In vitro antioxidant and hepatoprotective activities of *Paeonia emodi* (Wall.) rhizome methanol extract and its phenolic compounds rich fractions

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Abstract: The present study aimed to quantify the total phenolic content in *Paeonia emodi* rhizome methanol extract and its fractions and then evaluate the *in vitro* antioxidant and hepatoprotective activities of fractions rich in phenolic compounds. Maximum quantity of total phenolic content was observed in butanol (112.08±5.5 mg GAE/g dw) and chloroform fraction (107.0±3.5 mg GAE/g dw) followed by methanol extract (94.2±4.4 mg GAE/g dw), aqueous fraction (92.9±2.5 mg GAE/g dw), ethyl acetate (62.3±8.3 mg GAE/g dw) and n-hexane fraction (51.6±7.2 mg GAE/g dw). The fractions rich in total phenolic content were evaluated for *in vitro* antioxidant activity based on 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging assay. The butanol and chloroform fraction showed significantly ($P<0.05$) higher radical scavenging activity with IC_{50} values of 6.5 and 7.05±2.5 ppm respectively. Positive correlation (R square=0.95) was observed between total phenolic content and *in vitro* antioxidant activity. The fractions rich in phenolic compounds were also evaluated for their hepatoprotective activity in paracetamol intoxicated mice. Five days oral administration of these fractions at a dose of 300 mg/kg body weight restored the serum ALT, AST and ALP levels of paracetamol intoxicated mice to normal level. From the results of the present research it was concluded that the butanol and chloroform fractions of *P. emodi* rhizome methanol extract are rich in phenolic compounds and strong antioxidant and effective in attenuation of hepatotoxicity.

Keywords: Total Phenolic content, DPPH, antioxidant, liver.

INTRODUCTION

Liver is an internal vital organ associated with digestive system and responsible for a wide range of functions. It is involved in formation of coagulation factors, storage of vitamins and irons, filtration of toxin out of the blood, storage of blood, metabolism of foreign chemicals, proteins, carbohydrates, fats and hormones (Guyton and Hall, 1996). Liver is important for detoxification of xenobiotic agents (Muthulingam, 2010). Several factors such as excessive use of alcohol, hepatitis infections, fats accumulation, cancer and frequent exposure to toxic chemicals and drugs lead to liver injury. It has been reported that 50% of all acute hepatic failure is due to drug induced liver injury (Ostapowicz *et al*., 2002). Paracetamol (PCM) is one of the commonly known analgesic drugs used for relieving pain and fever (Rosa *et al*., 2006). Continuous use or overdosing of PCM leads to hepatotoxicity. Hepatotoxicity following PCM ingestion is due to the production of a highly reactive PCM metabolite, *N*-acetyl-p-benzoquinonimine (NAPQI). It is synthesized through the cytochrome $P4_{50}$ group of enzymes in the liver. NAPQI is usually converted to a non-toxic or safe form after its conjugation with glutathione (GSH) which is then removed by the kidney. NAPQI accumulation results in the excessive generation of reactive oxygen species (ROS) and ultimately liver damage (Ojo *et al*., 2006).

ROS are highly reactive radicals due to possession of unpaired electrons which include superoxide ion, hydroxyl radical and nitric oxide radical, and non-radicals such as peroxynitrite, hydrogen peroxide, hypochlorous acid and singlet oxygen (Vara and Pula, 2014). In living organisms, ROS are produced as a byproduct during oxygen metabolism (Devasagayam *et al*., 2004) or due to exposure to certain environmental factors such as sunlight, ultraviolet light, ionizing radiation and toxic chemicals (Gyamfi *et al*., 1999). ROS is important in regulation of various physiological functions such as growth, apoptosis, blood pressure, and cognitive and immune functions (Krause and Bedard, 2008). When in excess, ROS may oxidize and damage biological molecules such as DNA, proteins and lipids by extracting electron from them for attaining stability which may result in mutation, carcinogenesis and other degenerative diseases (Droge, 2002). Oxidation of the membrane proteins and or lipids of hepatocytes due to ROS may

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result in the rupturing of cellular membranes and in turn in the bulk release of cytosolic alkaline phosphatases (ALP) and transaminases such as alanine amino transferase (ALT) and aspartate amino transferase (AST) into the blood circulation (Poli, 1999). ROS can be stabilized or deactivated by endogenous antioxidant enzymes such as glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) and as well as by antioxidant compounds such as glutathione, α-tocopherol, ascorbic acid and other dietary antioxidants(Datta *et al*., 2000). The use of natural antioxidants of plant origin is now gaining attention (AidiWannes., 2010). Medicines derived from plant products are safer than their synthetic counterparts (Vongtau *et al*., 2005).

