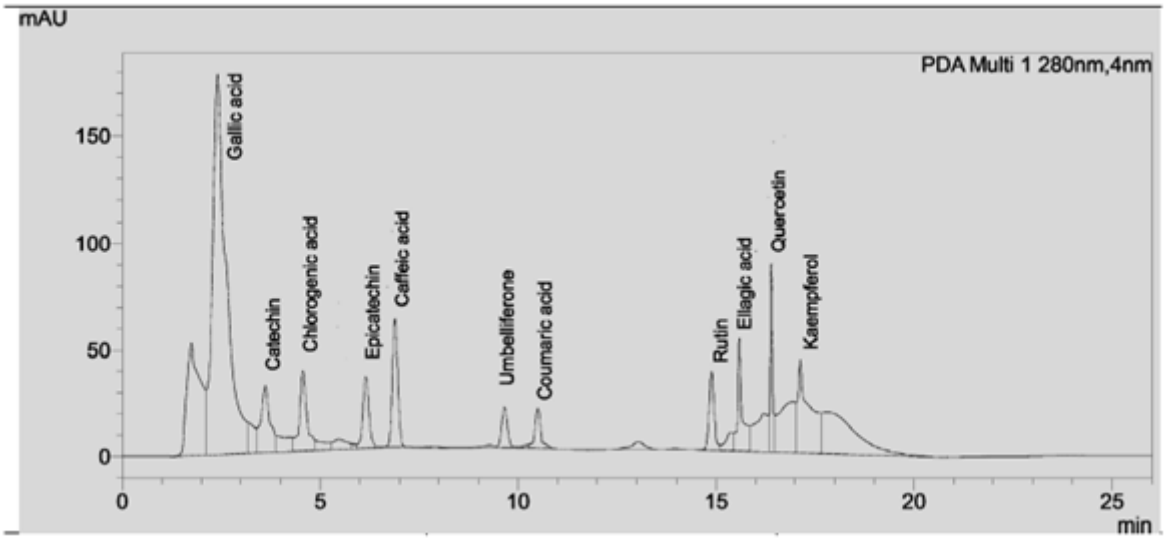


Figure 1 Development of multi-herbal combination MHC

Figure 1

Caption found in figure.

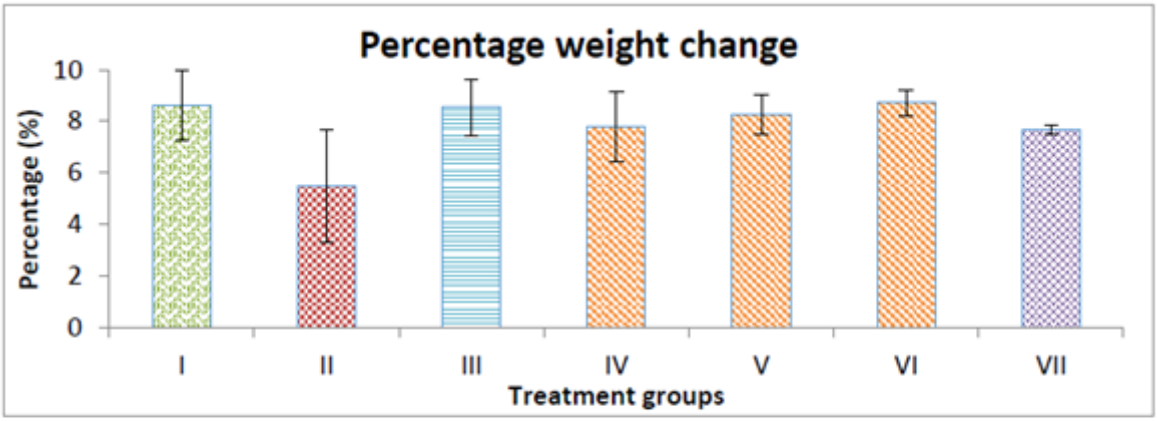


Retention time	Compound	MHC Concentration (µg/mg)
2.512	Gallic acid	8.29
3.912	Catechin	4.68
4.630	Chlorogenic acid	4.16
6.165	Epicatechin	6.11
6.889	Caffeic acid	2.73
9.761	Umbelliferone	1.77
10.360	Coumaric acid	1.25
14.915	Rutin	1.83
15.617	Ellagic acid	16.82
16.486	Quercetin	7.91
17.224	Kaempferol	40.68

Figure 2 HPLC-PDA chromatogram of multi-herbal combination (MHC)

Figure 2

Caption found in figure.



***Group I**: Normal control; **Group II**: Paracetamol (3 g/kg bw); **Group III**: MHC (200 mg/kg bw) per se; **Group IV**: MHC (50 mg/kg bw) + Paracetamol; **Group V**: MHC (100 mg/kg bw) + Paracetamol; **Group VI**: MHC (200 mg/kg bw) + Paracetamol; **Group VII**: Silymarin (50 mg/kg bw) + Paracetamol; Animals in each group (n = 6)*

Figure 3 Percentage change in body weight of animals in different treatment groups

Figure 3

Caption found in figure.

