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Original Article

CHEMICAL COMPOSITION AND HEPATOPROTECTIVE EFFECTS OF POLYPHENOLIC FRACTION FROM *RIVEA HYPOCRATERIFORMIS* IN PARACETAMOL INDUCED LIVER DAMAGE IN WISTAR ALBINO RATS

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ABSTRACT

Objective: This study was designed to chemical composition and hepatoprotective effects of a polyphenolic fraction of aerial parts of *R. hypocrateriformis* (PPFRH). It was shown to exhibit strong *in vitro* lipid peroxidation and scavenging activity against hydroxyl radical.

Methods: The chemical composition of a polyphenolic fraction of *R. hypocrateriformis* was analyzed by High-performance liquid chromatography method. Hepatocellular injuries induced by paracetamol were assessed by liver damage in Wistar albino rat; the hepatoprotective effect was evaluated by biochemical parameters in rat serum, antioxidant hydroxyl radical scavenging activity and lipid peroxidation in liver tissue.

Results: The polyphenolic fraction of aerial parts of *R. hypocrateriformis* for lipid peroxidation is significantly ($p<0.05$). In the hepatoprotective activity of liver enzymes and hepatic necrosis were significantly ($p<0.001$) closer to paracetamol. The correlation coefficient between the hydroxyl scavenging radical and total phenolic and flavonoid contents were found to be $R^2 = 0.9045$ and $R^2 = 0.8876$ suggesting the contribution of phenolic and flavonoid compounds of the polyphenolic fraction of aerial parts of *R. hypocrateriformis* by 90% and 88% to its radical scavenging activity.

Conclusion: The polyphenolic fraction of aerial parts of *R. hypocrateriformis* possesses a significant protective effect against acute hepatotoxicity induced by paracetamol and which may be due to the phenolic and flavonoid components.

Keywords: Antioxidant, Hepatoprotective, *Rivea hypocrateriformis*, HPLC, Phenolic

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INTRODUCTION

The liver plays a pivotal role in metabolism and detoxification of various components entering the body and hepatic injury associated with these metabolic dysfunctions can result in many disorders ranging from an ephemeral elevation of liver enzymes to life-threatening hepatic fibrosis, liver cirrhosis, and even hepatocellular carcinoma [1, 2]. Therefore, it is of prodigious significance to develop hepatoprotective agents to protect people from liver injury. However, the number of investigated medicines used successfully was limited, and some of them have potential adverse effects, especially when administered chronically or sub-chronically [3].

In modern beings, the use of active dietary ingredients and traditional herbs, which are believed to be harmless and free from serious adverse reactions, for the prevention and treatment of liver diseases has increased all over the world [2-6]. Within these natural phytoconstituents, dietary polyphenols such as flavonoids contain a number of phenolic hydroxyl groups and have been demonstrated to be the key ingredients responsible for the beneficial effect, which is mainly due to their scavenging activity against reactive oxygen species (ROS) [5, 7]. It is widely recognized that ROS can cause cell damage via the mechanism involving lipid peroxidation with subsequent tissue injury, especially live injury [7]. For this reason, it is of the highest priority to find natural antioxidants, especially polyphenolic fractions, for preventing or attenuating toxic liver injury today.

Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine [8]. Several studies were conducted in the field of drug discovery and development but due to the side effects of modern medicine, natural remedies are considered to be effective and safe alternative treatments for hepatotoxicity. Paracetamol induced toxicity in rats is one of the widely used experimental models to evaluate the hepatoprotective nature of herbal extracts [9, 10]. In recent years, the use of natural herbal products has enhanced worldwide attentions. Many herbal supplements are claimed to assist in a healthy lifestyle. Herbal drugs have made a significant contribution to the treatment of hepatotoxicity [11].