Many medicinal plants possess antioxidant properties as they possess antioxidant ingredients such asphenolic compounds (Nahak and Sahu, 2010). Phenolic compounds are a large class of plant secondary metabolites such as vanillin, gallic acid and caffeic acid, to complex high-molecular poly phenols such as stilbenes, flavonoids, and polymers. They contain an aromatic ring that bears hydroxyl groups (Andersen and Markham 2006). Phenolic compounds stabilize ROS, chelate transition metal ions and inhibit enzymes involved in oxidative stress (Dangles, 2012). The antioxidant activity of phenolic compounds depends on the structure, number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings (Balasundrama *et al*., 2005). The higher the concentration of phenolic compounds in a plant the stronger its antioxidant activity (Rekha *et al*., 2012).

Many plants have been screened for their *in vitro* and *in vivo* antioxidant activities. In *in vitro* studies, the antioxidant activities of medicinal plants are evaluated on the basis of percent scavenging of radicals. DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) scavenging assay is one of the well-known laboratory radical scavenging assays (Wojdyło *et al*., 2007). In *in vivo* studies, the antioxidant activities of medicinal plants are evaluated on the basis of their positive effects on the levels of antioxidant enzymes such as glutathione reductase, super oxide dismutase, catalase and per oxidase in the tissues of laboratory animals following administration of plant extracts (Jain *et al*., 2012).

Pakistan is rich in medicinal plants. *Paeonia emodi* Wall. (Paeoniaceae) is one of the important medicinal plants and known as Mamekh in Swat district of Khyber Pakhtunkhwa, Pakistan. The plant *P. emodi* has been used from long time in the traditional medical system. The roots and rhizomes are locally used as a remedy for backache, dropsy and epilepsy. It is also used traditionally as a tonic energizer, emetic, cathartic, blood purifier and colic. The seeds are used as purgative (Shinwari *et al*., 2003). The aerial part of this plant has been studied for total phenolic content and radical scavenging activity (Khan *et al*., 2005). Paeonins A and B and monoterpene galactosides have been isolated from the rhizome part of this plant which are potent chemical compounds with significant lipoxygenase inhibitory activity (Riaz *et al*., 2003b). There is also a requirement to screen the rhizome part of this plant for its total phenolic content and *in vitro* antioxidant and hepatoprotective activities. Therefore a study was arranged that aimed to measure the total phenolic content of *P. emodi* rhizome methanol extract and its fractions and then evaluate the phenolic content rich extracts for their *in*vitro antioxidant and hepatoprotective activities.

MATERIALS AND METHODS

The present research was conducted in two phases. The first phase involved plant collection, extract preparation and fractionation, estimation of total phenolic and evaluation of *in vitro* antioxidant activities of extract and its fractions on the basis of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging assay. During the second phase, the hepatoprotective effects of *P. emodi* rhizome methanol extract and its fractions were studied in paracetamol intoxicated mice.

Chemicals used

2, 2-diphenyl-1-icryl-hydrazyl (DPPH), Ascorbic acid, sodium pentobarbital, sodium carbonate (Na_2CO_3) and Folin-Ciocalteu were purchased from Sigma Co. (USA). Analytical grade methanol, n-hexane, ethyl acetate, chloroform and butanol used for plant extraction and fractionation were obtained from Merck Co. (Darmstadt, Germany).Commercially available kits (purchased from AMP Diagnostics, Austria) were used for estimating the levels of ALT, AST and ALP in serum on a UV visible light spectrophotometer (Agilent 8453).

Collection of plant material

The rhizome part of the plant *Paeonia emodi* (Wall.) was collected in Malam Jaba area of Swat, Pakistan and identified by an expert in Botany, University of Malakand.

Preparations of extract and fractionation

The rhizome part of *P. emodi* were rinsed with tap water and shade dried. The dried rhizomes were ground in electric chopper to get fine powder form and then 450 gram of powder was soaked in 2000ml of 95% methanol with occasional shaking for 72 hours. After soaking, theplant material was filtered through What man no. 1 filter paper and then evaporated through rotary evaporator at 40^{o}C. The extract in concentrated solution form was then transferred from the flask of rotary evaporator and placed in a beaker under running fan for evaporating the remaining solvent. Methanol extract of *P. emodi* was obtained in the form of thick paste yielding approximately 170 grams (37.77w/w) by weight. 42g of crude extract

was dissolved in 90 ml distilled water and then poured into a separating funnel and shaken well. Then 90ml n-hexane was added to the separating funnel. The upper layer of n-hexane was separated. Then 90ml of chloroform was added to the residue, mixed well, and the lower layer of chloroform was separated. To the residue, 90 ml ethyl acetate was added to obtain ethyl acetate fraction. Finally, butanol (90 ml) was added to the residue and lower layer of aqueous and upper layer of butanol fractions were separated. The fractions were evaporated through rotary evaporator at 45ºC. The percentage yield (w/w) of n-hexane was 7.4, chloroform 28.2, ethyl acetate12.16, butanol 11.24 and aqueous fraction was 38.08. The fractions were stored at 4ºC for future use.