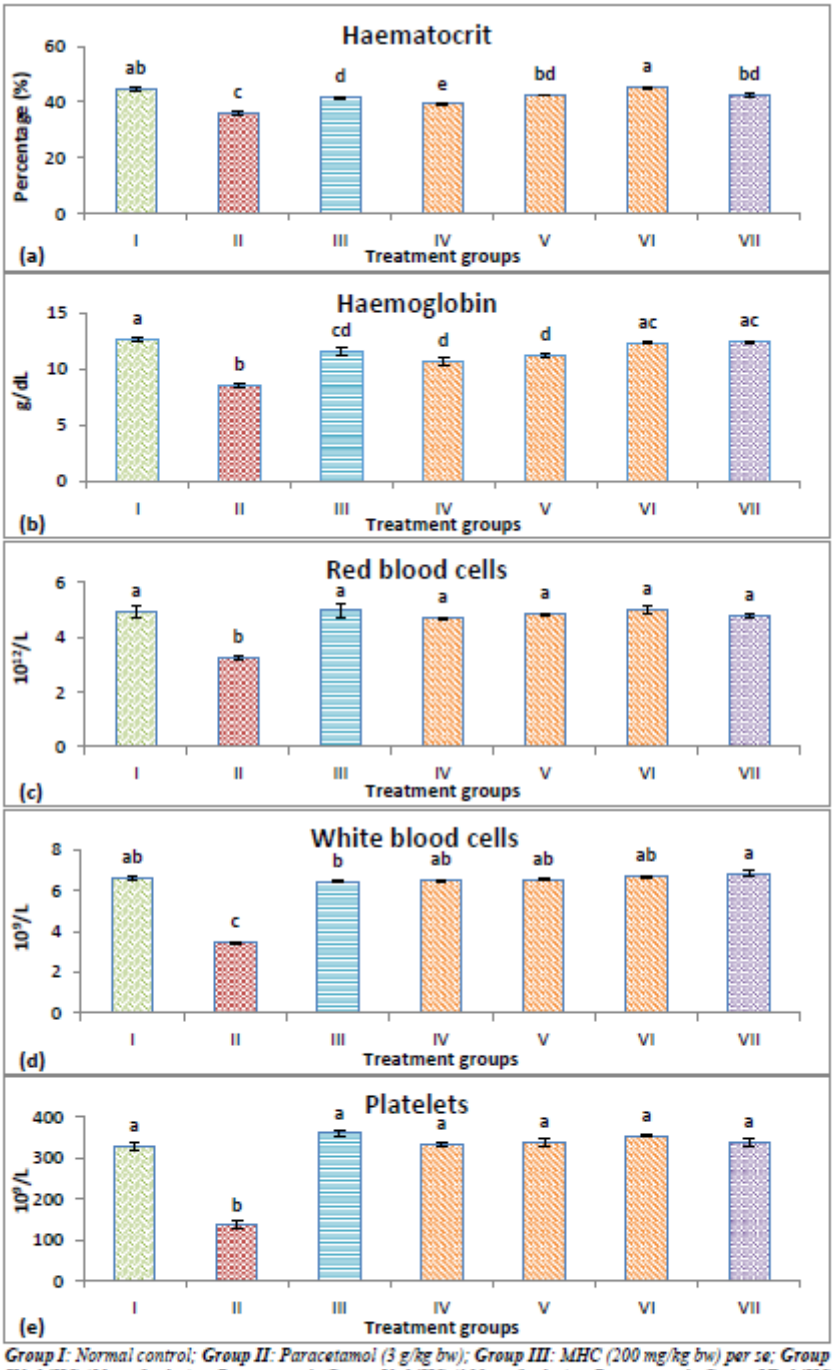


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Figure 4 Modulatory effect of MHC pre-treatment on paracetamol-mediated alterations in haematological indices

Figure 4

Caption found in figure.

