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In-vivo Hepatoprotective effect of *Malva parviflora* L. (Malvaceae) in isoniazid and rifampicin treated rabbits

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The purpose of this study was to evaluate the hepatoprotective effect of *Malva parviflora (M. parviflora)* in hepatic damage induced by the administration of isoniazid (INH) and rifampicin (RMP). Phytochemical investigation of *M. parviflora* was performed according to USP standards. In pharmacological investigation test animals (rabbits) were divided into four groups (n=6). Group I was control, kept on distilled water throughout the study. Group II was treated with INH (50 Mg/kg body weight) and RMP (100 Mg/kg body weight). Group III was treated with methanolic extract of *M. parviflora* (500 Mg/kg body weight) along with combination of INH (50 Mg/kg body weight) and RMP (100 Mg/kg body weight). Group IV was treated with a standard hepatoprotective drug silymarin (200 Mg/kg body weight) along INH (50 Mg/kg body weight) and RMP (100 Mg/kg body weight). The entire study was conducted for seven days. The hepatoprotective effect of the extract was judged by estimating the levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin. Phytochemical analysis revealed presence of polyphenolic compounds in *M. parviflora.* The *M. parviflora* extract produced significant ($p<0.05$) decrease in the serum levels of ALT, AST, ALP and bilirubin. The extract also yielded 40-50% decrease in ALT, AST, ALP and total bilirubin when compared with silymarin which shows that it has better hepatoprotective effect than silymarin. Methanolic extract of *M. parviflora* has better hepatoprotective activity when compared to silymarin. The extract could be useful in countering hepatic damage.

Keywords: *Malva parviflora*; Hepatotoxicity, Isoniazid, Rifampicin, hepatoprotective

INTRODUCTION

The liver is the largest organ in the body and is responsible for the synthesis of most of the serum proteins, and by this means controls oncotic pressure in the blood vessels. It is the site of storage of nutrients, energy derivation from the oxidation of the nutrients and a principal site for the detoxification of (xenobiotic) (Satdarshan and Monga, 2010). The liver functions are generally categorized as metabolic, synthetic, storage, catabolic and excretory functions (Reisner, 2013). In developing countries tuberculosis is one the prevailing disease and has a devastating effect. The strategies of WHO to eliminate tuberculosis worldwide are not enough to completely eradicate the disease (Bloom, 1994) Recent analyses suggest that improved diagnostic procedures, curative methods and additional preventive efforts are required to revolutionize tuberculosis (Lönnroth and Raviglione, 2008). The first line therapy for tuberculosis uses isoniazid (INH), pyrazinamide and rifampicin (RMP). These drugs can cause liver damage (drug-induced hepatitis) especially when given in combination (Thomas et al. 1981). N-acetyl –isoniazid formed by hydrolysis of INH causes bio alleviation by the Cytochrome P450 system to an acetyl radical (Bloom, 1994). Whereas the second important anti T.B drug i.e. RMP stimulates the metabolism of INH, resulting in the increased formation of hydrazine (a proven hepatotoxic agent). These drugs also decrease glutathione (GSH) levels resulting increased free radical injury (Ellard and Gamman, 1976; Kalra et al. 2007). Phytochemical analysis of drugs obtained from natural sources can give an idea of potential beneficial effects of the drugs in living systems. Plants containing phenols and flavonoids can aid in scavenging free radicals formed during hepatocyte damage in liver and removing free radicals formed during oxidative damage caused by drugs (Rice-Evans et al. 1997). In spite of tremendous advances in modern medicine, there are no effective and reliable drugs available that can stimulate liver function, for the hepatoprotection we use naturally obtained drug e.g silymarin (Benhmed et al. 2021; Steele et al. 1991).

Our present research focuses on the phytochemical analysis and evaluation of hepatoprotective properties of a locally-occurring weed, *Malva parviflora* in Pakistan.

MATERIALS AND METHODS

Plant collection

The plant was collected from Sheikhupura, Punjab, during January 2012, and was authenticated by Mr. M. Ajaib curator of Sultan Herbarium of Botany Department, Government College University (GCU), Lahore, Pakistan. A voucher specimen No. 1600 was deposited in the same herbarium. The plants were shade-dried for 15 days and then powdered. The resultant powder was stored in amber colored bottle and kept at dry and cool place.

Extraction

Three kilogram powder was subjected to macerate by placing in closed vessel and add 12 liter pure methanol with occasional shaking. The process takes 14 days for complete extraction. The extract was filtered and filtrate was dried in a rotary evaporator at 40^0C under reduced pressure, and weighed to calculate the percentage yield of extract.

