academicJournals

Vol. 7(17), pp. 1190-1200, 3 May, 2013
DOI: 10.5897/JMPR12.1196
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Evaluation of the hepatoprotective, nephroprotective and anti-malarial activities of different parts of *Bauhinia purpurae* and *Tipuana speciosa* grown in Egypt

Fahima F. Kassem[1], Saleh I. Alqasoumi[2,3], Shaimaa M. Sallam[1], Adnan A. Bekhit[4], Nagwa S. E. El-Shaer[1,5], Abdallah I. Farraj[6], Nabil A. Abdel-Salam[1] and Maged S. Abdel-Kader[1,3]*

[1]Department of Pharmacognosy, College of Pharmacy, Alexandria University, Alexandria 21215, Egypt.
[2]Department of Pharmacognosy, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh 11451, Kingdom of Saudi Arabia.
[3]Department of Pharmacognosy, College of Pharmacy, Salman Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Kingdom of Saudi Arabia.
[4]Department of Pharmaceutical Chemistry, College of Pharmacy, Alexandria University, Alexandria 21215, Egypt.
[5]Department of Natural Products, Faculty of Pharmacy, King Abdul Aziz University, P. O. Box 80260, Jeddah 21589, Kingdom of Saudi Arabia.
[6]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, P. O. Box 10219, Riyadh 11433, Kingdom of Saudi Arabia.

Accepted 7 March, 2013

In an attempt to explore, the hepatoprotective, nephroprotective and anti-malarial effects of some plants growing in Egypt, different parts of *Bauhinia purpurae* and *Tipuana speciosa* family Fabaceae were selected for the current study. Liver and kidney injury were induced in Wistar albino rats using paracetamol. The biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were estimated as reflections of the liver condition. Measured kidney parameters were; serum creatinine, serum urea, sodium and potassium level. Liver and kidney samples of rats treated with extracts showed marked improvement in the biochemical parameters were subjected to histopathological study. All the results were compared with the reference drug silymarin. The anti-malarial activity was evaluated *in vivo* using *Plasmodium berghei* Infected mice and chloroquine phosphate as positive control. The tested extracts were used in three dose levels and activity was expressed as reduction in blood parasitemia and increase in the mouse survival time. The ethanol extract of *B. purpurea* flowers (BPFE) was effective in the three used assays. *T. speciosa* leaves extract (TSLE) showed good nephroprotective and anti-malarial activities.

Key words: Hepatoprotection, nephroprotection, anti-malarial, *Bauhinia purpurae*, *Tipuana speciosa*, paracetamol, albino rats.

INTRODUCTION

In addition to their regulatory functions, both liver and kidney play an important role in detoxification and excretion of toxins; consequently they are exposed to high quantities of free radicals, which contribute to high oxidative stress (Abubaker et al., 2012; Pocock and Richards, 2006). Liver and kidney diseases represent two major

*Corresponding author. E-mail:mpharm101@hotmail.com. Tel: +96615886063. Fax: ++96615886001.

health problems in Egypt (Strickland, 2006; Gouda et al., 2011). Several protective compounds were discovered from plants such as silymarin (Morazzoni and Bombardelli, 1995; Mourelle et al., 1988; Chander et al., 1989), schisandrin B (Zhu et al., 1999; Maeda et al., 1981; Cyong et al., 2000), phyllanthin, hypophyllanthin (Ramachandra Row et al., 1966), picroside I and kutkoside (Ram, 2001; Ansari et al., 1988). Malaria is one of the most prevalent parasitic infections in the world and certainly the most detrimental. Each year, over two million people die from the disease, with the vast majority of the deaths in children under five years old in Sub-Saharan Africa (Snow et al., 2005). The natural alkaloid quinine and its dextroisomer quinidine are used for the treatment of malaria, especially in severe cases (Greenwood, 1992). Artemisinin discovered in the leaves of the Chinese traditional plant *Artemisia annua* was found to clear malaria parasites from patients' bodies faster than any other drug in history (Miller and Su, 2011). Artemisinin had become the choice of treatment for malaria, and the WHO called for an immediate halt to single-drug artemisinin preparations in favor of combinations of artemisinin with another malaria drug, to reduce the risk of parasites developing resistance (WHO, 2006, 2008, 2009).

Several therapeutic properties are assigned to *Bauhinia* species (Fabaceae), such as antiamoebic, anti-diabetic, anti-dysenteric, anti-inflammatory, anti-rheumatic, analgesic, and hypocholesterolemic (Duarte-Almeida et al., 2004; Fuentes and Alarcón, 2006). *Bauhinia* leaves are traditionally used for their anti-inflammatory and decongestant properties, whereas the bark is traditionally used in bronchitis, leprosy, tumors and ulcers (Rajkapoor et al., 2006). The methanol extract of *Bauhinia* leaves exhibited antioxidant activity attributed to the presence of cyclohexenone, lignans, and phenylethanoids derivatives (Sosa et al., 2002). Different parts of *Bauhinia purpurea* provided four *Bauhinia* statins as cancer cell growth inhibitors (Pettit et al., 2006). Treatment with the methanol extract of *Bauhinia racemosa* protects Wister albino rats against N-nitrosodiethylamine -induced hepatocarcinogenesis via suppressing nodule development and decreasing lipid peroxidation (Kumar et al., 2007). The alcohol extract of the stem bark of *Bauhinia variegata* showed a significant hepatoprotective activity against CCl_4 intoxicated Sprague-Dawley rats (Bodakhe and Ram, 2007). *Bauhinia guianensis* showed interesting *in vivo* antimalarial activity against *Plasmodium falciparum* (Kittakoop et al., 2000). Racemosol and demethylracemosol, isolated from the roots of *Bauhinia malabarica* exhibited moderate antimalarial activity (Silva et al., 2000).