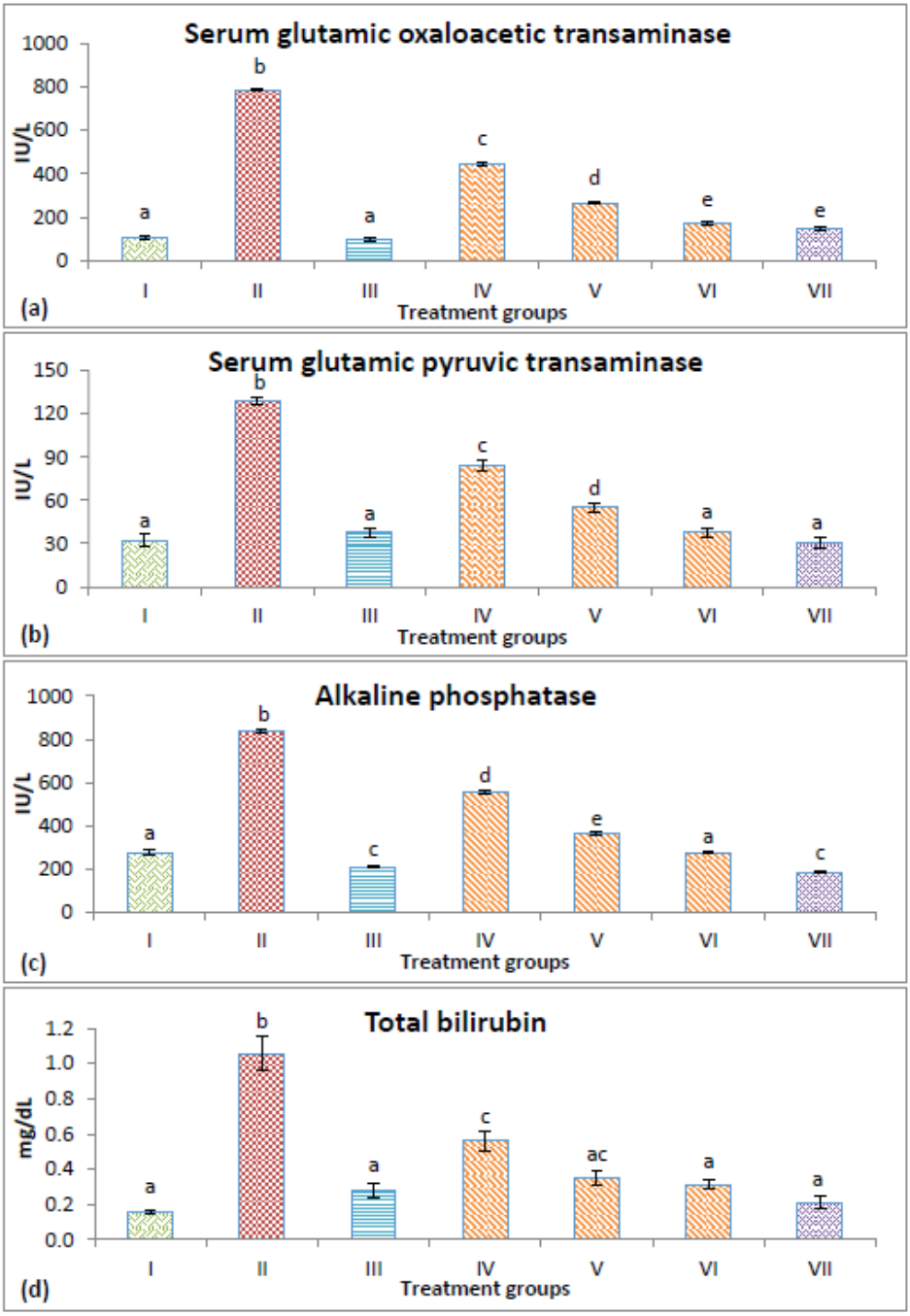

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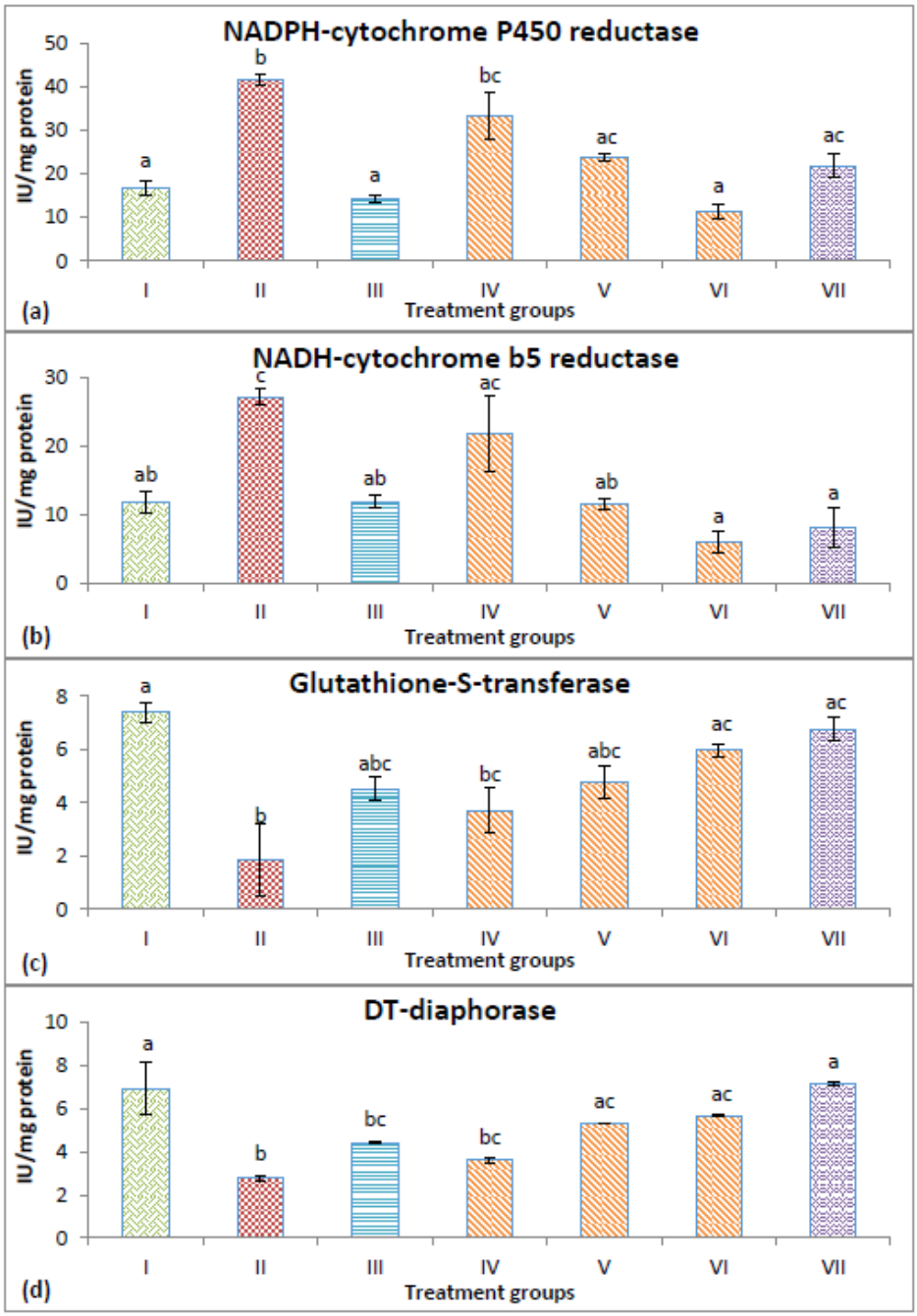
Figure 5 Modulatory effect of MHC pre-treatment on paracetamol mediated alterations on serum liver function biomarkers

Figure 5

Caption found in figure.



Group I: Normal control; Group II: Paracetamol (3 g/kg bw); Group III: MHC (200 mg/kg bw) per se; Group IV: MHC (50 mg/kg bw) + Paracetamol; Group V: MHC (100 mg/kg bw) + Paracetamol; Group VI: MHC (200 mg/kg bw) + Paracetamol; Group VII: Silymarin (50 mg/kg bw) + Paracetamol; Treatment groups with different lowercase letter(s) differ significantly at $p \leq 0.05$ (Tukey's HSD test); Animals in each group (n = 6)

Figure 6 Modulatory effect of MHC pre-treatment on paracetamol-mediated alterations in phase-I (a and b) and phase-II (c and d) reaction enzymes

Figure 6

Caption found in figure.

