

***Rosa brunonii* Lindely fruit as a new protective agent evaluated against Rif/INH induced toxicity in rats**

Ejaz Ahmad¹, Muhammad Jahangir*¹, Zahid Mahmood Akhtar², Saiqa Ishtiaq³, Hamid Mukhtar⁴, Hafiz Muhammad Faizan Haider¹ and Nadeem Irfan Bukhari³

¹Department of Chemistry, Government College University Lahore, Pakistan

²Department of Pathology, King Edward Medical University, Lahore, Pakistan

³Punjab University College of Pharmacy, University of Punjab, Lahore, Pakistan

⁴Department of Biotechnology, Government College University Lahore, Pakistan

Abstract: *Rosa brunonii* L., a less investigated plant contains flavonoid glycosides and is used to treat stomach ailments, heart problems, and diabetes in folk. The crude extract of the plant possesses antioxidant activity. The current work was aimed to investigate the presence of phytochemicals, antioxidative stress and protective potential of chloroform extract of the *Rosa brunonii* L. fruits (RBFCE) against liver and kidney toxicity induced by anti-tuberculosis drugs, rifampicin/isoniazid (Rif/INH) in *Wistar albino* rats. Animals were divided into six groups, each comprising 6 rats and fed with a standard pelleted diet. Normal control group was given only a standard pelleted diet. The vehicle control group received 0.5% carboxymethylcellulose (CMC) aqueous solution (vehicle). Negative and positive control groups were given Rif/INH (50+50 mg/kg, p.o) and silymarin (SILM) (200 mg/kg, p.o) in 0.5% vehicle for 30 days, respectively. Extract treated groups received low and high doses of RBFCE (500 mg/kg, p.o and 1000 mg/kg, p.o respectively) in 0.5% vehicle for 30 days. At a higher dose, animals showed significantly reduced Rif/INH induced toxicity in liver and kidney tissues as indicated by the normalized serum biochemical markers and histopathological investigations. The present exploration reveals the presence of strong antioxidant phytochemical constituents, antioxidative stress and protective potential of RBFCE against Rif/INH induced hepatic and renal damage.

Keywords: *Rosa brunonii* L. fruit, hepatotoxicity, nephrotoxicity, rifampicin / isoniazid.

INTRODUCTION

The use of antituberculosis drugs such as rifampicin and isoniazid (Rif/INH) have been proved to be associated with the production of certain toxic compounds which could induce hepato- and nephro-toxicity (Mahmoud *et al.*, 2014). Rif/INH treatment induces oxidative stress, and metabolic as well as morphological alterations in liver and kidneys as these organs are the main detoxifying and excretion sites for drugs (Santhosh *et al.*, 2007). Alteration in the serum biochemical marker in liver and renal function tests are supposed to be the key indicators for the hepatic and renal injury (Setty *et al.*, 2007). Conventional hepatoprotective drugs stimulate liver functions and regeneration of hepatic cells, but such drugs are also toxic at a particular dose (Gagliano *et al.*, 2007). Natural remedies are considered as more safer treatments for toxicity because these regulate the biochemical modification by promoting healing process and regeneration of liver cells (Ranawat *et al.*, 2010). Essential oils of various aromatic plants possess antioxidant and antimicrobial properties (Kiran *et al.*, 2007). Natural antioxidants are primarily plant phenolic compounds (Rice-Evans *et al.*, 1995, Kähkönen *et al.*, 1999), which besides having super antioxidative characteristics, also possess anticoagulant, free-radical

scavenging (Leenen *et al.*, 2000, Ng *et al.*, 2000), anticancer (Ren *et al.*, 2003), anti-inflammatory (Crespo *et al.*, 1999), anti-mutagenic (Miyazawa *et al.*, 2000) and antidepressant activities (Butterweck *et al.*, 2000).

Rosa brunonii L., commonly known as ‘Himalayan musk rose’, belongs to family *Rosaceae* and found in the western Himalayan region. Roots of the plant are locally named as ‘*Rajatarini*’ and are locally used to treat eye inflammation. Its fruit is locally used as a food and to prepare sweet preserve of flowers, called locally as *gulkand* which is used a laxative and in treatment for stomach ailments. Crude extract of *Rosa brunonii* L. flowers exhibits strong *in-vitro* antioxidant potential (Abbasi *et al.*, 2010). Some bioactive compounds isolated from the fruit of *Rosa brunonii* L. such as quercetin-3-O-rhamnoside, astragalin and tiliroside have strong antioxidant properties (Ishaque *et al.*, 2017). The presence of such antioxidant phytochemicals has increased the medicinal value of *Rosa brunonii* L. fruit. Keeping in view the strong *in-vitro* antioxidant potential of *Rosa brunonii* L. flowers, fruit as well as isolated compounds, the present study further investigated the hepatoprotective, nephroprotective and anti-oxidative stress potential of RBFCE against Rif/INH-induced hepatic and renal toxicity in *Wistar albino* rats. Medium polar chloroform fraction was selected for study due to ease of bioactive guided isolation and abundance of

*Corresponding author: e-mail: mjahangir.gcu@gmail.com

polyphenolic and flavonoid secondary metabolites in the solvent.

MATERIALS AND METHODS

Chemicals and reagents

Rifampicin (batch# 80201808017, Shenyung Pharma, China), Isoniazid (batch#18265/INH Amsal Chemicals, India) and carboxymethylcellulose (batch# 38591180306, India) were obtained from Schazoo Zaka (Pvt) Ltd. Lahore. While Silymarin (China) from Punjab Drug Testing and Research Centre, Lahore Pakistan. Methanol, chloroform, formalin, aluminium chloride (AlCl₃), sodium bicarbonate (NaHCO₃), Folin-Ciocalteu's phenol reagent (FCP), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 3-tert-butyl-4-hydroxyanisole (BHA) (Sigma-Aldrich), rutin (RU) (Fluka Chemie) urethane (Sigma-Aldrich), chloralose (Sigma-Aldrich) and gallic acid (GA) (Fluka Chemie) were procured from the local market. All the clinical diagnostic kits were purchased from Beckman Coulter and solvents used were of analytical grade.