Tipuana species were recommended for wound healing, GIT disorders, hemorrhoids, abdominal and rheumatic pains (Quiroga et al., 2012). Propolis from *Tipuana raco* exhibited antibacterial and free radical-scavenging activities due to the presence of flavones (Isla et al., 2001; Pereira and Neto, 2003). The volatile oil of the flowers showed a broad-spectrum antimicrobial effect and significant cytotoxic activity against breast, colon and cervix carcinoma cell lines. Pods extract was active against *Escherichia coli* and yeast (Kansoh et al., 2009).

EXPERIMENTAL

Plant materials

Fresh leaves, flowers, barks, roots and pods of both *B. purpurea L.* and *Tipuana speciosa Benth.* (Family Fabaceae) were collected from trees growing in Alexandria, Egypt in 2010. The flowers of *T. speciosa* were collected in May and other organs were collected in July. On the other hand, the flowers of *B. purpurea* were collected in April and other organs were collected in May. The plants were identified at Ornamental Trees Department, Faculty of Agriculture, Alexandria University, Egypt. Voucher specimens were deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University.

Extraction

From each plant organ, 500 g was chopped and exhaustively extracted with 90% ethanol by percolation at room temperature. The ethanol extracts were separately filtered and the solvent was distilled off under reduced pressure at 45°C to give the corresponding ethanol extracts. Another 500 g from *T. speciosa* leaves and *B. purpurea* flowers were similarly treated, the resultant ethanol extracts were dissolved in (1/2 L) mixture of ethanol/water (7:3) and successively extracted with petroleum ether, chloroform, ethyl acetate and *n*-butanol. Solvents were evaporated under vacuum to give the corresponding extracts as shown in Table 1.

Animals and chemicals

For hepatoprotective and nephroprotective activity, Wistar albino rats (150 to 200 g) of both sexes obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, were used. The animals were housed under constant temperature (22 ± 2°C), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow and access to drinking water *ad libitum*.

For anti-malarial activity, Swiss albino male mice (6 to 8 weeks, 20 to 32 g) were obtained from Leishmaniasis Diagnosis and Research Laboratory (LDRL), School of Medicine, Addis Ababa University. The animals were housed under constant temperature (22 ± 2°C), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow and access to drinking water *ad libitum*. All solvents used were of analytical grade. Silymarin and paracetamol were obtained from Sigma Aldrich (St. Louis, USA).

Malaria parasite strain

The rodent malaria parasite, *Plasmodium berghei* ANKA strain was obtained from the Bio-medical Laboratory, Department of Biology, Faculty of Science, Addis Ababa University and used to infect the mice for a four-day suppressive test.

Hepatoprotective and nephroprotective activity

Animals were divided into four groups, of five animals each. Group1 was used as control group, Groups II, III and IV received 500 mg of paracetamol (Pa) per kg body weight intraperitoneally for 3 days.

Table 1. Names, codes and weight of extracts and fractions obtained from different organs of *B. purpurea* and *T. speciosa*.

Extract or fraction	Plant and organ	Weight (g)	Code
Ethanol	*B. purpurea* bark	4	BPBE
Ethanol	*B. purpurea* leaves	13	BPLE
Ethanol	*B. purpurea* flowers	11	BPFE
Ethanol	*B. purpurea* roots	10	BPRE
Ethanol	*T. speciosa* bark	14	TSBE
Ethanol	*T. speciosa* leaves	16	TSLE
Ethanol	*T. speciosa* legumes	10	TSGE
Petroleum ether	*T. speciosa* leaves	4	TSLP
Chloroform	*T. speciosa* leaves	0.5	TSLC
Ethyl acetate	*B. purpurea* flowers	3.3	BPFEA
Ethyl acetate	*T. speciosa* leaves	7	TSLEA
n-Butanol	*B. purpurea* flowers	6.5	BPFB
n-Butanol	*T. speciosa* leaves	2.5	TSLB

Group II received only Pa. Group III was administered silymarin (Sily) at a dose of 10 mg/kg p.o. Group IV was divided into eleven subgroups (n=5). Subgroups 1 to 7 were treated with the ethanol extracts of different organs at 500 mg/kg. Subgroups 8 and 9 were treated with 300 mg/kg of BPFEA and BPFB, while Subgroups 10 and 11 were treated with 150 mg/kg of BPFEA and BPFB. Drug treatment was started 5 days prior to Pa administration and continued till day 7. After 48 h of the third Pa dose administration, the animals were sacrificed under ether anesthesia. Blood samples were collected by cardiac puncture and the serum was separated for determining the different bio-chemical parameters. The livers and kidneys were then immediately removed; small pieces were fixed in 10% formalin and kept for histopathological assessment.