Chemicals

All chemicals and solvents used were of analytical grade and were purchased as indicated:

Raw material of Rifampicin and Isoniazid (Pacific Pharmaceuticals), Silymarin (Abbot), Diagnostic kits of ALT, AST, ALP, and Total Bilirubin (Global Diagnostics UK), Analytical grade methanol), n-hexane, and chloroform, (Riedel-deHaën), formalin, hydrochloric acid, Sulphuric acid, Lead acetate, Sulphur powder, glucose, Zinc dust, Acetic acid, Ferric chloride, Pyridine (Merck), Bromine (RDH, Germany) and other laboratory prepared reagents in phytochemical analysis.

Phytochemical analysis of *Malvaparviflora*

For phytochemical analysis fresh plant parts i.e root, stem, leaves and fruit were separated and placed in a closed vessel and 70% methanol (men strum) is added and left for 7 days with occasional shaking The liquid is then strained off and the solid residue (Marc) is pressed to remove the solution as much as possible. The strained and expressed liquids are mixed and clarified by filtration. Phytochemical analysis was done (Akbar et al. 2021).

Experimental animals

Twenty four male albino rabbits (1.2-1.5 kg) were used for this study. The rabbits were purchased from Tolenton Market, Lahore, and kept in cages at the Animal House, College of Pharmacy, University of the Punjab, Lahore. The animals were kept at temperature of 25 ± 5˚C, fed with standard diet and water ad libitum. The study protocol was approved by Animal Ethical Committee, Punjab University College of Pharmacy, University of the Punjab (Approval No. AEC/UCP/012/4313) in compliance with the regulations of the National Research Council (NRC, 1996).

Experimental design

The rabbits were randomly assigned 4 groups, each comprising 6 animals. Group-I was control group, untreated, Group-II was negative control treated with INH 50 mg/kg/day + RMP 100 mg/kg/day (orally). Group-III was positive control treated with INH 50 mg/kg/day + RMP 100 mg/kg/day + silymarin 200 mg/kg/day (orally) and Group-IV was experimental group treated with INH 50 mg/kg/day + RMP 100 mg/kg/day + Extract 500 mg/kg/day (orally). Silymarin and extract were given every day for seven days, after the half hour administration of INH and RMP.

Biochemical investigations

The blood samples were collected in centrifuge tubes within 24 h after the last dose from all the rabbits through the marginal ear veins. The blood was allowed to clot for 30 min and then centrifuged at 2700 rpm for 10 min. The serum was separated and analyzed for ALT, AST, ALP and total bilirubin.

RESULTS

Phytochemical evaluations have revealed the presence of medicinally important groups that exhibit certain pharmacological actions. These groups are flavonoids, alkaloids, glycosides, terpenoids and sterols. These groups are responsible for multiple biological effects for example anti-inflammatory, anti-allergy, anti-haemorrhagic, antineoplastic and hepatoprotective activities (Benhmed et al. 2021).

Results of ALT, AST, ALP and Total bilirubin between Group I, II, III and IV are shown in Table 2. Administration of isoniazid (50 mg/kg) and rifampicin (100mg/kg) to Group II significantly raised ALT (%↑21.9), AST (%↑18.8), ALP (%↑48.9) and total bilirubin (%↑0.71) when compared with the normal values as shown in table 2. The value of ALT in Group I (treated with distilled water) increased from 23.97 U/L to 49.34 U/L (Group II treated with INH (50 mg/kg) and RMP (100 mg/kg). The levels of AST increased from 20.89 U/L (G-I) to 43.66 U/L (G-II) as shown in table 2. The mean of ALP and total bilirubin levels in Group I were 50.03 U/L and 2.13 mg/dl respectively and were raised up to 57 U/L and 3.03 mg/dl in Group II. Administration of INH (50mg/kg) and RMP (100mg/kg) along with methanolic extract of *M. parviflora* for 7 days resulted in significant ($p<0.05$) decrease in ALT (25.05 U/L), AST (25.44 U/L), ALP (47.15 U/L), and total bilirubin (1.81 mg/dl) when compared with Group II. Silymarin (250mg/kg) preceding the dose of isoniazid (50mg/kg) and rifampicin (100mg/kg) when compared with G-III, administered methanolic extract of *M. parviflora* preceding INH and RMP, better results were observed. Percentage decrease in ALT, AST, ALP and total bilirubin

was 43.08, 48.9, 56.2 and 2.43 respectively with *M. parviflora* extract whereas with Silymarin the percentage decrease in ALT (%↓24.3) , AST (%↓24.08), ALP (%↓46.21) and total bilirubin (%↓0.66) was less when compared.