Plant material

Rosa brunonii L. fruits were collected from Murree (latitude 33.78° N, longitude 73.39° E and an altitude of 2291 m), Pakistan during the 2nd week of October, 2016. After identification by an expert taxonomist, a voucher (GC. Herb. Bot. 3315) was deposited in the herbarium of the GC University Lahore for reference. No further specific permissions were required.

Preparation of plant extract

The fruits were washed with distilled water, followed by shade drying and then pulverized by mechanical means to get powder. Powder of *Rosa brunonii* L. fruit (250 g for each extraction) was extracted with 1L of n-hexane, chloroform and methanol respectively at 80°C for 6 h using Soxhlet extraction apparatus. After filtration through Whatman filter paper, solvent was evaporated from polyphenolic and flavonoid rich fraction by rotary evaporator (Laborota 4000-efficient, Heidolph Germany) at 40°C, dried, weighed and kept in amber color airtight container. The RBFCE was suspended in vehicle prepared as 1 ml of 0.5% carboxymethylcellulose (CMC) aqueous solution for oral administration to rats.

Phytochemical analysis and calculation of IC₅₀

The RBFCE was analyzed for the presence of different classes of phytochemicals, i.e., proteins, alkaloids, flavonoids, terpenoids, cardiac glycosides and phenolic contents using previously reported methods (Raaman, 2006). The sugars, fixed oils, anthraquinones, and steroids were tested by following the reported method (Brinda *et al.*, 1981). Phenolic and flavonoid contents were quantified by literature-cited methods (Singleton *et al.*, 1999) and (Quettier-Deleu *et al.*, 2000), respectively. The Value of IC₅₀ was calculated through DPPH free radical scavenging assay method (Takao *et al.*, 1994) by reading

absorption maxima on UV-Vis Spectrophotometer (UV-2450 Shimadzu, Japan).

Animals

This study was carried out at College of Pharmacy, University of the Punjab Lahore, using clinically healthy adult *Wistar albino* rats weighing between (170-210 g). The animals were retained under standard environmental conditions with free access to standard pelleted diet and water or where indicated otherwise or differently. All the experiments were carried out in accordance with ICH guidelines (Ohno, 2002) after the approval by Animal Ethical Committee of University of Punjab, College of Pharmacy (AEC/PUCP/1077).

Acute toxicity study

The acute toxicity study was performed by using the limit test procedure according to Organization for Economic Corporation and Development (OECD) test guidelines (Guideline, 2001) using 10 rats (5 males and 5 females) which were fasted for 3h before the commencement of study. Animals were given a single dose of RBFCE (2000 mg/kg, p.o) in 1ml of CMC and then observed uninterruptedly for 1h, then half hourly for the first 24h and daily thereafter for 3 days for any major behavioral change (Balogun *et al.*, 2016).

Subacute toxicity study

To conduct subacute toxicity experiment, thirty-six *Wistar albino* rats were divided into six different groups as per following detail (Sankar *et al.*, 2015):

Normal control group: Received standard pellet diet.

Vehicle control group: Administered with vehicle (0.5% CMC) orally for 30 days.

Negative control group: Given Rif/INH (50mg/kg+ 50mg/kg) in vehicle orally for 30 days.

Positive control group: Received Rif/INH (50mg/kg+ 50mg/kg) + 200 mg/kg Silymarin (SILM) in vehicle orally for 30 days.

Low dose RBFCE group: Received Rif/INH (50mg/kg+ 50mg/kg) and 500 mg/kg RBFCE in vehicle orally for 30 days

High dose RBFCE group: Given Rif/INH (50mg/kg+ 50mg/kg) and 1000 mg/kg RBFCE in vehicle orally for 30 days.

Mortality and general behavior of animals were recorded during the whole treatment period. Pre-study and post-study body weights of the rats were measured on the 1st and the 30th day (Sartorius balance TE214S, Germany). The net change in the rat body weights (W) was calculated by comparing the weights at 1st (W₁) and 30th day (W₂), respectively by the following equation:

$$W = W_2 - W_1$$

Collection and processing of blood and tissue samples

By the end of the study period, i.e., at 30 days, blood samples were collected in edentate and non-edentate glass

vials, followed by coagulation and centrifugation at 4000 rpm for 15 minutes to separate serum for assaying biochemical profiles and oxidative stress markers. Rats were sacrificed under anesthesia by i.p. administration of 1% chloralose (5ml/kg) in 25% urethane (w/v). Liver and kidney were immediately excised, weighed, washed with ice-cold saline and preserved in 10% formalin for histological processing. Before preservation for histopathological analysis, the weights of the liver and kidney were compared among the groups.

Assessment of serum biochemical profile

The hepatic biochemical markers i.e., serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, protein, bilirubin, cardiac markers, i.e., triglycerides, total cholesterol, high density lipoprotein (HDL) and renal biochemical markers i.e., urea, creatinine, blood urea nitrogen (BUN) and uric acid were estimated by routine chemistry analyzer (AU480 Beckman Coulter, USA) using commercially available kits by following instructions of the manufacturer.

Assessment of oxidative stress markers

Stress markers like malondialdehyde (MDA) and superoxide dismutase (SOD) in plasma were quantified according to the reported protocols (Yagi, 1987) and (Kakkar et al., 1984), respectively.

Histopathological investigation of liver and kidney tissues

All the preserved specimens of hepatic and renal tissues were subjected to histopathological investigations. After staining of tissue sections (5 μ m) with hematoxylin and eosin dye (H&E), microscopic examination was carried out using Nikon DS-Ri2 deca head microscope by an expert histopathologist.