Determination of biochemical parameters

Four biochemical parameters; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin were estimated as reported by Edwards and Bouchier (1991). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE). Serum creatinine and blood urea were assayed using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) by the reported method (Varley and Alan, 1984). Potassium level was measured using diagnostic strips (Reflotron®, ROCHE) while photometric determination of sodium level was done using Mg-uranylacetate method (Henry et al., 1974).

Histopathological study

The liver was immediately removed, fixed in 10% formalin, dehydrated with ethanol xylene mixtures and fixed with paraffin wax. Thin sections (3 µm) were stained in Mayer's hematoxylin solution followed by eosin-phloxine solution. Details of the experimental procedures were described by Alqasoumi et al. (2009).

In vivo anti-malarial activity

A donor mouse with parasitemia level of approximately 20 to 30% (that is, 20 to 30% of *P. berghei* ANKA strain) parasitized erythrocytes was used to infect mice. Infected blood from donor mouse was collected using a syringe containing trisodium citrate and was diluted in physiological saline to 10^7 parasitized erythrocytes/ml. Each experimental animal was subjected to inoculations of 0.2 ml (about 2×10^7 parasites) intra-peritoneal on day zero. Two hours after infection the mice were weighed and randomly divided into three groups of five mice. Group I received a vehicle containing 7% Tween 80 and 3% ethanol in distilled water that served as a negative control. Group II that served as positive control was given 25 mg/kg/day of Chloroquine Phosphate (ChlP). Group III was divided into 33 subgroups each of 5 animals received graded doses (100, 200 and 400 mg) of the different extracts and fractions dissolved in 7% Tween 80 and 3% ethanol through oral route (Peters and Robinson, 1999; Dominguez et al., 2009). Treatment was continued over four days. Twenty-four hours after the last treatment (5th day), blood smears were prepared from the tail of all mice, air dried, fixed with absolute methanol and stained with 6% Giemsa. The parasitemia was then determined microscopically by counting four fields of approximately 100 erythrocytes per field (Mesele, 2008). The efficacies of extracts and fractions were assessed by comparison of blood parasitemia and mouse survival time in treated and control groups (Trager and Jensen, 1976).

Statistical analysis

For each set of experiments where two or more than two groups were compared, an analysis of variance (ANOVA) test was used to determine the significance of the differences. Differences between the control and paracetamol-treated group were compared for significance using student's t-test for non-paired samples (Woolson and Clarke, 2002). All the values shown are the mean ± S.E.

RESULTS AND DISCUSSION

Hepatoprotective activity

Therapeutic doses of Pa eliminated mainly as sulfate and glucoronide (Eriksson et al., 1992) and only 5% of the dose is converted into N-acetyl-p-benzoquineimine (NAPQI). However, upon administration of toxic doses of Pa, higher percentages of the molecules are oxidized to highly reactive NAPQI by cytochrome p-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI are rapidly conjugated with glutathione (GSH),

a sulphydryl donor which results in the depletion of liver GSH pool (Remirez et al., 1995). Under conditions of excessive NAPQI formation or reduced of glutathione store, NAPQI covalently binds to vital proteins, the lipid bilayer of hepatocyte membranes and increases the lipid peroxidation (McConnachie et al., 2007).

Hepatic toxicity is reflected by increase in the biochemical parameter levels such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin. Treatment of rats with Pa resulted in severe damage of hepatocytes, biliary obstruction and transport inability across the liver as indicated by high levels of AST, ALT, GGT, ALP and bilirubin (Table 2) (Edwards and Bouchier, 1991). Pretreatment of rats with sily, significantly ($P < 0.001$) decreased the raised levels of AST, ALT, GGT, ALP and bilirubin induced by Pa (45.48, 47.28, 58.96, 37.84 and 67.2% respectively) (Table 2) indicating a good recovery from the hepatotoxic agent. The hepatoprotective effect offered by BPFE ethanol extract at 500 mg/kg doses, was found to be highly significant ($P < 0.001$) in all parameters studied with 35.36, 33.0, 41.7, 25.82 and 51.55% reduction in AST, ALT, GGT, ALP and bilirubin, respectively and was the best when compared to other extracts (Table 2). The serum levels of AST, ALT, GGT, ALP and bilirubin in the groups treated with 500 mg/kg body weight BPLE and BPBE showed moderate decreases in all measured parameters. Results were statistically significant except for BPBE reduction in bilirubin. BPLE did not show any effect on the level of ALP, however, its effect was superior to BPBE except in the reduction of AST level. Results obtained by treatment with BPRE were very weak and insignificant. Fractions obtained from BPFE were tested at 150 and 300 mg/kg body weight. The obtained results revealed that the activity was trapped to the BPFB. Animal treated with this fraction showed good significant reduction in the parameters studied. BPFB at 300 mg/kg body weight showed 39.66, 48.44, 49.32, 21.23 and 20.04% reduction in AST, ALT, GGT, ALP ($P<0.001$) and bilirubin ($P<0.01$). Phytochemical study of this fraction is highly recommended.