Determination of total phenolic content

The total phenolic contents of methanol extract and its various solvent fractions were evaluated by following the already reported procedure (Kim *et al*., 2003). Each extract in amount of 10 mg was dissolved in 20 ml of methanol to prepare stock solution of 0.5 mg/ml. One milliliter of stock solution was poured into 45 ml of distilled water. To this solution 1 ml of Folin-Ciocalteu's phenol reagent (FCR) was added. After five minutes, 3ml of Na_2CO_3 was added to the mixture. Four milliliter of distilled water was added and mixed thoroughly. The whole mixture was kept in the dark for incubation period of 90 minutes. After incubation, absorbance was measured at 760nm using UV spectrophotometer (1700 Shimadzu Japan). Milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw) was used as unit of total phenolic content. The methanol extract and the fractions that showed maximum phenolic content were then screened for DPPH radical scavenging and hepatoprotective activities.

DPPH Radical scavenging activity

The *in vitro* antioxidant activities of methanol extract and its chloroform, butanol and aqueous fractions were evaluated on the basis of scavenging of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) by using earlier procedures (Bursal and Gulcin, 2011). The concentration of solutions prepared for the activity were expressed as parts per million (ppm), equal to mg/L. Stock solutions of the extracts were prepared in methanol (each of 25 ml and of 500 ppm). From each stock solution a 5ml solution each of 20, 60 and 100 ppm was prepared in separate test tubes. Each concentration was taken in triplicate. The same procedure was repeated for ascorbic acid which was used as standard. To each test tube, 1 ml of DPPH was added. For keeping control, 1 ml of DPPH was added to some test tubes containing only 5 ml of methanol. After keeping the test tubes for an incubation period of 30 minutes at room temperature in dark, the absorbance of solutions was measured using UV spectrophotometer (1700 Shimadzu Japan)at 517 nm. For the calculation of antioxidant activity the following formula was used: % radical scavenging activity = (Ac – As / Ac) × 100, Ac represents the absorbance of control and as stands for the absorbance of extract/ascorbic acid solution.

Animals used

Adult healthy Swiss albino male mice weighing 24-28 grams were purchased from the National Institute of Health, Islamabad. A total of 28 mice were housed in seven cages, four in each cage (5"×9"×11" made of steel mesh). Standard rodent food and water were all time available to mice. Mice were maintained at 12:12 light/dark cycle of photoperiod. They were acclimatized for two weeks before starting experiment.

Hepatoprotective activity

During this research the institutional committee for animal ethics approved the animal study. The guidelines of Zimmermann (1986) for the care of experimental animals were followed during experiments on animals. The animals were divided into seven groups, each group comprising of four mice. For the identity of different mice groups various color tags were used. Group 1 served as normal control and was orally administered with normal saline, group 2 served as paracetamol control group, received paracetamol on day zero followed by normal saline, group 3 served as standard control group, received paracetamol on day zero followed by silymarin, a standard antioxidant and hepatoprotective drug, group 4 received paracetamol on day zero followed by methanol extract, group 5 received paracetamol on day zero followed by chloroform fraction, group 6 received paracetamol on day zero followed by butanol fraction and group 7 received paracetamol on day zero followed by aqueous fraction. The drugs and extracts were administered orally for 5 days. The following were the detail of dosing: paracetamol 1g/kg body weight (Sasidharan *et al*., 2012), silymarin 50 mg/kg body weight (Bak *et al*., 2012) and each plant extract 300 mg/kg body weight.

On day 6, all mice were starved for 12 hours and anesthetized with inhaled chloroform. Each rat restricted on the dissecting board was dissected and blood sample was drawn from the heart chambers into a 3 ml syringe with 21 Gauge needle. Blood was transferred into sterile tubes with coagulant and then centrifuged through Eppendorf 5702R centrifuge for 5 minutes at 3000 rpm for isolation of serum and stored at –20˚C until assayed. The serum was analyzed for biochemical markers of liver injury such as alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP).