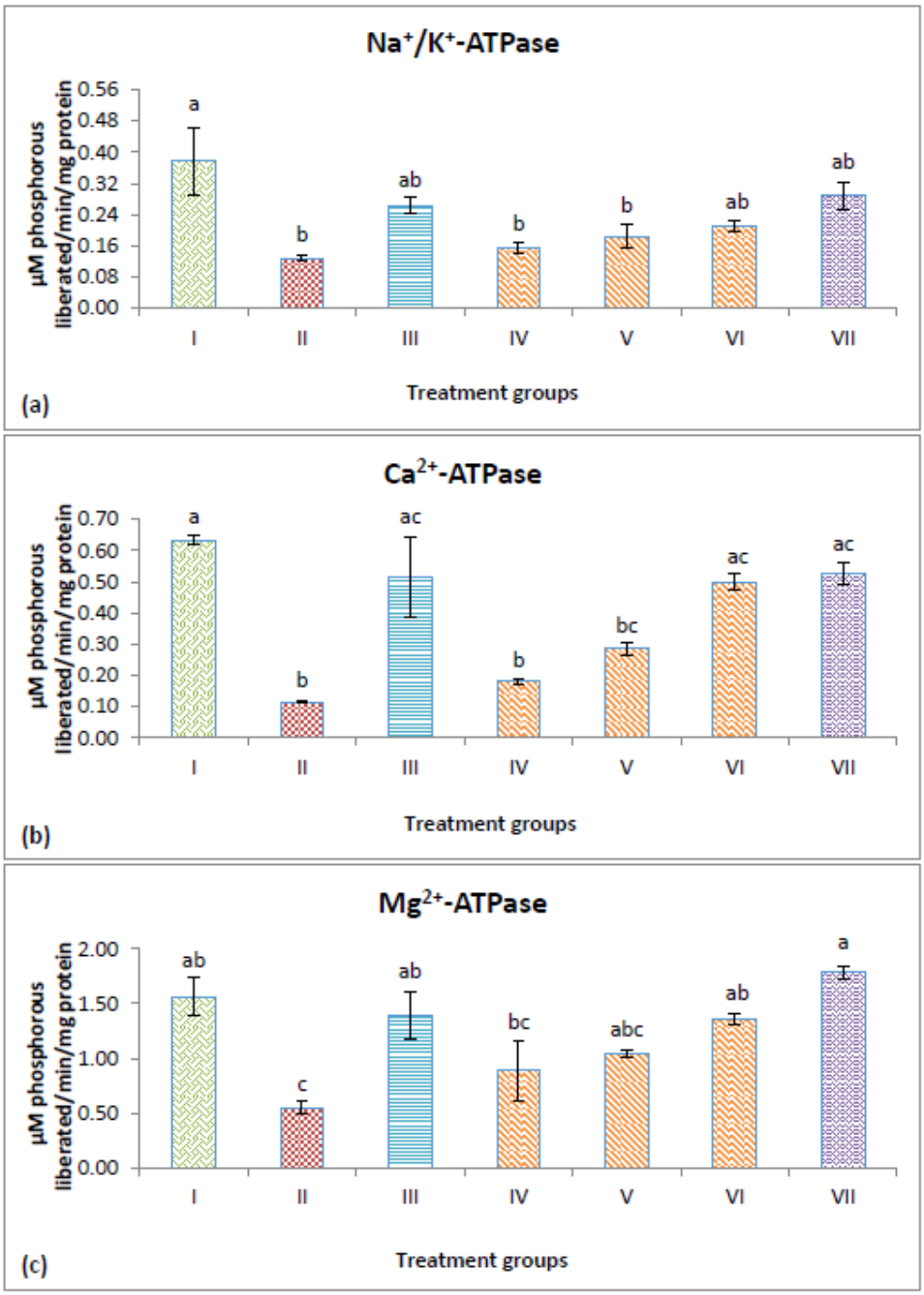


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Figure 7 Modulatory effect of MHC pre-treatment on paracetamol mediated alterations in membrane bound enzymes

Figure 7

Caption found in figure.