Rivea hypocrateriformis is a robust woody climbing shrub belonging to the family Convolvulaceae and is found in subtropical forests of India and Pakistan [12]. The plant is medicinally used by the indigenous population of Karnataka to cure various types of diseases, such as malaria, and to relieve pain. Evan though the plant is known for a large number of biological activities such as anti-diabetic, anti-implantation, in the treatment of burns and piles, pregnancy irruption, as antidepressant, as anticancer and as an analgesic [13-15]. Leaves and young shoots are eaten as a vegetable and roots are given after parturition. Cooked leaves of this plant are used as vegetable curry by the tribals of India. The plant has maximum vitamin An activity and has the capacity of maximum (75 to 98%) retention of β-carotene upon processing [16, 17]. Desmethyl bergenin hemihydrate is a naturally occurring isocoumarin isolated from this plant [18]. The phytochemical screening of mustai showed positive tests for steroids, alkaloids, glycosides, saponins, fixed oils/fats, tannins, and phenolic compounds [19]. Evaluation of pharmacological activities of *Rivea hypocrateriformis* in experimental animal models [20]. The great interest in these chemically varied compounds has resulted in the developments of novel therapeutic agents for the various treatments of diseases afflicted by human beings. Plant extract has a high level of biologically active components which exhibited most of the medicinal properties. No systematic biological and chemical investigation have been carried out. Therefore, the basic aim of our research was to determine the total phenolic and flavonoid contents in polyphenolic fraction of the species *Rivea hypocrateriformis* using

spectrophotometric methods and HPLC analysis, as well to examine in different ROS of hydroxyl radical scavenging activity and lipid peroxidation assays, in order to evaluate its natural antioxidant properties and evaluate the possible hepatoprotective effects of PPFRH in paracetamol-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Chemicals and reagents

All the materials used for this experiment were of analytical grade. Paracetamol (SD Fine Chemical, Mumbai), silymarin, Butylated hydroxyanisole (BHA) and thiobarbituric acid (Sigma-Aldrich Chemical, Bengaluru). Diagnostic kits (Swemed Biomedicals pvt. Ltd. Bengaluru) for the estimation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin estimation kits were purchased from Span Diagnostics, Surat, India. All other chemicals and reagents were of analytical grade.

Collection of plant material

The aerial parts of *R. hypocrateriformis* were obtained in and around Belur village, Basavakalyan taluka, Bidar district, Karnataka. Plant material was authenticated at the herbarium of the Laboratory of Botany, Gulbarga University, Kalaburagi with code number HGUG 90 voucher specimen of the titled plant has been deposited in the herbarium of the Department of Botany, Gulbarga University, Kalaburagi. It is dried under the shade, powdered in a grinder and powdered material was packed in paper bags and stored in an airtight container until use.

Preparations of polyphenolic fraction of *Rivea hypocrateriformis* (PPFRH)

Dried aerial parts of *R. hypocrateriformis* (500 g) were finely powdered, extracted in a Soxhlet apparatus with ethanol for 72 h. After 3 d it was filtered, and the solvent was concentrated under reduced pressure to obtain a dark green viscous mass (50 g). The green viscous mass was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 ml/l). Extraction of polyphenols was carried out for 36 h at room temperature and the combined ethyl acetate layer was concentrated. The residue was lyophilized and stored at 70 °C.

Phytochemical analysis

A small portion of the extract was analyzed chemically to determine the presence of phytoconstituents such as flavonoids, saponins, phenolic compounds, alkaloids, glycosides, steroids and tannins using standard chemical tests [21]. The total polyphenolic and flavonoid contents of the extract were assayed using the standard methods [22, 23].

Determination of total phenolics and total flavonoids

Total phenolic compounds in the various extracts were estimated by the Folin–Ciocalteu method as tannic acid equivalents (TAE), expressed as milligrams of tannic acid per gram of extract [22]. Briefly, aliquots of 2 ml extracts or standard solutions were mixed with 1 ml of Folin-Ciocalteu reagent and allowed to react for 3 min. After the addition of 1 ml of 10% Na_2CO_3, the mixture was allowed to stand at 25 °C for 2 h. Absorbance was measured at 760 nm, and total phenolic contents were calculated as TAE from a calibration curve, y = 0.7728x+0.0368 (R^2 = 0.8981, 20-100 µg of tannic acid). The data were presented as the average of triplicate analyses.