Ethical approval

Rats were handled according to the Ethical Guidelines for Laboratory Animals of College of Pharmacy, University of Punjab, Lahore Pakistan and the study protocol for animal data was approved by the Animal Ethical Committee, College of Pharmacy, University of Punjab (AEC/PUCP/1077), prepared by National Institute of Health.

STATISTICAL ANALYSIS

All the results were displayed as the mean \pm standard error of the mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* Tuckey's multiple comparison test. A p-values < 0.05 were considered significant. Graph Pad Prism® (Version 8.0.1(244) for Windows) was used for statistical data analysis.

RESULTS

Phytochemical characteristics and calculation of IC₅₀

The phytochemical screening revealed the presence of phenolic compounds, flavonoids, terpenoids, saponins, sugars, fixed oils, proteins and cardiac glycosides in RBFCE. Total phenolic contents were expressed as gallic acid equivalents (32.0 mg GAE/g) while flavonoids were expressed as rutin equivalents and found to be 14.0 mg of RUE/g. fig. 1(a, b). The antioxidant activity of RBFCE by DPPH assay was found to be 59.40% while 50% inhibition (IC₅₀) was observed at the concentration of 62.5 μ g/ml.

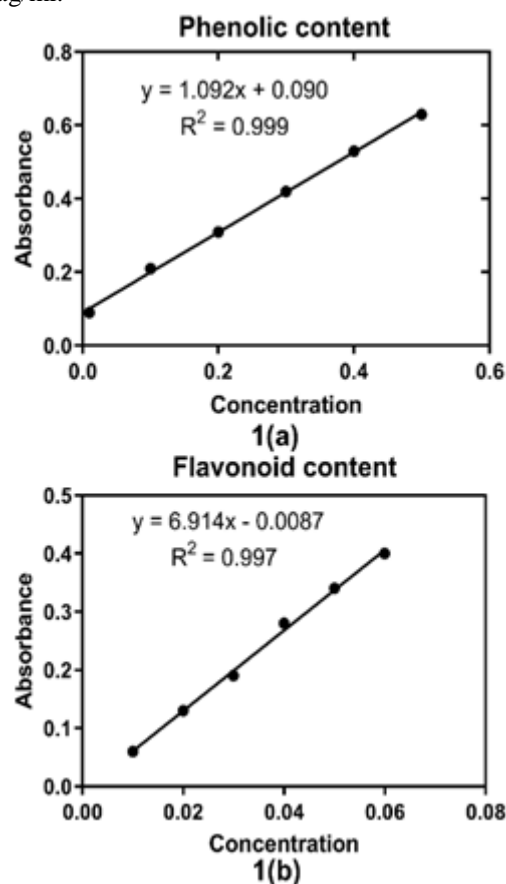


Fig. 1: (a) Calibration curve of gallic acid for Phenolic content estimation in RBFCE (b) Calibration curve of rutin for flavonoid content estimation in RBFCE

Acute toxic profile

In acute toxicity study, animals were cautiously observed for behavioral changes and development of symptoms of toxicity at different time intervals for a period of 3 days. No signs of drowsiness, salivation, piloerection, lacrimation, convulsions, tremors, and mortality was observed during the acute toxic study period.

Subacute hepatoprotective and nephroprotective profile

The subacute study was conducted at doses of 500 mg/kg, p.o and 1000 mg/kg, p.o of RBFCE for 30 days for

normal, vehicle, Rif/INH treated, low dose RBFCE (500 mg/kg, p.o), high dose RBFCE (1000 mg/kg, p.o) and SILM treated groups. The parameters focused were alteration in weight (both body and tissue), hepatic biochemical markers, renal function markers, lipid profile and oxidative stress enzymes as well as histopathological changes in the hepatic and renal tissue sections of all groups. No major behavioral changes and mortality of animals were observed during the whole study period of 30 days.

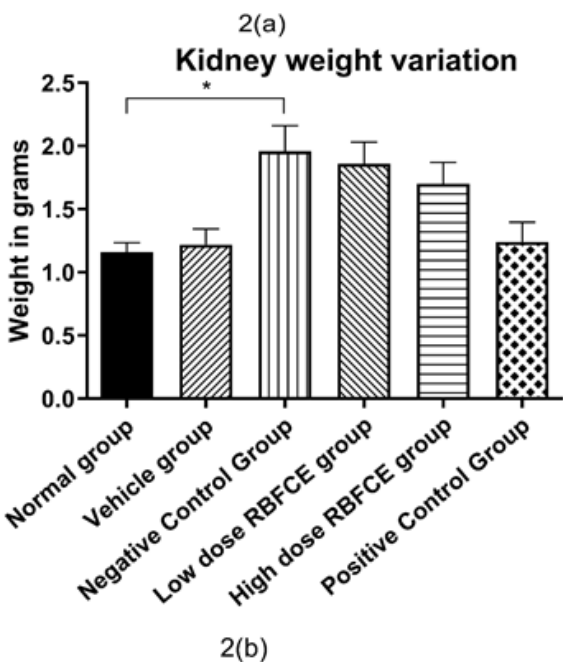
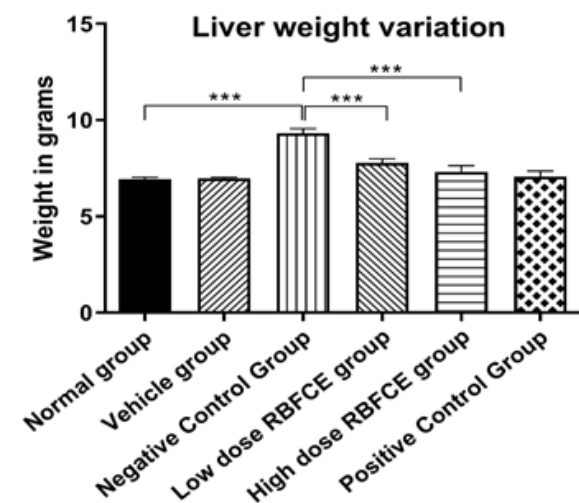