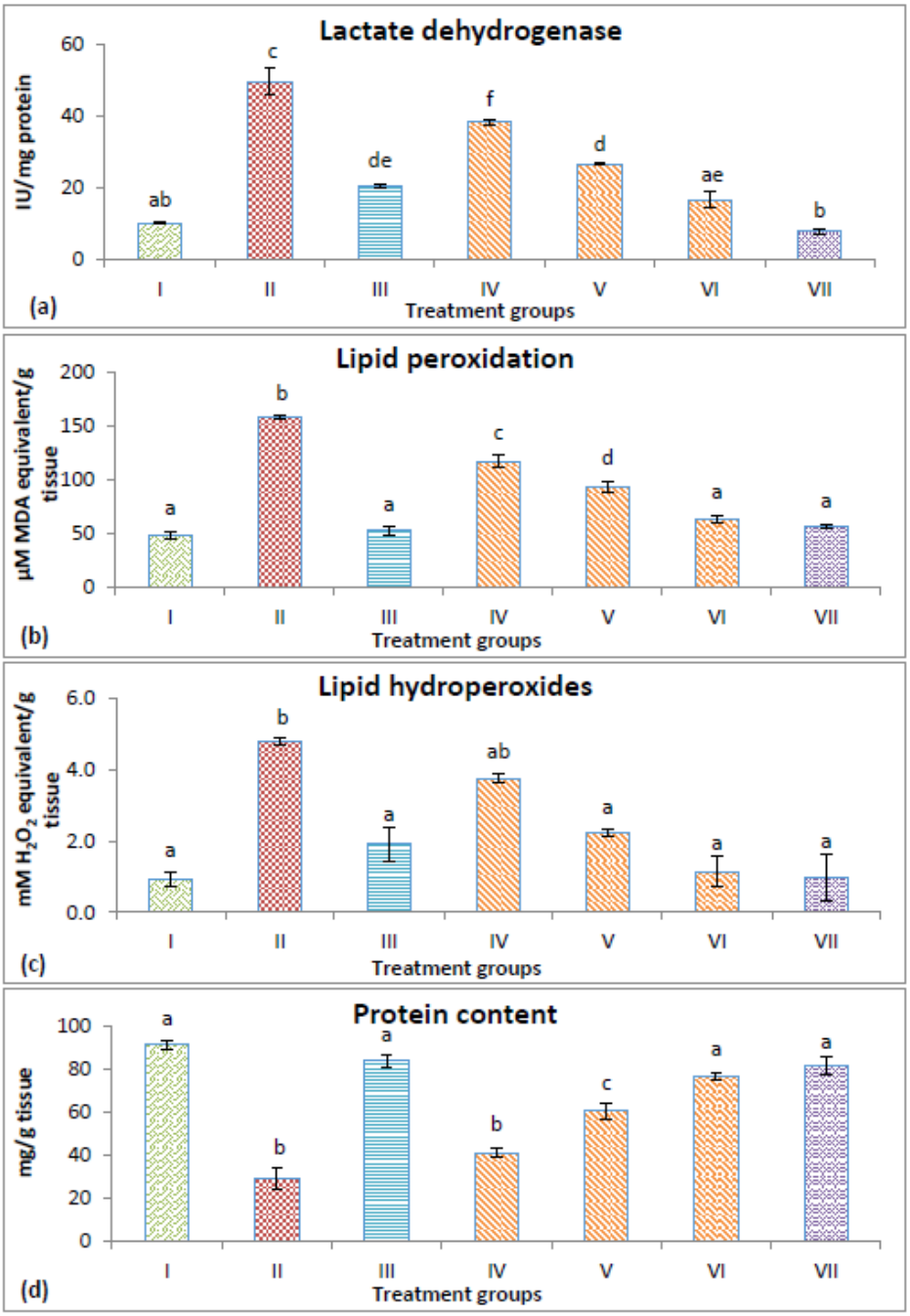

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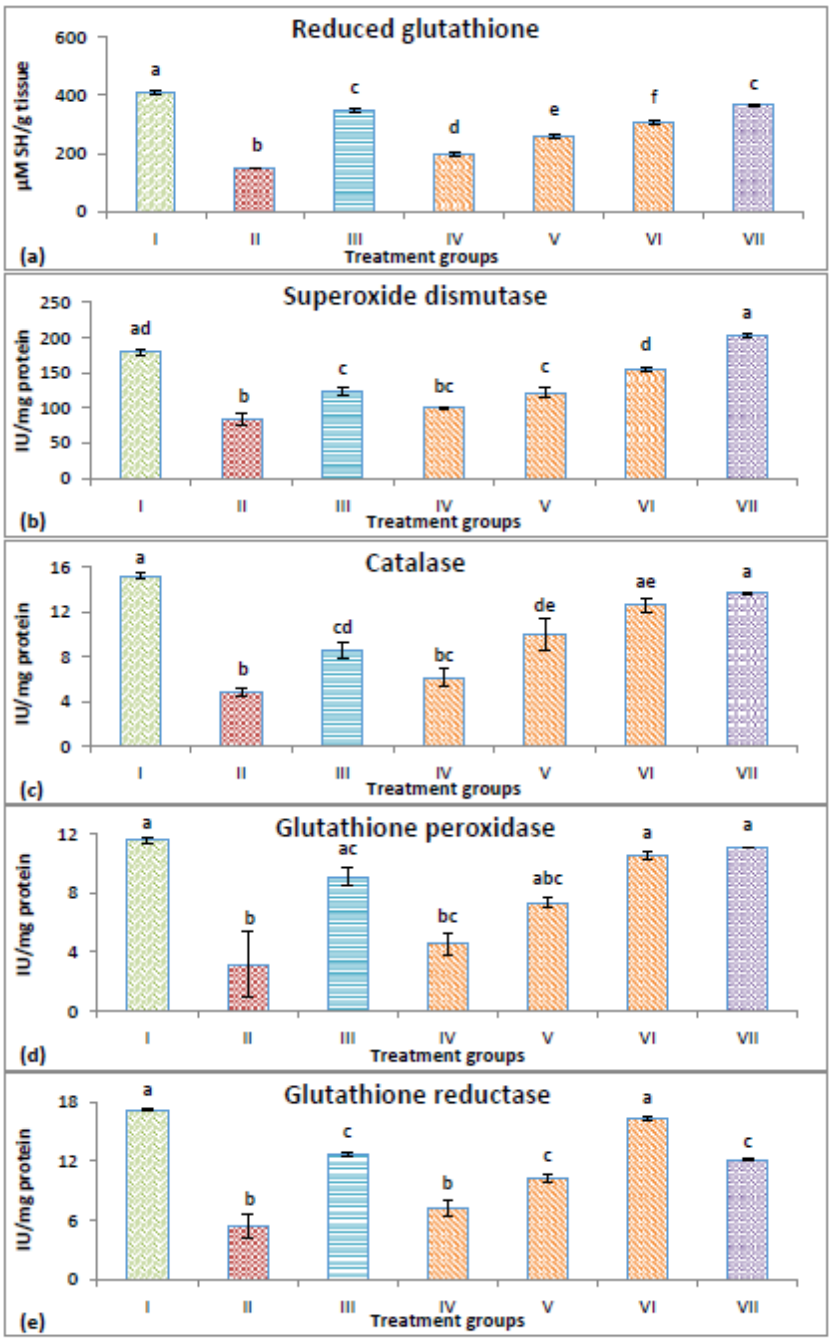
Figure 8 Modulatory effect of MHC pre-treatment on paracetamol-mediated alterations in oxidant biomarkers

Figure 8

Caption found in figure.