In addition, the total flavonoids of the extracts were measured as quercetin equivalents (QAE) using a modified colorimetric method [23]. Appropriately diluted extracts or standard solutions (1 ml) were mixed with 5% $NaNO_2$ solution (0.2 ml). After 6 min, 0.2 ml of 10% $AlCl_3$ solution was added and allowed to stand for another 6 min. Subsequently, the reaction solution was mixed with 0.6 ml of 4% NaOH solution, and 60% ethanol was immediately supplied to the final volume of 10 ml, followed by a thorough mixture and a further wait for 10 min. The absorbance of the mixture was determined at 510 nm versus blank water, and all determinations were carried out in triplicate. Quercetin calibration curve was prepared in ethanolic solutions with the same procedure. The concentration of flavonoids was calculated as QAE according to the following linear equation based on the calibration curve y = 1.2444x-0.011, R^2 = 0.9434 (0.02 −0.1 mg of quercetin).

HPLC analysis of flavonoids

The quantification of the component quercetin in PPFRH was carried out using an AGILENT LC-1200 SERIES high-performance liquid chromatography (HPLC). HPLC system equipped with a quaternary pump, multi-wave UV/VIS detector, autosampler, fraction collector and 5 mm Zorbax RX-C18 (150 X 4.6 mm, Agilent Technologies) column was used. The multi-wave UV–VIS detector was set at 254 nm and, by a 100% methanol as the mobile phase at a flow rate of 1.0 ml/min with a run time of 30 min and injection volume is 50 µl. The standard solution is prepared at a concentration of 1.0 mg/ml in methanol. Samples were also prepared by the same procedure. Before injection, all samples were filtered through a 0.22 µm Millipore membrane. Flavonoid compounds were identified by comparison of their chromatograms and UV-Vis spectroscopic data with a standard compound. Concentrations of the investigated flavonoids were determined based on the chromatographic data of the standard compounds. The calibration curves (peak area Vs concentration) for individual standards were obtained for a wide concentration range.

Experimental animals

Adult male albino Wistar rats (180-220 g) of either sex were procured from Central animal house M. R. Medical College, Gulbarga, Karnataka. The rats were divided into five groups of six animals each. They were kept under a temperature of (20±2) °C, humidity of 50% and light and dark cycles of 12 h: 12 h. They were fed with commercial pellet diet (Amrut Laboratories Pranava Agro Industries Ltd., Sangli, India) and water was provided *ad libitum*. The protocol was approved by Institutional Animal Ethics Committee (IAEC) (No. HKES COP/IAEC/2012/54).

Acute toxicity

Wistar albino mice and rats weighing (180-220 g) of either sex were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and no adverse effects or mortality was detected in mice and rats up to 4000 mg/kg, p. o., during the 24 h observation period, with special attention given during the first 4 h and daily thereafter, for a total of 14 d. During this period the mortality and/or the moribund status of the animals were noted.

Paracetamol-induced hepatotoxicity

Paracetamol (acetaminophen), a widely used antipyretic analgesic drug an overdosage, produces acute hepatic damage. The covalent binding of an oxidation product of paracetamol, i.e., N-acetyl-p-benzoquinone imine and sulphydryl groups of protein results in cell necrosis and lipid peroxidation which causes hepatotoxicity leading to increased levels of serum marker enzymes like AST, ALT, ALP and total bilirubin (TB) [24-27].

Hepatoprotective studies

Wistar albino rats of either sex were divided into five groups of 6 animals each and are given the following treatment orally for three days. Group I served as normal control, and they received 2% gum acacia (0.1 g/200 g bw) p. o. Group II received paracetamol at a dose of (2 g/kg) p. o. (paracetamol control). Group III received both silymarin (100 mg/kg bw, p. o.) and paracetamol dose. Group IV received paracetamol and PPFRH (300 mg/kg p. o.). Group V received paracetamol and PPFRH (600 mg/kg p. o.). After 24 h of the last treatment, blood was collected from retro-orbital plexus, allowed to clot for 1 h at room temperature and serum was separated by centrifugation at 3000 rpm at 30 °C for 15 min. The serum was then collected and analysed for various biochemical parameters.

Assessment of liver function

The serum collected after centrifugation was analysed for various biochemical parameters like SGOT/AST, SGPT/ALT, ALP and TB.