Fig. 2: (a) Liver relative weight variation & (b) Kidney relative weight variation of normal group, vehicle group, negative control group, low dose RBFCE group, high dose RBFCE group and positive control groups. Values are expressed as means, with their standard errors symbolized by vertical bars. *represents $p < 0.05$, ***represents $p < 0.001$

Effect of RBFCE on body, liver and kidney relative weight variation

As shown in figs. 2(a, b) and 3, the liver, kidney and relative body weights were altered by, 33.96% (Mean (M) =9.31, $p < 0.001$), 68.97% (M = 1.96, $p < 0.05$) and by -39.16% (M=131, $p < 0.001$), respectively in Rif/INH treated group compared to the normal group on 30th day of study. Negative value indicates reduction in weight. No significant ($p > 0.05$) effect of 0.5% CMC on body 5.6% (M=227), liver 0.43% (M=6.98) and kidney weights 5.2% (M=1.22) relative to normal group was observed in vehicle group. Alterations in body, liver and kidney relative weights for low dose RBFCE treated rats were found to be 10.6% (M = 145, $p > 0.05$), -16.5% (M=7.8, $p < 0.001$) and -5.1% (M=1.86, $p > 0.05$), respectively as compared to the negative control. On the other hand, significant alterations in body, liver and kidney relative weights for high dose RBFCE treated rats were found to be 17.9% (M = 154, $p < 0.01$), -21.6% (M=7.3, $p < 0.001$) and -13.27% (M=1.7, $p > 0.05$), respectively when compared to negative control. No significant difference ($p > 0.05$) in rat body -2.4% (M = 154, liver 2.82% (M= 7.3) and kidney weights 37.1% (M=1.7) of high dose RBFCE treated group were observed as compared to positive control. Insignificant alterations ($p > 0.05$) in body, and tissue weights were observed in high dose treated group as compared to low dose.

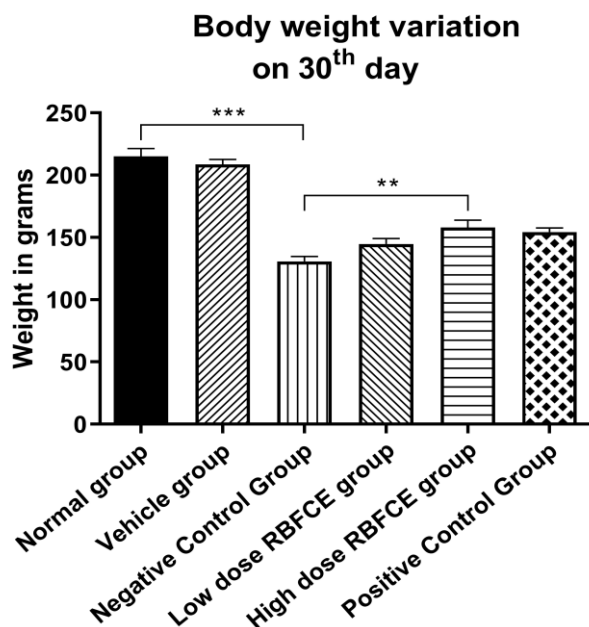


Fig. 3: Body relative weight variation of normal group, vehicle group, negative control group, low dose RBFCE group, high dose RBFCE group, and positive control groups. Values are expressed as means, with their standard errors displayed by vertical bars. **represents $p < 0.01$ and ***represents $p < 0.001$