Group I: Normal control; Group II: Paracetamol (3 g/kg bw); Group III: MHC (200 mg/kg bw) per se; Group IV: MHC (50 mg/kg bw) + Paracetamol; Group V: MHC (100 mg/kg bw) + Paracetamol; Group VI: MHC (200 mg/kg bw) + Paracetamol; Group VII: Silymarin (50 mg/kg bw) + Paracetamol; Treatment groups with different lowercase letter(s) differ significantly at $p \leq 0.05$ (Tukey's HSD test); Animals in each group (n = 6)

Figure 9 Modulatory effect of MHC pre-treatment on paracetamol mediated alterations in antioxidant biomarkers

Figure 9

Caption found in figure.

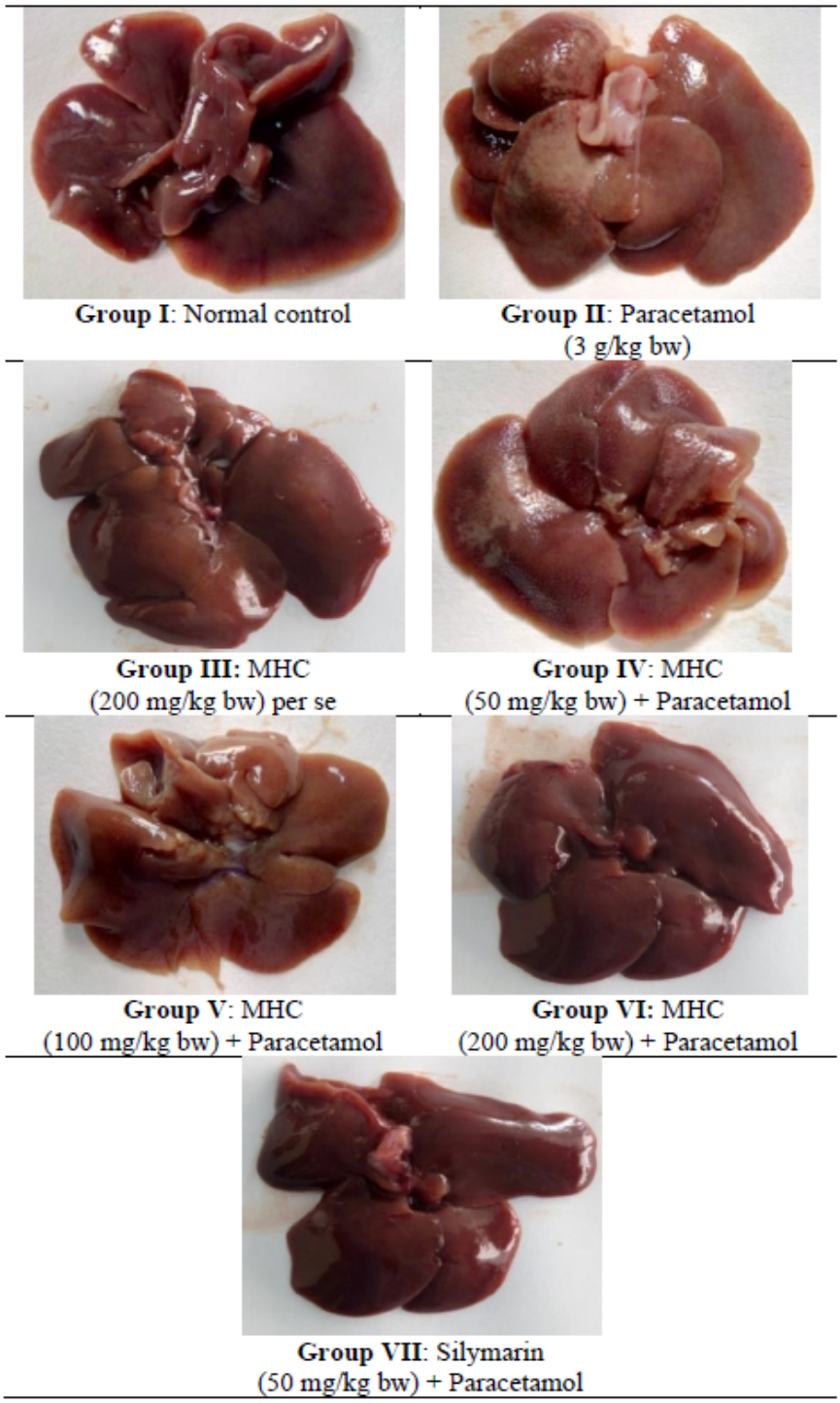


Figure 10 Gross morphological appearance of animal livers in different treatment groups

Figure 10

Caption found in figure.