Animal treated with 500 mg/ kg body weight of TSBE showed significant ($P < 0.05$ to $P < 0.001$) reduction in the levels of AST, ALT, GGT, ALP and bilirubin (22.87, 20.35, 26.83, 26.25, and 38.17%). Extracts obtained from other plant parts were less effective in reduction of liver biochemical parameters with low or insignificant results (Table 2). However, highly significant ($P<0.001$) 29.85% reduction in ALP level was observed in animal treated with 500 mg/ kg body weight of TSGE.

Nephroprotective activity

The kidney regulates plasma ionic composition including sodium, potassium, calcium, magnesium, chloride. It is also concerned with the removal of nitrogenous metabolic waste products such as urea, creatinine and uric acid (Pocock and Richards, 2006). Elevations of serum electrolytes, urea and creatinine are considered reliable parameters for investigating drug-induced nephrotoxicity in animals and man (Adelman et al., 1981). Pa exhibits a significant rise in the biochemical markers of kidney function like serum urea, serum creatinine, sodium and potassium level. Pretreatment with Sily (10 mg/kg p.o) decreased the raised levels of serum urea, serum creatinine, percentage of sodium and potassium (35.23, 51.9, 31.7 and 52.4%) induced by Pa (Table 3). Extracts obtained from different organs pf *B. purpurea* showed variable degrees of nephroprotection. BPFE was the most effective extract and resulted in highly significant ($P<0.001$) reduction in the serum urea, serum creatinine, sodium and potassium levels (45.35, 43.88, 27.61 and 40.67%) respectively. Although BPBE treated group showed some decrease in all the measured parameters, however the results were not statistically significant. Animals in the group treated with BPRE lowered only the level of serum creatinine (17.19%). The serum levels of urea and creatinine in the group treated with BPLE showed significant decreases ($P <0.01$ and $p <0.001$) by 17.32 and 35.31% respectively.

In case of *T. speciosa* extracts, most effective nephroprotective results were observed in the group of animals that received 500 mg/kg body weight of TSLE. The reduction in serum urea, serum creatinine, sodium and potassium level were 22.03, 32.37, 25.42 and 42.09% respectively and the results were highly significant ($P< 0.01$, $P< 0.001$). TSLE was fractionated and the obtained fractions were tested at 150 and 300 mg/kg. TSLC showed highly significant ($P< 0.001$) reduction (21.92, 24.01, 26.09 and 32.9%) in all the tested parameters (serum urea, serum creatinine, sodium and potassium level, respectively) at 300 mg/kg dose (Table 3), however, reduction did not exceed that obtained with original extract TSLE. TSLP and TSLEA treated animals showed 14.06, 35.57, 32.6, 30.07% and 10.07, 31.13, 29.3, 34.31% decrease in the levels of serum urea, serum creatinine, sodium and potassium level respectively. Results were statistically significant ($P<0.05$ and $P<0.001$).

The serum levels of creatinine, sodium and potassium in the group treated with TSBE showed significant ($P < 0.01$ and $p <0.001$) decreases by 49.77, 26.81 and 41.52% respectively, at the dose of 500 mg/kg body weight (Table 3). TSGE at the doses of 500 mg/kg failed to reduce the raised level of the biomarkers indicating that the extract is free from any nephroprotective effect.

Histopathological study

The histological appearance of the hepatocyte reflects their conditions (Prophet et al., 1994). Extracts that gave good protection in the biochemical parameters were subjected to the histopathological study as well. Liver cells

Table 2. Effect of different extracts of *B. purpurea* and *T. speciosa* parts on Wistar albino rats liver serum biochemical parameters (n-5).

Treatment	Dose (mg/kg)	Bio-chemical parameters									
		Bilirubin (mg/dl)		ALP (U/L)		GGT(u/l)		AST (U/L)		ALT (U/L)	
		(% Decrease)	(Mean ±)	(% Decrease)	(Mean ±)	(% Decrease)	(Mean ±)	(% Decrease)	(Mean ±)	(% Decrease)	(Mean ±)
Normal			0.56±0.05		276.5±10.09		4.29±0.32		39.00±2.87		80.03±4.37
Pa	500		3.21±0.1***		532.5±19.27***		15.46±0.89***		218.66±11.40***		245.5±12.03***
Sily	10	67.32	1.05±0.04***	37.84	331.00±17.67***	58.96	6.34±0.24***	47.28	115.26±7.08***	45.48	133.83±6.27***
BPBE + Pa	500	10.06	2.89±0.08	13.36	461.33±11.91*	21.44	12.15±0.87*	21.11	172.5±10.97*	21.18	193.5±7.79**
BPFE + Pa	500	51.55	1.55±0.05***	25.82	395.00±10.10***	41.70	9.01±0.37***	33.00	146.5±5.85***	35.36	158.66±8.93***
BPRE + Pa	500	7.57	2.97±0.15	6.57	497.5±9.11	8.08	14.21±0.70	11.81	192.83±10.95	-	276.16±13.29
BPLE + Pa	500	21.88	2.51±0.11**	-	522.83±11.30	42.99	8.81±0.34***	28.96	155.33±8.11***	18.80	199.33±9.81*
TSBE+ Pa	500	38.17	1.98±0.13***	26.25	392.66±9.74***	26.83	11.31±0.45**	20.35	174.16±7.80*	22.87	189.33±10.24**
TSLE + Pa	500	19.08	2.6±0.15*	18.52	433.83±19.40**	10.45	13.85±0.42	25.15	163.66±6.67**	-	266.5±11.37
TSGE + Pa	500	22.76	2.48±0.19*	29.85	373.5±19.44***	13.57	13.36±0.57	17.37	180.66±14.50	4.00	235.66±9.67
Normal			0.54±0.01		360.83±9.44		3.46±0.20		37.35±2.88		86.4±4.25
Pa	500		2.86±0.08***		633.33±13.35***		13.48±0.31***		274.16±6.03***		292.83±9.00***
Sily	10	57.98	1.20±0.10***	31.39	434.5±17.40***	63.41	4.93±0.21***	62.75	102.1±3.78***	48.03	152.16±6.24***
BPFEA + Pa	150	9.20	2.59±0.06*		633.16±57.03	7.29	12.5±0.32	3.22	265.33±11.49	31.98	199.16±22.76**
BPFEA + Pa	300	17.83	2.35±0.12**	4.26	606.33±23.71	7.66	12.45±0.18*	13.06	238.33±12.56*	18.15	239.66±9.57**
BPFB + Pa	150	15.15	2.42±0.12*	9.52	573.00±14.29*	15.57	11.38±0.50**	16.04	230.16±12.27**	34.03	193.16±6.16***
BPFB + Pa	300	20.04	2.28±0.13**	21.23	498.83±9.49***	49.32	6.83±0.22***	21.23	498.83±9.49***	20.04	2.28±0.13**