Serum transaminase activity was measured as per the standard procedure described by the manufacture's instruction manual provided in the kit using semi-auto analyzer according to the method [28]. The ALP and the serum bilirubin were determined using the method of Scand [29].

Effect on *in vitro* lipid peroxidation

Preparation of rat homogenates

Rats were handled according to international regulations and maintained under standard conditions of humidity, food, circadian cycles, and temperature. Liver homogenates were obtained from 3-month-old male Wistar rats weighing (180-220 g). For the ferrous ion oxidation with xylenol orange (FOX) method, 40% (w/v) homogenates were prepared in HPLC-grade methanol.

Lipid peroxidation by FOX method

Lipid peroxidation was conducted for a 60 min interval at 37 °C. The mixture for lipid hydroperoxide generation contained 0.01 ml of Fenton's reagent (0.05 ml of 5 mmol manganese chloride and 0.05 ml of 50 mmol hydrogen peroxide), 0.1 ml of the PPFRH, and 0.1 ml of each homogenate 0.9 ml of FOX reagent (49 mg of ferrous ammonium sulphate in 50 ml of 250 mmol H_2SO_4, 0.397 g of butylated hydroxytoluene and 0.038 g of xylenol orange in 950 ml of HPLC grade methanol was added to each sample and left to react for 30 min at room temperature. The absorbance was read at 560 nm [30].

$$Inhibition\ of\ LPO\ (\%) = \frac{Acontrol - Bsample}{Acontrol} \times 100$$

Where A is absorbance Control and B is absorbance sample

Effect on hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extracts was determined by the method [31]. The reaction mixtures contained 0.8 ml of phosphate buffer solution (50 mmol/l, pH 7.4), 0.2 ml of PPFRH/standard at different concentrations, 0.2 ml of ethylene diamine tetra acetic acid (1.04 mmol/l), 0.2 ml of $FeCl_3$ (1 mmol/l) and 0.2 ml of 2-Deoxy-D-ribose (28 mmol/l) were taken in the test tubes. The mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 ml of ascorbic acid (2 mmol/l) and 0.2 ml of H_2O_2 (10 mmol/l). After incubation at 37 °C for 1 h, 1.5 ml of cold thiobarbituric acid (10 g/l) was added to the reaction mixture followed by 1.5 ml of HCl (25%). The mixture was heated at 100 °C for 15 min and then cooled down with water. The absorbance of the solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging activity was evaluated with the inhibition of percentage of 2-Deoxy-D-ribose oxidation on hydroxyl radicals. The percentage of hydroxyl radical scavenging activity was calculated according to the following formula:

$$Hydroxyl\ radical\ scavenging\ activity(\%) = \frac{[Ao - (A1 - A2)]}{Ao} \times 100$$

Where, A_0 is the absorbance of the control without a sample, A_1 is the absorbance after adding the sample 2-Deoxy-D-ribose, A_2 is the absorbance of the sample without 2-Deoxy-D-ribose. Then the percentage of inhibition was plotted against concentration.

Data and statistical analysis

The data were expressed as mean±SEM and the statistical significance was determined using analysis of variance (ANOVA) followed by Student's t-test. Values were considered to be significant at P<0.001.

RESULTS AND DISCUSSION

Phytochemical investigation

The qualitative phytochemical composition of a polyphenolic fraction of aerial parts of *R. hypocrateriformis* showed the relatively high concentration of bioactive compounds such as flavonoids, saponins, phenolic compounds, alkaloids, glycosides, steroids, and tannins.

Total phenolic and flavonoid contents

Polyphenols are the most abundant as well as an important class of compounds in the plant kingdom due to their antioxidant nature and various disease curing abilities [32]. Phenolic compounds are the most active antioxidant derivatives in plants [33]. They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are also stable radical intermediates [34]. Flavonoids and phenolic acids are important contributing factors to the antioxidant and hepatoprotective effects of the human diet. However, most of the phenolics suffer extensive biotransformation before absorption in the human body. So they act as hepatoprotective and antioxidants. The total phenolic and flavonoid contents of PPFRH were determined by Folin–Ciocalteu method and the flavonoid content determined by two independent colorimetric methods. The assays were performed in the PPFRH, as that could be more beneficial than isolated constituents because of the additive and synergistic effects. Considering that a bioactive individual component can change its properties in the presence of other compounds present in the extracts [35]. A calibration curve was first plotted using different concentrations of tannic acid. The results were determined from the regression equation of the calibration curve (A= 10.97 B-0.4098, R^2 = 0.998) as tannic acid equivalents in milligram per gram of extract (mg TAE/g extract).