STATISTICAL ANALYSIS

Results were expressed as means and standard deviation of mean of replicates. Relationship between total phenolic content and antioxidant activity was calculated by

applying linear regression of statistics. Probit regression was applied for calculating IC_{50}. Results were compared by applying Tukey Test. SPSS 16.0 was used for all these analysis.

RESULTS

Total Phenolic Contents

The total phenolic contents of *P. emodi* rhizome methanol extract and its fractions was measured (table.1). The values of total phenolic content were expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw). Maximum amount of total phenolic content was observed in butanol (112.2±5.5 mg GAE/g dw) and chloroform fraction (107.0±3.5 mg GAE/g dw) followed by methanol extract (94.2±4.4 mg GAE/g dw), aqueous fraction (92.9±2.5 mg GAE/g dw), ethyl acetate fraction (62.3±8.3 mg GAE/g dw) and n-hexane fraction (51.6±7.2 mg GAE/g dw).

DPPH radical scavenging assay

The methanolic extract and its phenolic content rich fractions such as chloroform, butanol and aqueous extracts were screened for their *in v*itro antioxidant potentialon the basis of DPPH radical scavenging assay (table 1). The radical scavenging activities of methanol extract and its fractions increased with concentration. Butanol and chloroform fractions showed significantly higher radical scavenging activityat all concentrations when compared with methanol extract and aqueous fraction ($P>0.05$). At lowest concentration (20 ppm), maximumradical scavenging activity was shown by ascorbic acid (72.15±4.902%) followed by butanol fraction (67.8±2.32%), chloroform fraction (65.18 ±3.87%), methanol extract (41.82±2.68%) and aqueous fraction (37.37±2.24%). The same pattern of antioxidant activity was also observed at higher concentrations i.e. 60 and 100 ppm. Table 1 also shows the concentrations (IC_{50}) of ascorbic acids, methanol extract and its phenolic compounds rich fractions that caused 50 % inhibition of DPPH radical. The IC_{50} values were in the following decreasing order: ascorbic acid (3.07±1.1 ppm) > butanol fraction (6.5±2.1 ppm) >chloroform fraction (7.1±2.5 ppm) > methanol extract (40.83±2.9 ppm) > aqueous fraction (38.2± 2.24%). Lowest IC_{50} value ($IC_{50=}$ 3.0) was shown by ascorbic acid. After ascorbic acid, butanol fraction showed lowest IC_{50} value (6.5±2.1) followed by chloroform fraction (7.1±2.5 ppm), methanol extract (38.2 ±3.2) and aqueous fraction (40.8±2.9). Statistically, ascorbic acid, butanol fraction and chloroform fraction showed homogeneity in radical scavenging activity ($P> 0.05$).

In order to point out a relationship between the IC_{50} values and total phenolic content, the data were subjected to linear regression analysis. Significantly strong correlation (R square=0.95) was found between total phenolic content and IC_{50} values for scavenging DPPH radical (fig. 1). Significantly higher amount ($P<0.05$) of total phenolic content was noted in butanol and chloroform fraction and the same fractions showed significantly higher radical scavenging activity with IC_{50} values of 6.5±2.13 and 7.1±2.5 ppm respectively (table1).

Hepatoprotective activity

During the present study, *P. emodi* rhizome methanol extract and its butanol, chloroform and aqueous fractions which were rich in total phenolic content were also screened for hepatoprotective effects in mice with paracetamol induced hepatotoxicity (table 2). Silymarin was used as a standard hepatoprotective drug during this study. The biochemical indicators of liver injury such as serum levels of ALT, AST and ALP were evaluated.

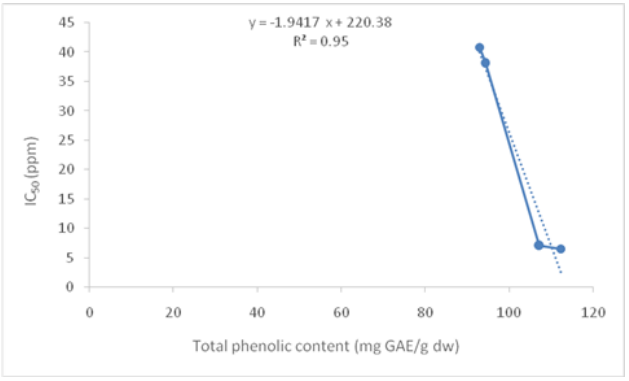

Fig. 1: Correlation between total phenolic content and IC_{50} values for DPPH inhibition in *P. emodi* rhizome methanol extract and its fraction

Serum ALT level: Paracetamol intoxication caused significant increase in the serum ALT level of mice groups when compared with the healthy control mice group (<0.05). Those paracetamol intoxicated animal groups which received Silymarin and plant extracts showed significantly lower serum ALT level when compared to paracetamol control group ($P<0.05$). The mice group treated with butanol fraction showed maximum reduction (60.3%) in serum ALT level followed by the mice groups treated with chloroform fraction (48.8%), methanol extract (45.5%), Silymarin (37.9%) and aqueous fraction (37.4%).