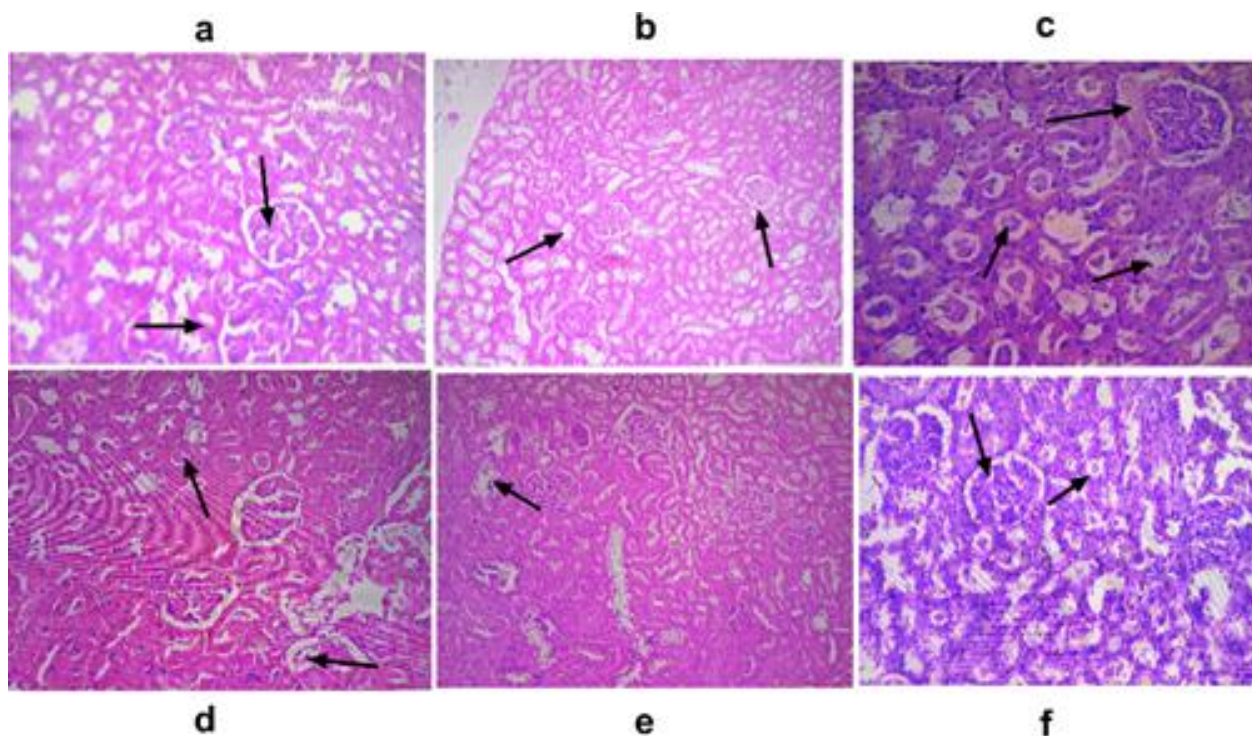


Fig. 4: Photomicrographs of stained kidney sections: (a) Normal group showing normal glomerulus and tubules; (b) Vehicle group showing normal tubules and glomeruli. (c) Negative control group showing extensive thyroidization of renal tubules, along with focal tubular atrophy and focal glomerular sclerosis (d) Low dose RBFCE group showed normal looking glomeruli with focal thyroidization and atrophic changes in the tubules. (e) High dose RBFCE group showed normal glomeruli and tubules with only focal tubular atrophy. (f) Positive control group showing normal looking glomeruli and focal thyroidization of tubules.

Effect of RBFCE on hepatocyte integrity markers

Rif/INH administration to negative control group significantly ($p < 0.001$) increased the serum ALT, ALP and AST levels by 353.57% ($M = 127$), 383.2% ($M = 247.4$) and 168.10% ($M = 415.6$), respectively, compared to the normal group. There was no significant ($p > 0.05$) effect of vehicle on serum ALT, ALP and AST markers which were altered by merely 4.3% ($M = 29.2$), 3.52% ($M = 53$) and -8.8% ($M = 141.4$), respectively. Oral treatment of low dose of RBFCE decreased ($P < 0.001$) the serum ALT to -52.3% ($M = 60.6$), ALP to -57.2% ($M = 106$) and AST to -44% ($M = 232.8$) compared to the Rif/INH treated group. On the other hand, oral treatment of high dose of RBFCE decreased ($P < 0.001$) the serum ALT to -64.6% ($M = 45.0$), ALP to -63.6% ($M = 90$) and AST to -56.5% ($M = 180.8$) compared to the negative control group. No significant ($p > 0.05$) rise in serum ALT 5.63% ($M = 45.0$), AST 8.01% ($M = 180.8$) and ALP 6.13% ($M = 90.0$) in high dose RBFCE treated animals were observed as compared to the positive control group (table 1).

Effect of RBFCE on serum total protein, albumin and bilirubin Profile

Serum total protein, albumin and bilirubin profiles were significantly altered ($p < 0.01$) by -18.50% ($M = 4.84$), -25.9% ($M = 2.96$), and 350% ($M = 0.9$, $p < 0.001$),

respectively in Rif/INH intoxicated group as compared to the normal control animals. Vehicle did not show a significant ($p > 0.05$) effect on serum total proteins, albumin and bilirubin profiles by only altering -0.34% ($M = 5.92$), -2.81% ($M = 3.88$), 10.0% ($M = 0.22$), respectively in the above parameters in vehicle group. Treatment with low dose of RBFCE altered ($p > 0.05$) the serum total proteins 10.74% ($M = 5.36$), albumin 6.8% ($M = 3.16$) and bilirubin level -33.33% ($M = 0.6$) in rats as compared to the negative control. However, the higher dose of RBFCE increased the serum total proteins by 15.7% ($M = 5.60$, $p < 0.05$) and albumin by 25.0% ($M = 3.7$, $p < 0.001$) while decreased ($p < 0.01$) the bilirubin level -51.1% ($M = 0.44$) as compared to the negative control. A small depletion ($p > 0.05$) in protein -3.78% ($M = 5.60$) and albumin level -4.15% ($M = 3.7$) was observed along with the rise in bilirubin content 22.22% ($M = 0.44$) in high dose RBFCE treated animals as compared to the positive control. Significant difference ($p < 0.01$) was observed for albumin regulation in high dose treated group as compared to low dose (table 1).

Effect of RBFCE on serum total cholesterol, triglyceride and HDL profile

Serum total cholesterol, triglycerides, and HDL profiles were significantly ($p < 0.001$) raised by 173.8% ($M =$

Table 1: Sub-acute study to investigate the protective effect of RBFCE on Wistar albino rats for 30 days

Parameters	Animal Groups					
	Normal	Vehicle	Negative control	Low dose RBFCE	High Dose RBFCE	Positive control
ALT (IU/L)	28±2.8	29.2±2.2	127±8.5 ^{***}	60.6±7.2 ^{†††}	45±5.8 ^{†††}	42.6±4.9 ^{†††}
ALP(IU/L)	51.2±3.7	53±3.6	247.4±23.4 ^{***}	106±13.1 ^{†††}	90±8.9 ^{†††}	84.8±8.4 ^{†††}
AST (IU/L)	155±22.1	141.4±18.5	415.6±18.1 ^{***}	232.8±36.9 ^{†††}	180.8±29.4 ^{†††}	167.4±18.6 ^{†††}
Total Protein (g/dL)	5.94±0.1	5.92±0.1	4.84±0.3 ^{**}	5.36±0.21	5.64±0.2 [†]	5.82±0.2 ^{††}
Albumin (g/dL)	3.99±0.1	3.88±0.1	2.96±0.1 ^{***}	3.16±0.1	3.7±0.1 ^{†††}	3.86±0.2 ^{†††}
Bilirubin (mg/dL)	0.2±0.03	0.22±0.03	0.9±0.1 ^{***}	0.6±0.1	0.44±0.12 ^{††}	0.36±0.1 ^{††}
Cholesterol (mg/dL)	80.8±6.2	84.6±4.7	221.2±9.1 ^{***}	148.8±11.3 ^{†††}	126±6.5 ^{†††}	92.2±9.7 ^{†††}
HDL (mg/dL)	90.2±4.14	85.6±12.4	148.4±14.8 [*]	136.8±13.0	94.6±10.9 [†]	85.2±12.7 [†]
Triglycerides (mg/dL)	68.2±9.3	66.0±5.4	132.4±10.1 ^{***}	91.2±12.2 [†]	82.4±3.9 ^{††}	74.4±7.4 ^{†††}
Creatinine (mg/dL)	0.7±0.1	0.8±0.1	2.86±0.1 ^{***}	1.56±0.14 ^{†††}	1±0.2 ^{†††}	0.88±0.1 ^{†††}
Urea (mg/dL)	36.4±3.8	33.4±2.5	75±4.7 ^{***}	62.4±5.7	47.8±4.9 ^{††}	42.2±4.0 ^{†††}
BUN (mg/dL)	17±1.1	16.6±1.2	31.6±2.2 ^{***}	24±1.7 [†]	19.6±2.1 ^{†††}	17.8±0.8 ^{†††}
Uric acid (mg/dL)	1.65±0.1	1.77±0.1	2.7±0.2 ^{***}	2.32±0.23	2.02±0.1 ^{†††}	1.8±0.1 ^{†††}
MDA (nmol/100ml)	1.17±0.2	1.5±0.3	2.9±0.5 [*]	2.46±0.2	2.1±0.4	1.7±0.4
SOD (U/ml)	0.06±0.004	0.05±0.007	0.03±0.008 [*]	0.034±0.1	0.05±0.003	0.05±0.004