The results were determined from the regression equation of the calibration curve (A= 10.97 B-0.4098, R^2 = 0.998) as quercetin equivalents in milligram per gram of extract (mg QAE/g extract). This quantitative assay demonstrated that PPFRH contained the highest amount of total phenolics (0.170 µg TAE/mg fraction) and total flavonoids (0.193 µg QAE/mg fraction), suggesting that PPFRH is a polyphenol enhanced fraction as shown in the (fig. 1).

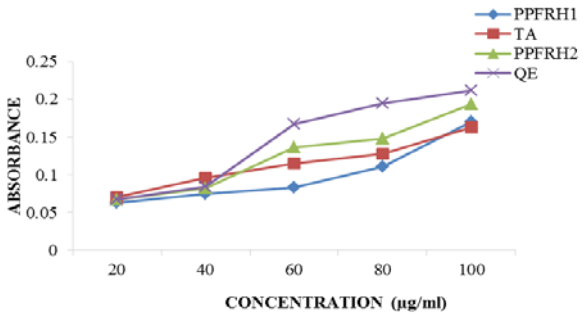

Fig. 1: Total phenolic and flavonoid contents of a polyphenolic fraction of *R. hypocrateriformis* (PPFRH1 and 2). Values expressed are mean, total phenol and flavonoid contents are expressed as mg of tannic acid equivalent (TAE) and quercetin equivalent (QAE) per gm of fraction

Therefore, the high content of phenolics and flavonoids in extracts of the *R. hypocrateriformis* might indicate the strong antioxidant and hepatoprotective properties. The results of the present study strongly suggest that phenolics and flavonoids are important components of the plant and some of their pharmacological effects could be attributed to the presence of these constituents.

Characterization of polyphenolic composition of PPFRH

The main polyphenolic compounds present in PPFRH were identified and quantified to gain an insight into the compounds responsible for antioxidant and hepatoprotective effects. HPLC profiles of polyphenolic compounds present in PPFRH are shown in (fig. 2A) and retention time (t_R) and quercetin were baselines separated from other with t_R is 3.108 min, respectively, as shown in (fig. 2B) was identified to be present in the highest level in PPERH, followed by quercetin.