*P<0.05, **P<0.01, ***P<0.001

as well. Liver cells of rats treated with 500 mg kg^{-1} Pa (Figure 1B) showed great damage represented by extensive focal necrosis, lymphocytic infiltrate, extensive hydropic swelling with rosette formation, lymphocytic exudates and dilated congested vessels in portal tracts.

Liver cells treated with 10 mg kg^{-1} of the standard drug Sily (Figure 1C) prior to Pa administration showed improvement in the liver cell histopathology with granular cytoplasm, mild congestion in central veins, mild portal tract infiltration and few focal necrosis. Histopathological appearance of liver cells obtained from subgroup treatment with 500 mg kg^{-1} BPFE before intoxication by Pa (Figure 1D) showed the best degree of protection obtained in the current study with normal lobules, mild central focal necrosis, mild congestion in central veins and mild infiltration in portal tracts. Subgroup treated with 300 mg kg^{-1} BPFB (Figure 1E) showed mild infiltrated congested portal tracts, dilated congested central vein, dilated congested sinusoids and focal necrosis. Administration of 500 mg kg^{-1} TSBE (Figure 1F) showed moderate protection represented by normal lobule, moderate portal tract dilation, congestion and central vein congestion.

Histopathological study revealed the normal renal architecture in control group (Figure 2A). Pa treated rats showed sever damage in the kidney cells (Figure 2B) appeared as variable size and atrophic cellular glomeruli, marked cloudy swelling in tubules and narrow lumens.

The protective standard drug Sily at 10 mg kg^{-1} helped indecreasing the cellular damage induced by Pa. Cellular appearance showed mostly nearly normal glomeruli with few variable size atrophic glomeruli, mild tubular degeneration, necrosis and cloudy swelling. Kidneys of animal treated with 500 mg kg^{-1} BPFE showed less protective effect than that exerted on the liver cells. Marked congestion, tubular dilation, chronic inflammatory exudates in the cortex, hemorrhage and blood

Table 3. Effect of different extracts of *B. purpurea* and *T. speciosa* parts on Wistar albino rats kidney bio-chemical parameters (n=5).

Treatment	Dose (mg/kg)	Bio-chemical parameters							
		Potassium (mmol/l)		Sodium (mmol/l)		Creatinine (mg/dl)		Urea (mg/dl)	
		(% Decrease)	(Mean ±)	(% Decrease)	(Mean ±)	(% Decrease)	(Mean ±)	(% Decrease)	(Mean ±)
Normal			5.33±0.28		82.38±4.09		2.95±0.17		47.43±10.32
Pa	500		11.80±0.90***		167.16±8.05***		10.95±0.67***		152.00±3.70***
Sily	10	52.40	5.61±0.23***	31.70	114.16±4.26***	51.90	5.26±0.29***	35.23	98.45±3.97***
BPBE + Pa	500	15.39	9.98±0.70	8.97	152.16±5.24	12.78	9.55±0.39	3.94	146.00±4.22
BPFE + Pa	500	40.67	7.00±0.19***	27.61	121.00±4.75***	43.88	6.15±0.23***	45.35	83.06±16.77**
BPRE + Pa	500	8.19	10.83±0.45		166.83±5.14	17.19	9.06±0.32*		152.33±6.60
BPLE + Pa	500	13.55	10.2±0.49	8.07	153.66±3.22	35.31	7.08±0.24***	17.32	125.66±5.53**
TSBE+ Pa	500	41.52	6.90±0.16***	26.81	122.33±4.99**	49.77	5.5±0.20***		155.00±5.80
TSLE + Pa	500	42.09	6.83±0.25***	25.42	124.66±4.69***	32.37	8.5±0.22**	22.03	118.5±3.59***
TSGE + Pa	500	17.04	9.78±0.42		177.66±5.18		11.50±0.60		151.16±4.71
Normal			6.40±0.25		73.45±2.01		3.3±0.22		49.86±1.69
Pa	500		14.13±0.44***		182.00±4.77***		13.11±0.51***		150.5±4.37***
Sily	10	40.09	8.46±0.43***	44.58	100.85±3.22***	67.72	4.23±0.27***	37.22	94.48±4.93***
TSLP + Pa	150	8.72	12.90±0.57	27.74	131.5±***	12.70	11.45±0.31*	8.63	137.5±5.45
TSLP + Pa	300	30.07	9.88±0.31***	32.60	122.66±4.23***	35.57	8.45±0.31***	14.06	129.33±5.14*
TSLC + Pa	150	8.37	12.95±0.57	10.07	163.66±4.58*	11.43	11.61±0.41*	9.74	135.83±3.77*
TSLC + Pa	300	32.90	9.48±0.38***	26.09	134.50±***	24.01	9.96±0.39***	21.92	117.5±3.73***
TSLEA + Pa	150	15.33	11.96±0.29**	13.55	157.33±4.69**		13.06±0.60		151.33±5.11
TSLEA + Pa	300	34.31	9.28±0.21***	29.30	128.66±5.16***	31.13	9.03±0.30***	10.07	135.33±4.34*