The whole data displayed as mean ± S.E.M. For statistical analysis, *post hoc* Tuckey's multiple comparison test used to compare the mean of all groups. * as compares to the normal, † as compares to negative control. *, † shows p<0.05, **, †† represents p<0.01 and ***, ††† represents p<0.001.

221.2), 94.13% (M=132.4) and 64.52% (M=148.4, p<0.05) respectively in Rif/INH treated group paralleled to the normal control animals. Insignificant alteration (p>0.05) in the serum total cholesterol 4.7% (M=84.6), triglycerides -3.23% (M = 66.0) and HDL profiles -5.1% (M =85.6) were seen in vehicle treated animals. Oral treatment of low dose of RBFCE decreased (p<0.001) the elevated serum total cholesterol up to -32.7% (M=148.8), triglycerides to -31.10% (M=91.2, p<0.05) and HDL to -7.82% (M =136.8) as compared to the negative control. Besides this, treatment with high dose of RBFCE decreased (p<0.001) the serum cholesterol upto -43.0% (M=126), triglycerides to -37.8% (M = 82.4, p<0.01) and HDL to -36.3% (M=94.6, p<0.05) as compared to the Rif/INH treated group. A notable rise observed in serum cholesterol 36.7% (M=126), while triglycerides and HDL levels raised up to 10.8% (M=82.4) and 11.03% (M=94.6) respectively in high dose RBFCE treated group compared to positive control (table 1).

Effect of RBFCE on serum creatinine, urea and blood urea nitrogen (BUN)

Serum creatinine, urea and BUN levels were increased (p <0.001) by 308.6% (M=2.86), 106.1% (M=75) and 85.9% (M=31.6), respectively in negative control group as compared to the normal animals. With altering only 14.3% (M=0.8), -8.24% (M=33.4) and -2.4% (M=16.6), respectively the serum creatinine, urea and BUN profiles, vehicle did not affect (p>0.05) on the above parameters. Oral treatment of low dose of RBFCE decreased (p<0.001) the serum creatinine to -45.5% (M = 1.56), urea to -16.8% (M=62.4) and BUN to -24.1% (M =24)

(p<0.05) as compared to the Rif/INH group. On the other hand, high dose of RBFCE decreased (p<0.001) the serum creatinine to -65.0% (M=1), urea to -36.3% (M=47.8, p<0.01) and BUN to -38% (M=19.6, p<0.001) in comparison to the negative control. A small rise (p>0.05) in creatinine, 13.64% (M=1) urea, 13.3% (M = 47.8) and BUN levels, 10.11% (M=19.6) was observed in high dose RBFCE treated group when compared to the positive control. A statistically significant difference (p<0.05) was observed for creatinine normalization in high dose treated group as compared to low dose (table 1).

Effect of RBFCE on uric acid profile

Variation in uric acid level was found to be significant (p <0.001) as 62.7% (M=2.70) in Rif/INH treated group when compared to the control animals. No significant (p > 0.05) consequence of vehicle was observed on uric acid level 7.3% (M=1.8) in vehicle group. Oral treatment of low dose of RBFCE decreased (p>0.05) the serum uric acid level to -13.4% (M=2.32) as compared to Rif/INH group. Besides this, treatment with high dose of RBFCE significantly (p<0.001) decreased the serum uric acid level up to -24.72% (M=2.02) when compared to the Rif/INH treated animals. As compared to positive control animals, significant rise (p<0.05) in uric acid profile 30.87% (M=2.02) was seen in high dose RBFCE treated animals.

Effect of RBFCE on oxidative stress markers

Oxidative stress markers, i.e., MDA and SOD were changed significantly (p<0.05) by 146.0% (M=2.9) and -48.3% (M= 0.03), respectively in negative control group as compared to that of normal group. Vehicle did not