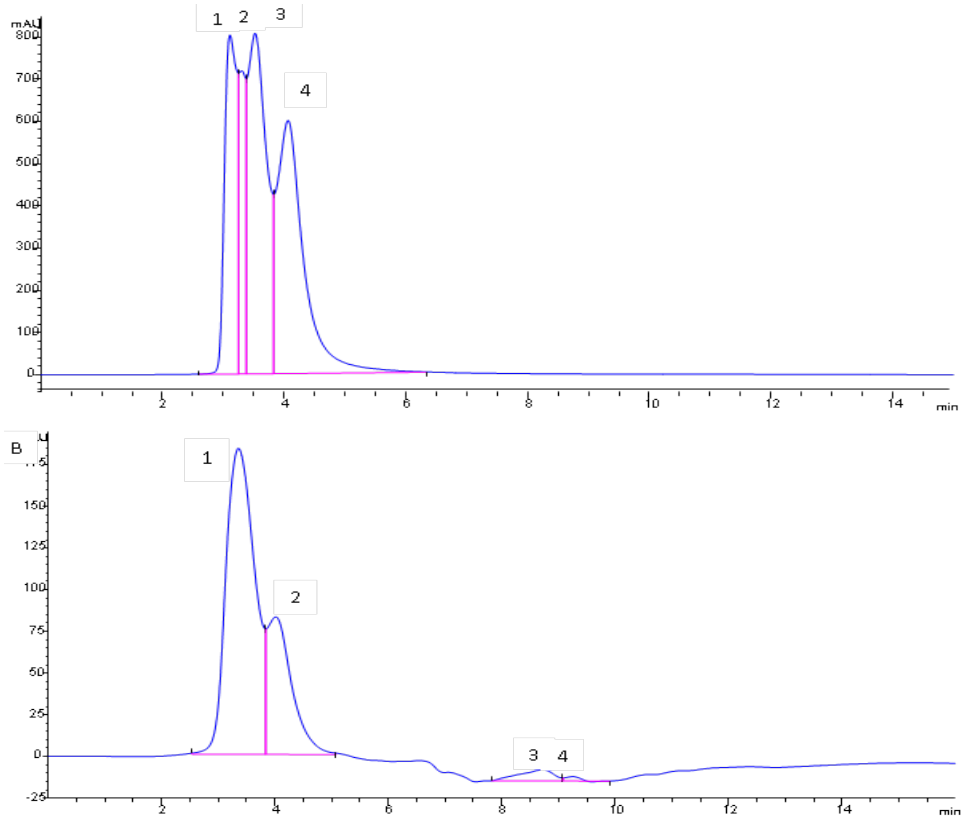

Fig. 2: RP-HPLC profiles of the standard flavonoid quercetin (A) and the flavonoid compounds in PPFRH (B) at 254 nm. Peaks: 2, quercetin

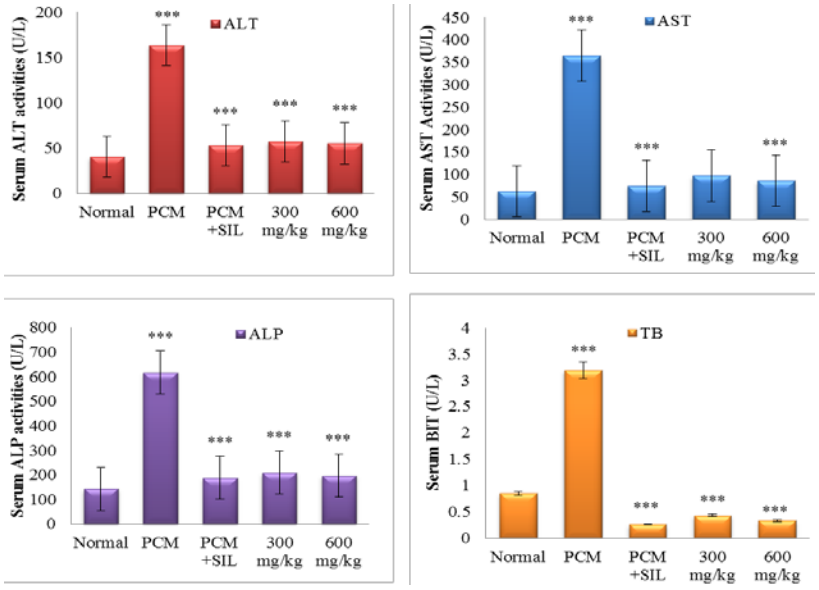

Fig. 3: Effects of PPFERH on serum ALT, AST, ALP and TB. Rats were administrated paracetamol with PPFERH at 300, and 600 mg/kg body weight once daily for 4 consecutive days prior to the single administration of paracetamol (1%). Values are expressed as means±SD of 6 rats in each group. ***p<0.001, as compared to the paracetamol intoxicated group

Quercetin was reported to be effective in several models of liver damage including paracetamol induced necrotic cells in rats [36-38].

Quercetin was demonstrated to be the main compound in hepatoprotective extracts from the plant. Therefore, the presence of quercetin and other antioxidants in PPFRH may be the main contributing factor toward its hepatoprotective activity as a dietary supplement. The results obtained in this analysis clearly indicate that *R. hypocrateriformis* is a good source of quercetin as a natural antioxidant and hepatoprotective agent.

Acute toxicity

The toxicity study of PPFRH indicated, there was no adverse effect on mortality detected in Swiss albino mice and Wistar albino rats that were administered up to 4000 mg/kg, orally. This was observed during 24 h period, and the extract was found to be safe at the given dose.