*P<0.05, **P<0.01, ***P<0.001.

casts in the tubules, cellular glomeruli with variable sizes (few of them atrophic) were all observed. Treatment with 500 mg kg^{-1} BPLE prior to Pa intoxication showed cells with cortical vascular dilation and congestion, chronic inflammation and destruction of glomeruli, focal cortical degeneration, glomerular atrophy and chronic inflammatory exudates in the cortex around glomerul. Treatment with 500 mg kg^{-1} TSLE, 300 mg kg^{-1} of both TSLP and TSLC were not effective in improving the histopathological appearance of the renal cells. Congestion and hemorrhage at corticomedullary area, glomerular changes, cloudy swelling in tubules, vessels congestion and dilation. Best histopathological nephroprotection was observed in subgroup treated with 300 mg kg^{-1} TSLEA where cells showed normal medulla and few small atrophic glomeruli with mild cloudy swelling.

Anti-malarial activity

Different extracts were evaluated for their *in vivo* anti-malaria activity at three dose levels (100, 200, 400 mg/kg) on *P. berghei* infected mice using ChlP as positive control.

Treatment of infected mice with ChlP resulted in suppression of parasitemia to non-detectable levels.

All the tested extracts and fraction showed variable levels of significant, dose dependent protection against *P. berghei* parasite. Survival rates were very low with both BPLE and TSGE reflecting a very poor protection.

Although BPFE and TSLE did not achieve 100% suppression of parasitemia like ChlP, however, 100% survival was obtained at the doses of 200 and 400 mg/kg. The effect of these two extracts is

Table 4. Anti-malarial activities of different extracts of *B. purpurea* and *T. speciosa* parts at three dose levels*.

Treatment	Dose (mg/kg)	% Parasitaemia	% Suppression	Survival % on Day 10
Normal		43.03	0.0	0.0
ChIP	25	0.0	100	100
	100	33.36 ± 1.42	22.47	10
BPBE	200	30.11 ± 0.94	30.02	20
	400	26.38 ± 1.05	38.69	20
	100	35.12 ± 1.36	18.38	0.0
BPLE	200	31.80 ± 0.78	26.09	0.0
	400	28.92 ± 0.52	32.79	10
	100	12.01 ± 0.18	72.08	60
BPFE	200	7.96 ± 0.46	81.50	100
	400	4.83 ± 0.38	88.77	100
	100	29.91 ± 0.24	30.49	20
BPRE	200	22.68 ± 0.33	47.29	60
	400	18.09 ± 0.68	57.95	60
	100	26.82 ± 0.31	57.70	60
TSBE	200	18.20 ±0.52	37.67	60
	400	15.86 ±0.22	63.14	60
	100	13.00 ±0.91	69.78	80
TSLE	200	9.72 ± 0.62	77.41	100
	400	5.25 ± 0.21	87.79	100
	100	39.17 ± 0.22	11.29	0.0
TSGE	200	36.29 ± 0.64	15.66	0.0
	400	35.84 ± 0.80	16.70	0.0
	100	32.81 ± 0.63	23.75	20
TSLP	200	29.76 ± 0.77	30.83	40
	400	26.93 ± 1.24	37.41	40
	100	36.12 ± 0.80	16.05	20
TSLC	200	31.27 ± 0.73	27.32	20
	400	28.96 ± 0.86	32.69	40
	100	28.18 ± 0.62	34.51	20
TSLEA	200	24.46 ± 0.32	43.15	40
	400	17.26 ± 0.58	59.88	40
	100	24.93 ± 0.23	42.06	40
TSLB	200	18.77 ± 0.41	56.37	40
	400	14.03 ± 1.22	67.39	60

*Values are Mean ± SD, P<0.05.