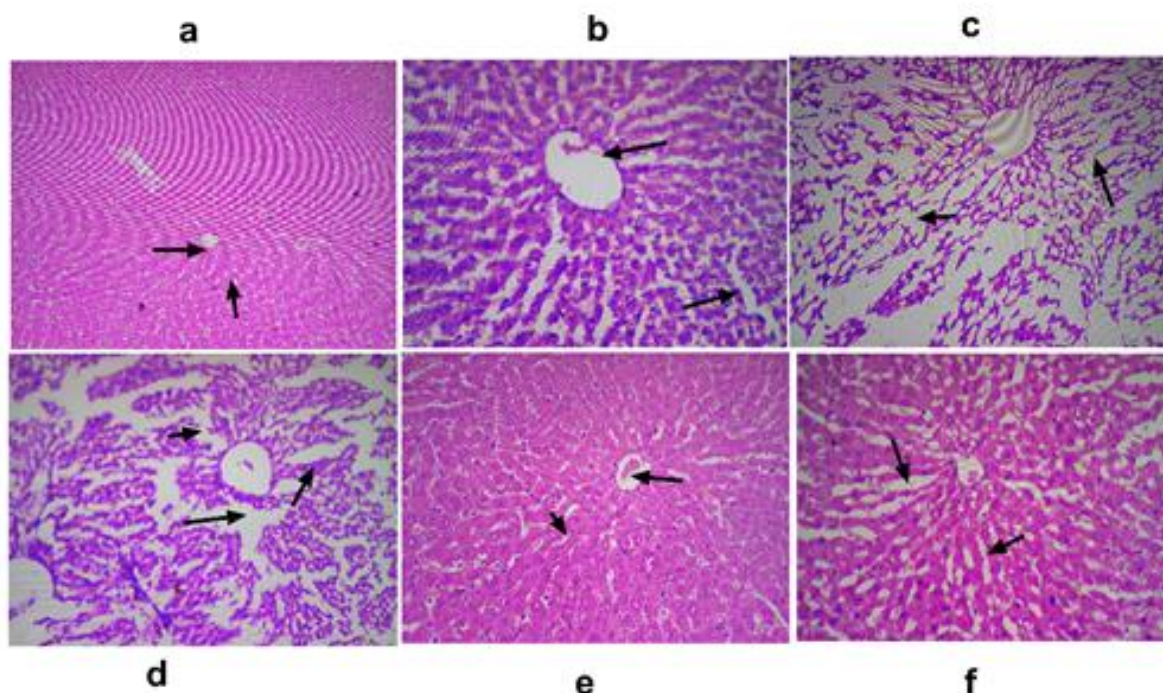


Fig. 5: Photomicrographs of stained liver sections: (a) Normal group showing central vein surrounded by normal hepatic plates. (b) Vehicle group showing normal central vein and hepatic cell plates along with focal sinusoidal dilatation (c) Negative control group showing markedly dilated sinusoids and extensive damage of liver cell plates.(d) Low dose RBFCE group showing central vein with surrounding mild to moderate distorted trabecular pattern and sinusoidal dilatation in the liver tissue (e) High dose RBFCE group showing one to two cells thick liver cell plates surrounded by thin sinusoids (f) Positive control group showing regular architecture and hepatic cell plates arranged in a trabecular pattern with mild sinusoidal dilatation

show significant ($p > 0.05$) effect on MDA and SOD profiles by increasing and decreasing 26.7% ($M = 1.5$) and -20.7% ($M = 0.05$) respectively, in comparison to the control animals. Oral treatment of low dose of RBFCE to Rif/INH-intoxicated rats altered the MDA by -14.5% ($M = 2.46$) and SOD by 13.3% ($M = 0.034$) as compared to the negative control group. However, as compared to negative control animals, high dose of RBFCE altered MDA by -28.1% ($M = 2.1$) and SOD by 66.7% ($M = 0.05$) ($p < 0.05$). Small difference in MDA and SOD profiles 20.5% ($M = 2.1$) and 4.2% ($M = 0.05$) were observed, respectively in high dose RBFCE treated animals as compared to the positive control (table 1).

Histopathological observations

The histopathological investigations of kidney tissue of both normal and vehicle control groups showed normal glomeruli and tubules (fig. 4). No inflammation and fibrosis were observed in the interstitium. Rif/INH treated toxic animals showed extensive thyroidization of renal tubules, along with focal tubular atrophy. Glomerulus showed mild sclerosis. Renal tissue showed normal looking glomeruli with focal thyroidization and atrophic changes in the tubules of low dose RBFCE treated group. The, high dose extract treated group showed normal glomeruli and tubules with only focal tubular atrophy.

Focal thyroidization of tubules and normal glomeruli could be observed in positive control animals. Signs of inflammation and fibrosis cannot be appreciated.

In the histopathological investigations of liver tissue of normal and vehicle control groups, no signs of inflammation, fibrosis, neoplasm, and necrosis were observed (fig. 5). Regular hexagonal architecture was well maintained with normal portal area and thin sinusoidal spaces. Liver tissue with central vein surrounding one to two cells thick hepatic cell plates arranged in a trabecular pattern and only focal sinusoidal dilatation could be appreciated in the vehicle control group. On the other hand, negative control group showed markedly dilated sinusoids and haphazardly arranged broken hepatic cell plates. Hepatocytes undergoing individual cell necrosis could also be observed. Low dose RBFCE treated group showed partially preserved hepatic cell plates. Mild to moderate sinusoidal dilatation was also revealed. Moreover, liver tissue of high dose RBFCE treated animals showed no inflammation and fibrosis with preservation of central vein and liver tissue. Liver cell plates showed one to two cells thick trabecular plates surrounded by thin sinusoids. Moreover, the liver tissue of the positive control group showed regular architecture and hepatic cell plates arranged in a trabecular pattern without

any signs of inflammation, fibrosis, and necrosis. Only mild sinusoidal dilatation was noted. The histopathological changes have been shown by the arrow marks on the figs.

DISCUSSION

Drug-induced toxicity occurs through a variety of mechanisms. Drugs and their metabolites interact with the cellular proteins leading to the formation of new adducts that activate immunological reactions causing cellular necrosis (Ramaiah *et al.*, 2001). This study has a couple of strengths as in this work, the presence of phytochemicals, *in-vivo* hepato- and nephro-protective and anti-oxidative stress potential of RBFCE was investigated. IC₅₀ was calculated first time for RBFCE by using DPPH free radical method. The IC₅₀ value depicts the promising anti-oxidant potential of RBFCE. Moreover, qualitative analysis showed the presence of various classes of phytochemicals, i.e., phenols, flavonoids, terpenoids, saponins, proteins and cardiac glycosides in the RBFCE. Current *in-vivo* research work is in-line with the preceding *in-vitro* antioxidant activity of *Rosa brunonii* L. fruit extract (Ishaque *et al.*, 2017, Shan *et al.*, 2019).