Table 1: Phytochemical analysis of *M. parviflora* L

Group	Methanolic extract of leaf	Methanolic extract of fruit	Methanolic extract of stem	Methanolic extract of root
Terpenoids	+	+ + +	+ +	+
Sterols	+	+ + +	+ +	+ + +
Glycosides	+	+ +	+	+ + +
Flavonoids	++	++	+	_
Alkaloids	+ + +	+ +	+	++
Proteins	_	_	_	_
Carbohydrates	+	+ + +	+ + +	+ + +
Saponin	+	+ +	+ +	+
Lipids	_	_	_	_
Tannins	_	_	_	_
'+' /-' indicates the presence/absence of a particular phytochemical respectively				

Table 2: Levels of AST, ALT, ALP and total Bilirubin in Rifampicin-treated rabbits

Parameters	G-I	G-II	G-III	G-IV	Statistical Comparison of G-I with G-II	Statistical Comparison of G-II with G-III	Statistical Comparison of G-II with G-IV
AST U/L	20.89±1.02	43.66±4.20 (%↑18.8)	25.44±1.69 (%↓43.08)	29.09±0.83 (%↓24.3)	t =5.26 p=<0.05*	t= 4.02 p<0.05*	t= 3.40 p<0.05*
ALT U/L	23.97±1.73	49.34±3.62 (%↑21.9)	25.05±2.36 (%↓48.9)	24.21±1.16 (%↓24.08)	t = 6.34 p <0.05*	t=5.62 p<0.05*	t= 6.61 p<0.05*
ALP U/L	50.03±1.03	57.00±0.72 (%↑48.9)	47.15±1.20 (%↓56.2)	44.34±4.68 (%↓46.21)	t = 5.54 p<0.05*	t= 7.03 p<0.05*	t= 2.67 p<0.05*
Total Bilirubin mg/dl	2.13± 0.40	3.03 ±0.12 (%↑0.71)	1.81± 0.15 (%↓2.43)	2.09± 0.25 (%↓0.66)	t= 2.15 p<0.05*	t= 6.35 p<0.05*	t= 7.66 p<0.05*

Values are expressed as mean ± SEM; n = 6; t is test of significance, *p < 0.05 is level of significance level by applying student t. test. Comparison of biochemical parameters of GI (distilled water), GII (INH+RMP), GIII (*M. parviflora*+ INH + RMP) and GIV (Silymarin + INH + RMP) after treatment.
% change is shown in parentheses

DISCUSSION

Tuberculosis occurs in almost one third of world population. According to one estimation carried out in 2001 about 2 million people die every year from this infectious disease (Lönnroth and Raviglione, 2008). In 1997 WHO declared tuberculosis as global health emergency (Anon, 1997). Isoniazid and rifampicin are the first line drugs in treatment of tuberculosis. In Pakistan every year tuberculosis causes approximately 70,000 deaths and around 270,000 people fall sick due to this infections disease per year. According to WHO Pakistan is among the 27 most affected states (Anon, 1997). For the evaluation of hepatoprotective effect of *Malva parviflora*, we used to measure change in serum levels of ALT, AST, ALP and total Bilirubin over antitubercular drugs which were responsible for oxidative stress and liver damage. INH and RMP co-treatment results in hepatic oxidative stress, apoptosis of hepatocytes and steatosis (Gutierrez and Navarro, 2010).

Phytochemical analysis of *M. parviflora* had indicated the presence of polyphenolic compounds i.e. flavonoids, terpenoids and glycosides, alkaloids. Due to presence of these phenolic groups *M. parviflora* possess antioxidant potentials. The antioxidant properties of phenol containing

compounds arise from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions. Free redicals were responsible for oxidative stress and cause of liver damage. So the dugs which showed antioxidant effect, had hepatoprotection.in our present study we proved hepatoprotective activity of *Malva parviflora* which already had reported antioxidant potential. Due to the antioxidant potentials, methanolic extract of *M. parviflora* have shown hepato-protection in damage induced by anti–tuberculosis drugs by reversing the formation of reactive oxygen species and regenerating anti-oxidant enzymes like glutathione peroxidase, catalase and superoxide dismutase (Maqbool et al. 2021). Apart from the phenolic compounds that are responsible for the antioxidant activity, there might be some other active compounds that also exert some effects (Friedman and Keeffe, 2011; Arthur et al. 1984).