significant and dose dependent. At the dose of 400 mg/kg, TSLE and BPFE produced 87.79 and 88.77% suppression of parasitemia respectively (Table 4). Treatment with BPFE and TSLE confer a good antiplasmodial activity against *P. berghei* in mice. Further investigation of the clinical and toxicological potential of the two extracts as well as exploring their chemical constituents could result in the use of them as a remedy

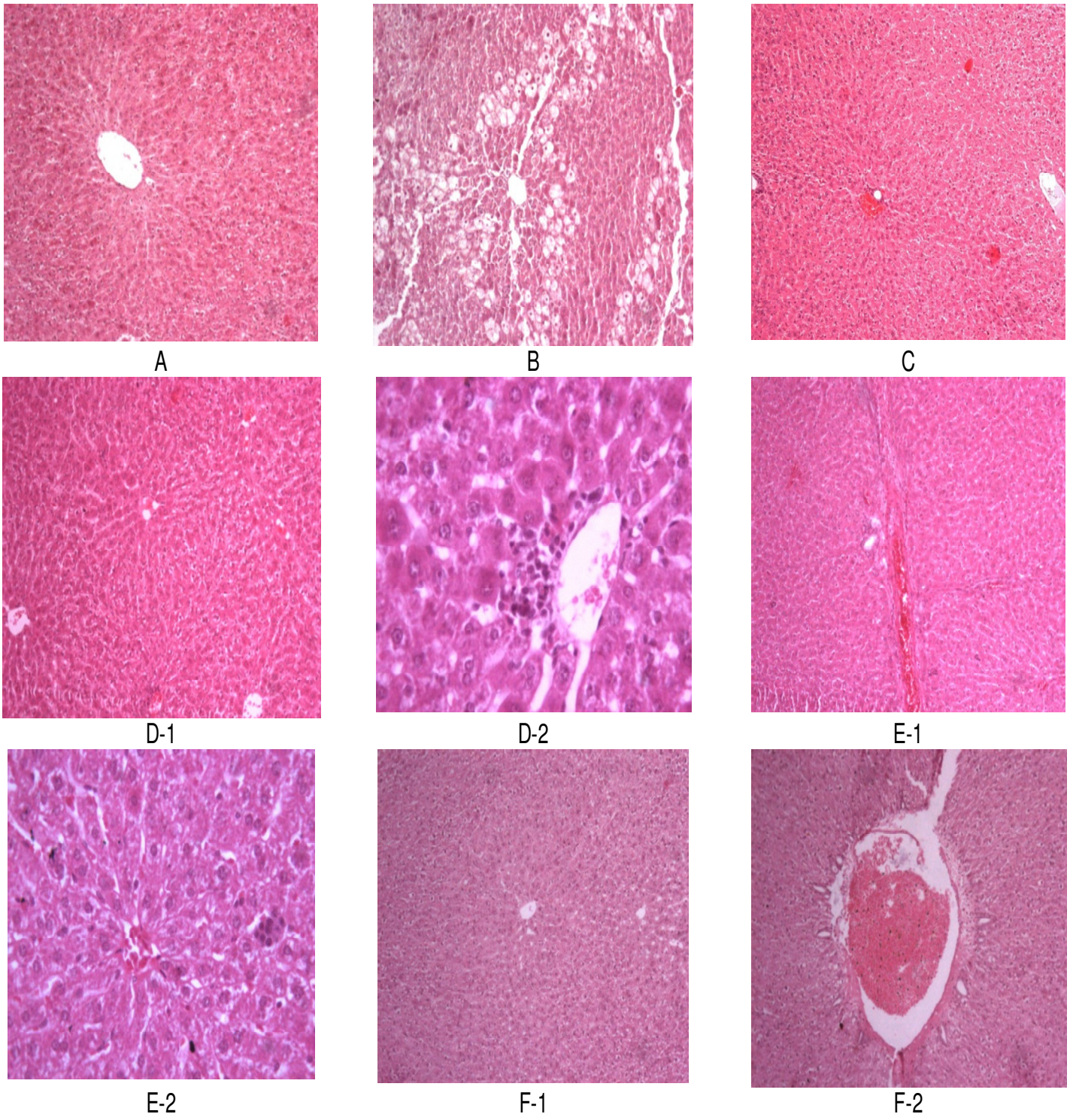

Figure 1. Histopathological study of liver cells; (A) normal cells; (B) liver cells of rats treated with Pa; (C) liver cells of rats treated with Pa and Sily; (D) liver cells of rats treated with Pa and 500 mg kg^{-1} of BPFE, (E) liver cells of rats treated with Pa and 300 mg kg^{-1} of BPFB; (F) liver cells of rats treated with 500 mg kg^{-1} of TSBE.