To the best of our knowledge, this is the first investigation of the protective potential of RBFCE against hepato- and nephro-toxicity induced by Rif/INH using *in-vivo* model. The liver has transaminases for synthesis and breakdown of amino acids and to convert into energy storage molecules. Significant changes in morphology and physiology of the liver were observed in Rif/INH treated group. Increase in the serum hepatic enzymes (ALT, AST, ALP) and bilirubin seems to be a biochemical warning for wide disturbances in structure and functions of hepatocytes in Rif/INH treated group (Ramappa *et al.*, 2013). High level of transaminases may be associated with the permeability of hepatocytes, so that more enzymes leak out into the bloodstream (Das *et al.*, 2008). A significant rise in the serum ALP indicates hepatic cellular damage and also bile duct obstruction. The serum bilirubin level is a key marker for hepatic functions. Destruction of sub-cellular filaments of bile duct disturbs bile secretions, leading to jaundice and cellular damage. The present findings showed that a low dose RBFCE normalized the serum ALT, AST and ALP remarkably and the high dose RBFCE restored the above liver enzymes and serum bilirubin significantly ($p < 0.05$), exhibiting hepatoprotective effect of RBFCE against Rif/INH induced liver toxicity. RBFCE treatment normalized the hepatocytes functions by improving the hepatic clearance of bilirubin. Lipid peroxidation (LPO) is an essential cause for tissue damage (Kitts *et al.*, 1998). MDA, an end product of LPO is involved in the formation of highly reactive oxygen species (ROS). Consumption of non-protein sulfhydryl (NP-SH) content also induce

damage to the hepatic tissue, and displays an additional indicator of hepatotoxicity (Li *et al.*, 2015).

Elevated plasma MDA in Rif/INH treated animals indicated hepatotoxicity. A significant decrease in MDA level was observed in the high dose RBFCE treated group as compared to the negative control. Probable protection of liver cells by RBFCE was due to a decrease in the Rif/INH-mediated LPO through deterrence in the generation of free radical species. A significant rise in the SOD levels in the high dose RBFCE treated group strengthened the anti-oxidative stress potential of the plant which is in-line with the strong anti-oxidant potential of *Rosa brunonii* L. fruit. RBFCE treatment normalized the MDA and SOD level in hepatic tissues, thus indicating a protective effect of the extract.

The depletion of total protein and albumin content in Rif/INH treated group also considered to be marker of liver toxicity (Maiti *et al.*, 2019). It could be either due to defective protein synthesis or increased free radical formation. Significant restoration to the normal levels of total protein and albumin in high dose RBFCE group exhibited the protective potential of the RBFCE.

The Rif/INH toxicity leads to the increased cholesterol and triglyceride level (Akpovi *et al.*, 2013). Significant restoration of total cholesterol, triglycerides and HDL levels to the normal range in high dose RBFCE treated group were noted as compared to low dose treated animals.

Rif/INH toxicity also induces significant changes in the morphology and physiology of kidney tissues (Muzika *et al.*, 2016) and are thought to be responsible for acute kidney injuries (AKI) as evident from the rise in creatinine, urea, BUN and uric acid levels (Beebe *et al.*, 2015, Chogtu *et al.*, 2016). In line with the above, the levels of the renal function parameters were elevated in the Rif/INH treated group.

Alterations in serum renal function markers, as well as histopathological investigations in Rif/INH treated animals indicated irregular kidney functions as compared to normal control group. Drug toxicity has also induced the capillary proliferation and thyroidization in kidneys. Congested blood vessels in interstitium and edema were also seen in some Bowman spaces. A low dose of RBFCE restored the kidney functions to some extent as compared to the negative control group. Low dose RBFCE group showed normal looking glomeruli with focal thyroidization and atrophic changes in the tubules. No thyroidization with mild focal tubular atrophy was observed in high dose RBFCE as compared to the low dose. Presence of normal glomeruli and tubules with only focal tubular atrophy proves the better protective effect of high dose RBFCE treated group.

Rif/INH therapy strongly influenced the histopathology of liver tissues (Daher *et al.*, 2013, Kim *et al.*, 2017). The present histopathological investigations of liver tissue of negative control animals showed disturbed regular hexagonal structure with overall loss of liver lobule architecture in the form of broken hepatic plates and dilated sinusoidal spaces. Histopathological examination of the liver tissue of low dose treated animals showed partially preserved hepatic cell plates with mild sinusoidal dilatation. Whereas, high dose of RBFCE treated animals showed no inflammation and fibrosis with a well-maintained hexagonal structure. Normal portal area, thin sinusoidal spaces and no interstitial inflammation in liver tissues of high dose treated group prove the promising protective potential of the plant at high dose.

CONCLUSION

RBFCE possess strong anti-oxidative stress and protective potential against Rif/INH induced liver and kidney toxicity as evident from normalized serum biochemical and histopathological profiles which were likely be due to the strong antioxidant and anti-inflammatory properties of RBFCE.

Current *in-vivo* study was in-line with the previous *in-vitro* research work, indicating the promising anti-oxidative stress potential of *Rosa brunonii* L., which might be due to the strong free radical quenching abilities of phenolic and flavonoid compounds present in RBFCE. Consequently, RBFCE possesses considerable protective effect against hepato- and nephro-toxicity induced by Rif/INH. High dose of plant extract is more protective than the low dose but less than the positive control. Findings of the present work might play an important role towards the discovery of new plant-based protective remedies.

Availability of data and materials

We have presented all our data in the form of figs. and tables. The datasets supporting the conclusions of this article are presented in this main paper. The plants were collected from Murree, Punjab, Pakistan and identified by an expert taxonomist at Department of Botany, GC University, Lahore, Pakistan. A voucher specimen (GC.Herb.Bot.3315) was deposited in the Herbarium of GC University, Lahore, Pakistan for future reference.

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