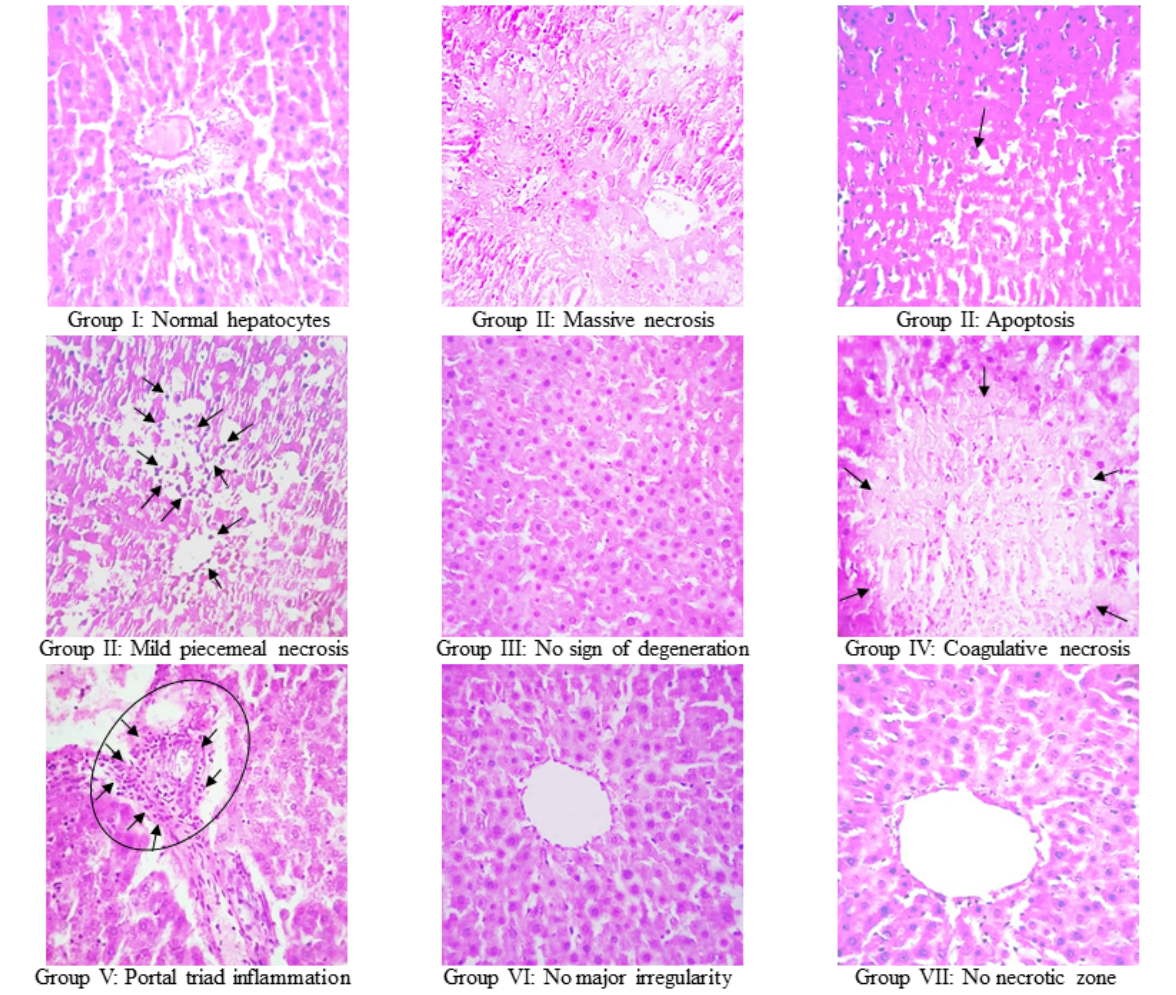


Figure 11 Microscopical examination of Hematoxylin and Eosin stained sections of liver tissues (original magnification: 400×)

Figure 11

Caption found in figure.