Effects of PPFRH on levels of serum ALT, AST, ALP and TB activities

The results of the hepatoprotective effects of PPFRH on the enzymatic activities of serum ALT and AST are shown in (fig. 3).

Paracetamol induced serum rise of ALT was protected by 100 mg/kg body weight dose of silymarin and 300 and 600 mg/kg doses of PPFRH. In the paracetamol group, serum ALT and AST activities were 163±3.99 and 365±5.60 IU/l, respectively.

Paracetamol induced serum rise of ALT and AST were protected by 100 mg/kg body weight dose of silymarin, 300 and 600 mg/kg doses of PPFRH serum showed remarkable decreases to 53.51±1.90, 57.4±1.16 and 55.3±0.69 IU/l ($p<0.001$) and 74.78±1.18, 97.53±2.44 and 85.95±1.79 IU/l ($p<0.001$) respectively, the paracetamol-induced increases were significantly reduced by pre-treatment, and this reduction was dose-dependent. (fig. 3) shows in the paracetamol induced a significant increase in serum ALP and TB levels sharply increased by comparison with that of the paracetamol group (617.56±1IU/l and 3.18±0.21 mg/dl), respectively ($p<0.001$). The pretreatment of PPFRH dose-dependently reduced the ALP and TB levels caused by paracetamol, especially when the dosage increased to 100 mg/kg bw silmarin, 300 and 600 mg/kg body weight, 3. 188.28±5.27, 208.79±5.42 and 196.72±2.45 IU/l ($p<0.001$) and 0.26±0.06, 0.43±0.10 and 0.33±0.12 mg/dl ($p<0.001$), respectively.

Paracetamol-induced hepatotoxicity is the most commonly used screening method for testing the hepatoprotective nature of plant extracts. The hepatic damage leads to increased serum levels of enzymes damage and loss of functional integrity of cell membrane in liver [39]. Due to damage of hepatocytes, i.e., cell necrosis (NC). The increased production of serum enzymes in blood stream was associated with central/submissive necrosis of liver which causes severe hepatic injury. The hepatoprotective effect of PPFRH may be due to phytoconstituents like polyphenols [40-43]. The *in vitro* lipid peroxidation assay and hydroxyl radical scavenging assay suggest the ability of PPFRH to reduce biological oxidative stress [44]. Hence, the hepatoprotective effect of that PPFRH extract may be achieved by the scavenging free radical activity of the oxidative stress [45]. Moreover, the increased levels of these serum enzymes were significantly decreased by treatment with PPFRH at 300 mg/kg and 600 mg/kg, implying that the extract prevented the liver damage. The PPFRH treatment showed dose-dependent activity, PPFRH at 600 mg/kg showed good result than 300 mg/kg which is shown in the (fig. 3) for the measured levels of different serum enzymes. Phytochemical screening of PPFRH showed the presence of flavonoid and phenolic compounds. These were further confirmed by quantitative and HPLC analysis and these antioxidant phytochemicals of PPFRH strength subsidize to its hepatoprotective activity. Hence, from this study, it was concluded that PPFRH possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

The results of the effect of Polyphenolic extract of *R. hypocrateriformis* to prevent lipid peroxidation are shown in (fig. 4).

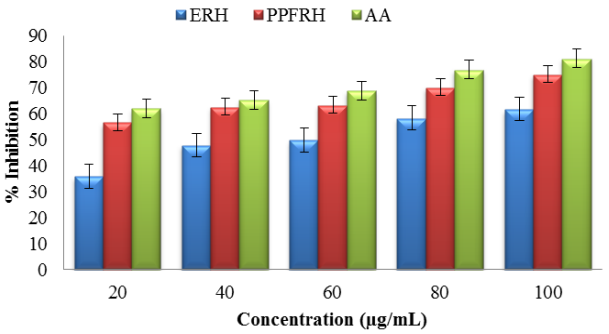

Fig. 4: Determination of lipid peroxidation inhibition activity of PPFRH Data expressed as mean±SD (n = 3) for all tested dosages