M. parviflora significantly reduced liver enzymes in animals treated with INH + RMP preceding a dose of *M. parviflora* for seven days. *M. parviflora* contains flavonoids and glycosides which probably inhibited activation of mono acetyl hydrazine by cytochrome P450. Mono acetyl hydrazine is a metabolite of INH which binds covalently with the liver cell macromolecules, causing hepatocyte injury (Thomas et al. 1981). RMP increases INH toxicity by enhancing the production of metabolites from acetyl hydrazine (Lönnroth and Raviglione, 2008; Bloom, 1994). The results of *M. parviflora* were better when compared with reference anti-oxidant drug Silymarin as can be seen in table 2.

The present investigation demonstrated that ALT, AST, ALP and total Bilirubin levels of *M. parviflora* to become normal. It can be recommended as good hepatoprotective agent in patients being treated for tuberculosis.

CONCLUSION

The present research work has provided us the base line for the utilization *Malva parviflora* extract as hepatoprotective agent which is already utilized as a folk medicine.

CONFLICT OF INTEREST

We declare that we have no conflict of interest

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AUTHOR CONTRIBUTIONS

SA wrote the manuscript and performed the experimental work, SI rechecked the manuscript and SK designed the experimental work. NA and UR helped in data compilation, and assisted throughout the experimental as well as theoretical work.

REFERENCES

Akbar S, Ishtiaq S, Sikandar K, Rizwan MY, Rehman U, Rehman S, Rasool S, Rehman S, Fatima SF, Arshad N, Khan MN, Munir A (2021). Phytochemical analysis and antioxidant studies of leaf, stem, root and fruit of Malvaparviflora L. *BIOSCIENCE RESEARCH*, 18(4): 2626-2634.

Anon X (1997). The resurgence of tuberculosis: a call for commitment. General policy topics. WHO Drug Information, 5: 39-40.

Arthur MJ, Bentley IS, Tanner AR, Saunders PK, Millward-Sadler GH, Wright R (1985). Oxygen-derived free radicals promote hepatic injury in the rat. *Gastroenterology*, 89(5): 1114-1122.

Benhmed F, Kerroum F, BenZakour S, Djire A, Kharoubi O (2021). Antioxidant and Hepatoprotective effect of Aqueous extract of *Pistaciaathlentica*Desf on Rats exposed to Mercury. *BIOSCIENCE RESEARCH*, 18(2): 1238-1247.

Bloom, Barry R (1994). Tuberculosis: Pathogenesis, Protection and Control. American Society for Microbiology. ASM Press: 634 p.

Ellard GA, Gammon PT (1976). Pharmacokinetics of isoniazid metabolism in man.*Journal of Pharmacokinetic and Biopharmaceutics*, 4(2): 83-113.

Friedman LS, Keeffe EB (2011).Handbook of Liver Disease.Elsevier Health Sciences, 536 p.

Gutierrez RMP, Navarro, YTG (2010). Antioxidant and hepatoprotective effects of the methanol extract of the leaves of *Saturejamacrostema*. *PharmacognosyMagzine*, 6(22): 125-131.

Kalra BS, Aggarwal S, Khurana N, Gupta U (2007). Effect of cimetidine on hepatotoxicity induced by isoniazid-rifampicin combination in rabbits. Indian *Journal of Gastroenterology*, 26(1): 18-21.

Lönnroth K, Raviglione M (2008). Global Epidemiology of Tuberculosis: Prospects for Control. *Saminars in Respiratory and Critical Care Medicine*, 29(5): 481-491.

Maqbool M, Ajaib M, Ishtiaq M, Bhatti KH, Mushtaq W, Mazhar MW, Hussain T, Mazhar M (2021). Ethnobotanical, Phytochemical, Antioxidant Study of Persicariabarbata (L.) Hara (PBH) from District

Bhimber (AJK), Pakistan. *BIOSCIENCE RESEARCH*, 18(2): 1189-1197.

NRC (1996). Guide for the care and use of laboratory animals. Washington DC, USA: National academy press.

Pietta PG (2000). Flavonoids as antioxidants. Journal of Natural Products, 63(7): 1035-1042.

Reisner H (2013). Essentials of Rubin's pathology.Lippincott Williams & Wilkins 310 p.

Rice-Evans C, Sampson J, Bramley PM, Holloway DE (1997). Why do we expect carotenoids to be antioxidants *in-vivo*. Free Radical Research, 26(4): 381-398.

Satdarshan P, Monga S (2010). Signaling Pathways in Liver Diseases.Springer 526 p.

Steele MA, Burk RF, DesPrez RM (1991). Toxic hepatitis with isoniazid and rifampicin.*A meta-analysis: Chest*, 99(2): 465-471.

Thomas BH, Wong LT, Zeitz W, Solomonraj G (1981). Isoniazid metabolism in the rabbit, and the effect of rifampin pretreatment.*Research Communications in Chemical Pathology and Pharmacology*, 33(2): 235-247.