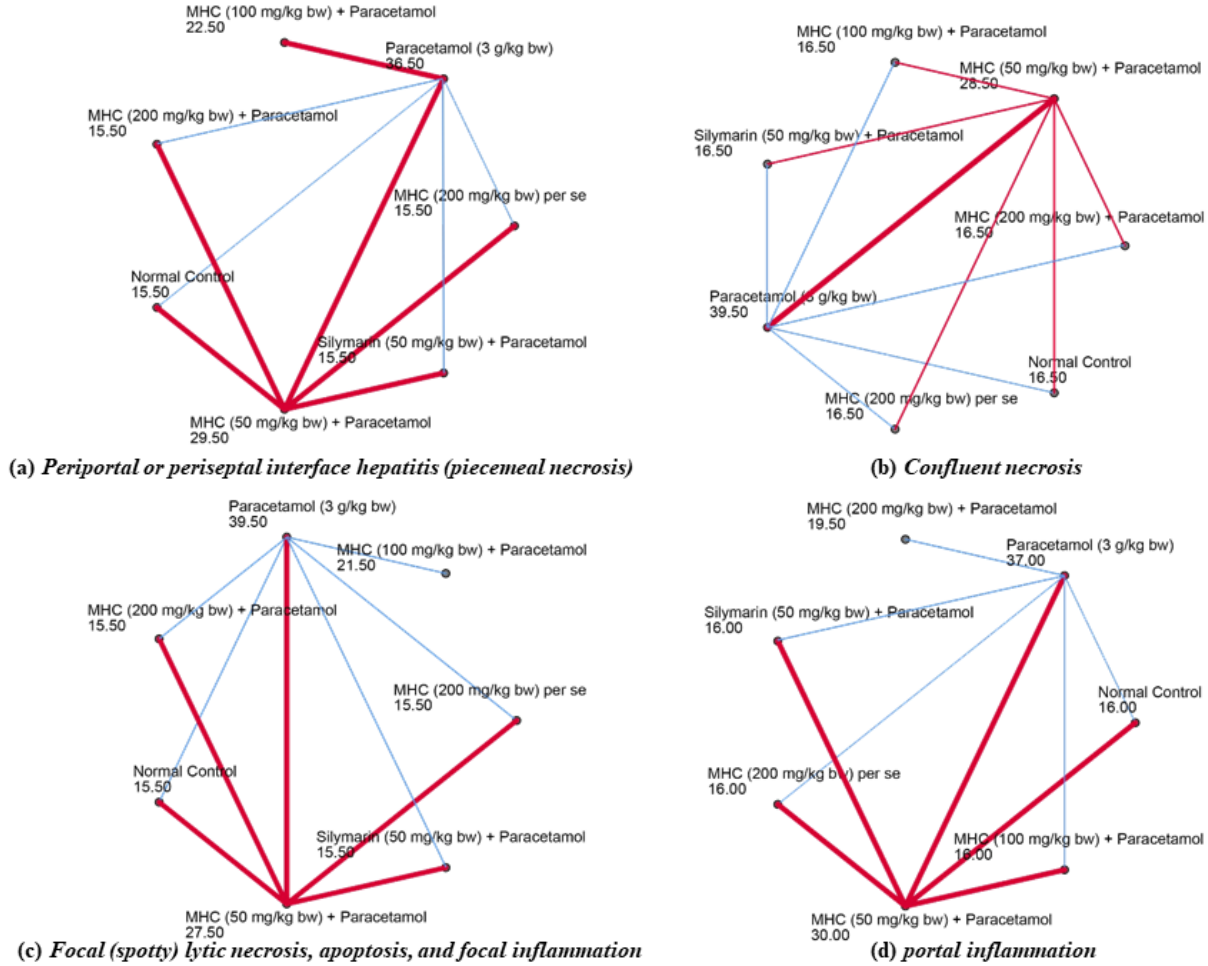


Figure 12 Dunn-Bonferroni pairwise comparisons with each node showing sample average rank of group [Blue lines indicate statistically significant differences at $p \leq 0.05$]

Figure 12

Caption found in figure.

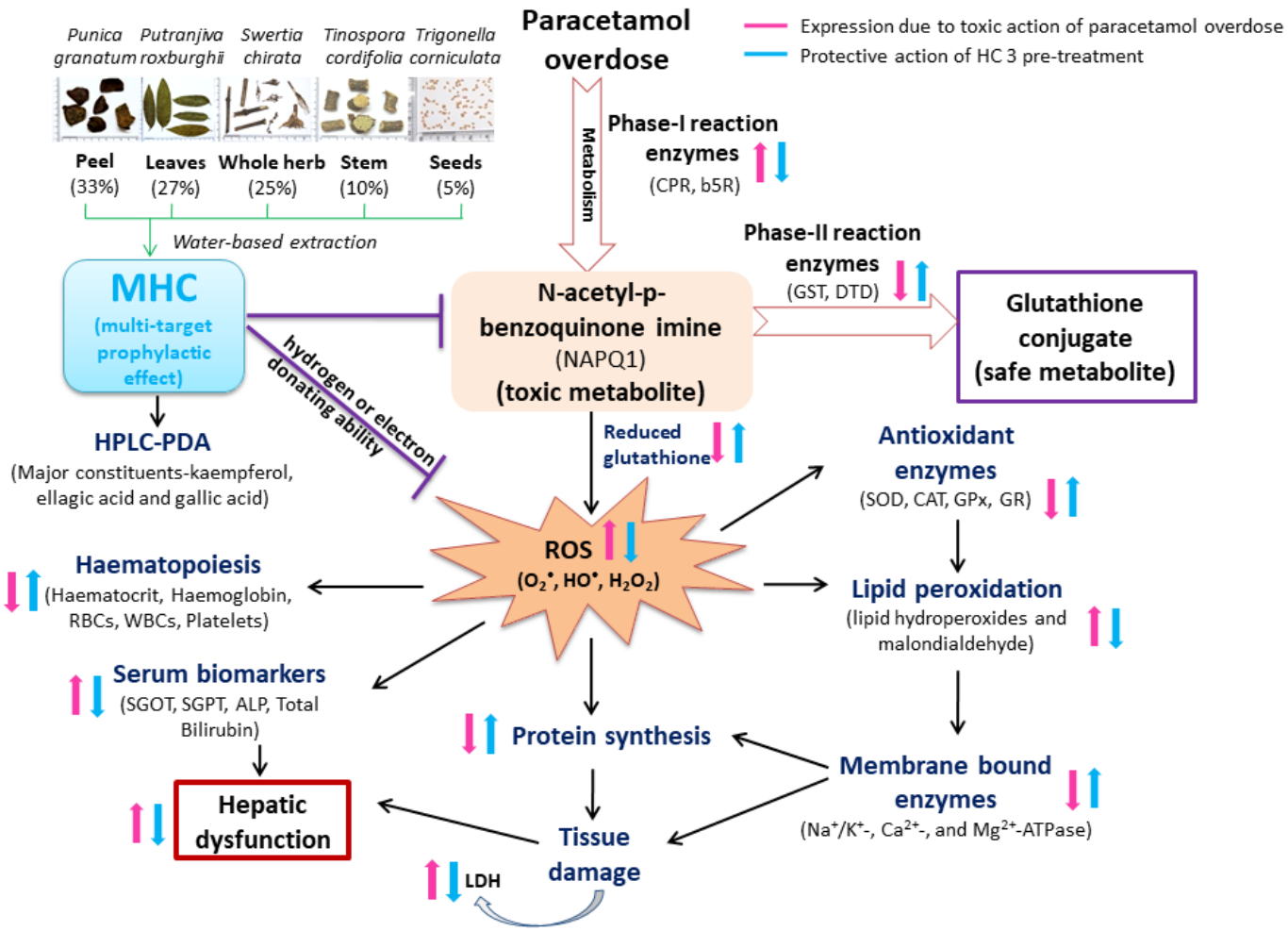


Figure 13 Modulatory effect of MHC against paracetamol induced acute hepatotoxicity in female Wistar rats

Figure 13

Caption found in figure.

Supplementary Files

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- SupplementaryTables.docx