At a 100 µg/ml, the extract showed a 76% scavenging activity by this method. In biological systems, MDA is a very reactive species and takes part in cross-linking of DNA with proteins and also damaging the liver cells [46]. Lipid peroxidation has been broadly defined as the oxidative deterioration of polyunsaturated lipids. Initiation of a peroxidation sequence in a membrane or polyunsaturated fatty acid is due to abstraction of a hydrogen atom from the double bond in the fatty acid. The free radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which then easily reacts with an oxygen molecule to give a peroxy radical [47]. Peroxy radicals can abstract a hydrogen atom from another molecule, or they can abstract a hydrogen atom to give a lipid hydroperoxide, R-OOH. A probable alternative fate of peroxy radicals is to form cyclic peroxides; these cyclic peroxides, lipid peroxides, and cyclic endoperoxides fragment to aldehydes including MDA and polymerization products. MDA is the major product of lipid peroxidation and is used to study the lipid peroxidation process in rat liver homogenate. Determination of the lipid peroxide content was carried out indirectly by means of derivatizing MDA with TBA at high temperature and acidic conditions [48]. After have been suggesting that phenolics and flavonoids are major contributing compounds toward the lipid peroxidation of the PPFRH and polyphenol-flavonoid rich of PPFRH might have the latent to provide significant natural defense against oxidative damage.

Fig. 5 shown in the hydroxyl radical scavenging activity of PPFRH was dose dependent. The fraction, PPFRH is higher activity than that of the crude extract (ERH). At a concentration of 100 mg/ml, the scavenging activity of ERH and PPFRH was found to be 68.36% and 76.5%, respectively, whereas, at the same concentration, the standard BHA was 79.79%. The demonstrating inhibitory effect of hydroxyl radical scavenging activity of standard BHA is higher than that of PPFRH.

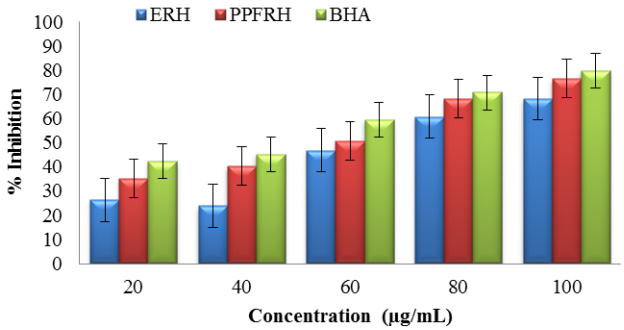

Fig. 5: Determination of hydroxyl radical scavenging activity of PPFRH. Data are expressed as mean±SD (n = 3) for all tested dosages

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [49, 50]. When hydroxyl radical generated by the Fenton reaction attacks deoxyribose, the deoxyribose degrades into fragments that react with TBA on heating at low pH to form a pink colour. In this assay, iron ions are equally available to both the deoxyribose and the compounds under test. Only compounds that are able to interfere with the non-binding capacity of sugar and withdraw the iron ions and render them inactive or poorly active in the Fenton reaction can bind iron ions strongly enough to inhibit deoxyribose degradation in the absence of EDTA [51]. At the same concentration, the inhibition of deoxyribose degradation in the ascorbate was greater, which implied that PPFRH was a strong metal chelator and a moderate scavenger of hydroxyl radical. The ability of the PPFRH to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be a good scavenger of active oxygen species, thus reducing the rate of a chain reaction.

CONCLUSION

The results clearly demonstrate that polyphenolic-rich PPFRH has a protective effect against paracetamol-induced acute hepatotoxicity in rats, as evidenced by the lowered tissue lipid peroxidation and

elevated levels of enzymatic and nonenzymatic antioxidants in liver. The predominant individual polyphenolics in PPFRH were found to be quercetin, responsible for antioxidant and hepatoprotective activities. The results indicate that the polyphenolic-rich PPFRH has a significant potential to allow future exploitation as a natural antioxidant and dietary source for the mitigation of oxidative stress-induced liver injury. The isolation of secondary metabolites from the plant will help us further in understanding the mechanism of action and identification of lead compounds of clinical utility.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest

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