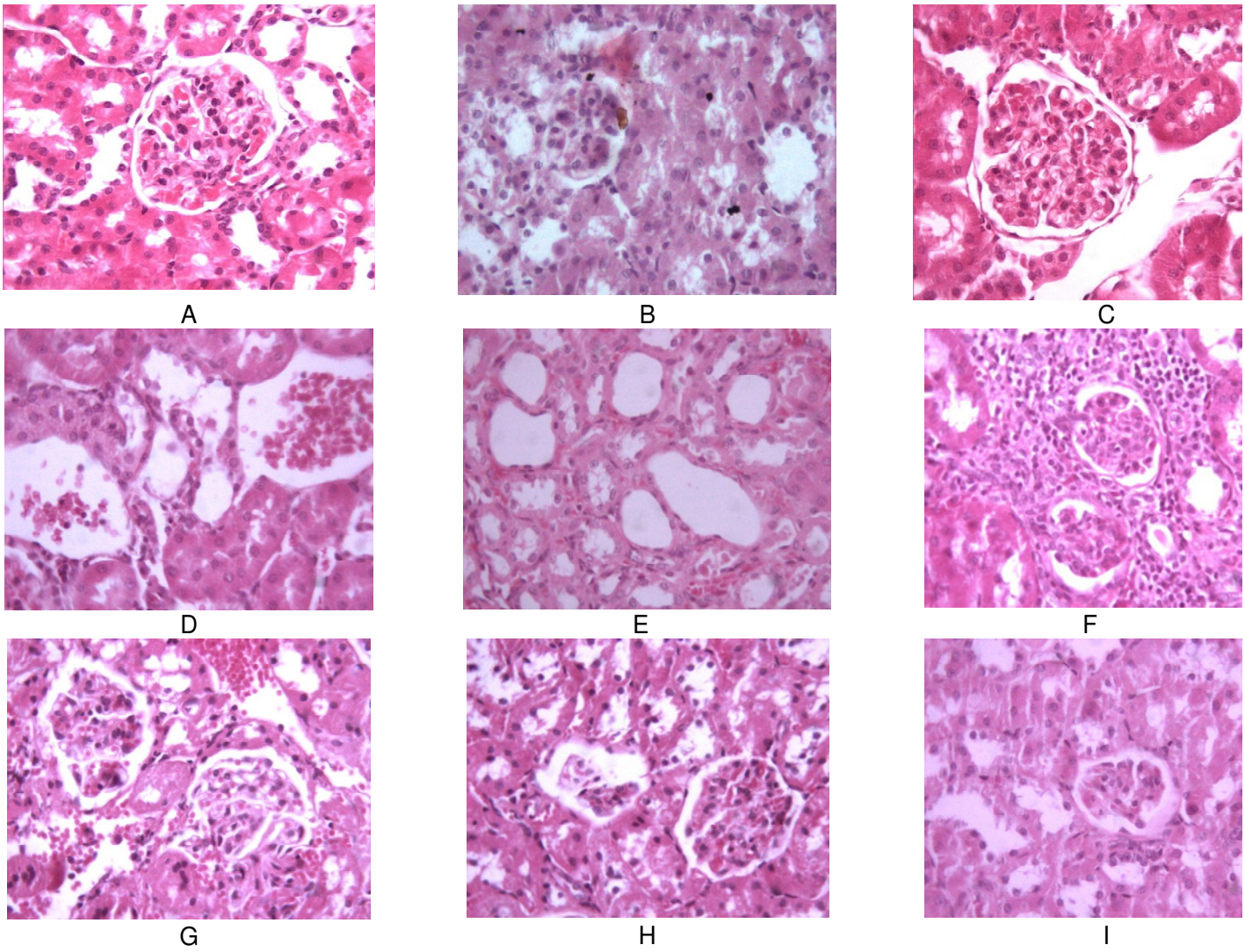

Figure 2. Histopathological study of kidney cells; (A) normal cells; (B) kidney cells of rats treated with Pa; (C) kidney cells of rats treated with Pa and Sily; (D) kidney cells of rats treated with Pa and 500 mg kg^{-1} of BPFE, (E) kidney cells of rats treated with Pa and 500 mg kg^{-1} of BPLE; (F) kidney cells of rats treated with 500 mg kg^{-1} of TSLE; (G) kidney cells of rats treated with 500 mg kg^{-1} of TSLP; (H) kidney cells of rats treated with 500 mg kg^{-1} of TSLC; (I) kidney cells of rats treated with 500 mg kg^{-1} of TSLEA.

for the treatment of malaria in human.

Conclusion

Promising results were obtained from the pharmacological study of the hepatoprotective, nephroprotective and *in vivo* anti-malarial effects of different parts of two fabaceous trees *B. purpurea* and *T speciosa*. The ethanol extract of *B. purpurea* flowers extract (BPFE) was effective in the three used assays. Reduction in elevated biochemical parameters and improvement in the histopathological appearance in liver cells and to less extent in renal cells were good indicators for hepatoprotective, nephroprotective activity. Survival rate of 100% observed in animals treated with 200 mg kg^{-1} dose and 88.77% suppression of parasitemia with 400 mg kg^{-1} dose in the anti-malarial assay reflects an effective protection against *P. berghei* parasite. Biologically directed phytochemical study of this extract and its fractions is highly recommended.

In case of *T. speciosa* the leaves extract (TSLE) and its fractions were the best in nephroprotective assay as indicated by biochemical parameters and histopathological appearance. It also showed best anti-malarial activity where 100% survival rate and 87.79% suppression of parasitemia were observed in animals treated with 200 and 400 mg kg^{-1} respectively. The bark extract (TSBE) showed a moderate protection in the hepatoprotective study.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Malik Sawood at the Research Center, College of Pharmacy, King Saud University for technical assistance.

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