Serum AST level: Paracetamol intoxication caused significant increase in the serum AST level of mice groups when compared with the healthy control mice group (<0.05). The paracetamol intoxicated animal groups that received Silymarin and plant extracts showed significantly lower serum AST level when compared to paracetamol control group ($P<0.05$). The mice group treated with butanol fraction showed maximum reduction (68.9%) in serum AST level followed by the mice groups treated with chloroform fraction (66.3%), methanol extract and aqueous fraction (64.7% each) and Silymarin (58.7%). Silymarin and methanol extract and its fractions showed similar effect on serum AST level ($P>0.05$).

Table 1: Antioxidant activities (% scavenging) of *P. emodi* rhizome methanol extract and its fractions at various concentrations

Samples	Total Phenolic content (mg GAE/g dw)	Concentration (ppm)			IC_{50}
		20	60	100	
Methanol	94.2± 4.4[b]	41.8 ±2.7[b]	53.9±2.1[b]	64.8±3.5[b]	38.2±3.2[a]
Chloroform	107.0±3.5[a]	65.2±3.9[a]	69.9 ±2.3[a]	84.4±2.6[a]	7.1±2.5[b]
Butanol	112.2±5.5[a]	67.9 ±2.3[a]	74.6±2.3[a]	87.8±2.7[a]	6.5+ 2.1[b]
Aqueous	92.9± 2.5[b]	37.4 ±2.2[b]	59.8± 2.2[b]	61.9±6.5[b]	40.8+ 2.9 [a]
Ascorbic acid	----------	72.2±4.9[a]	74.3±4.9[a]	87.6±4.4[a]	3.0±1.1 [b]

Each value represents mean and standard deviation of three replicate (n=3). The alphabetical order is according to decreasing mean value. Values in the same row with different superscript letter are significantly different ($p<0.05$).

Table 2: Effects of *P. emodi* rhizome methanol extract and its phenolic content rich fractions on some liver related serum parameters of paracetamol intoxicated mice

Animal Groups	Liver related serum enzymes		
	ALT	AST	ALP
Normal control	15.25±3.3[e]	8.0 ± 2.58[c]	64.75 ± 5.7[g]
Paracetamol control	203±10[a]	65.33±4.5[a]	216.74±10.5[a]
Silymarin	126.2±5 (-37.9 %)[b]	27.04±5.0 (-58.7 %)[b]	171.53±6.3 (-20.9 %)[b]
Methanol extract	110.67±5.5 (-45.5 %)[b]	23.00±5.4 (-64.7 %)[b]	141.44±7.7 (-34.74 %)[c]
Chloroform fraction	104.3±5 (-48.8 %)[bc]	22.08±3.6 (-66.3 %)[b]	126.33±5.1 (-41.7 %)[cd]
Butanol fraction	80.6±6.5 (-60.3 %)[d]	20.33±2.1 (-68.9 %)[b]	120.75±7.2 (-44.3 %)[de]
Aqueous fraction	127.1± 5.7 (-37.4 %)[b]	23.00±[b]2.6 (-64.7%)	114.32±6.7 (-47.3 %)[def]

Each value represents a mean +standard deviation (n=4). The alphabetical order is according to decreasing mean value. Values in the same column followed by a different letter are significantly different ($p<0.05$). Values in parentheses indicate percent decrease in parameters of silymarin and extracts treated animal groups from paracetamol control animal group

Serum ALP level: The serum level of alkaline phosphatase (ALP) was also studied. Silymarin and each extract caused significant reduction ($P<0.05$) in serum ALP level of paracetamol intoxicated mice group when compared to paracetamol control group. Remarkable reduction in serum ALP level was caused by aqueous fraction (47.25 %) followed by butanol fraction (44.28%), chloroform fraction (41.71%), methanol extract (34.74%) and silymarin (20.85%). The serum ALP level of mice treated with plant extracts was significantly lower than shown by mice group treated with silymarin ($P<0.05$).

DISCUSSION

The present research was conducted to quantify the total phenolic content in *P. emodi* rhizome methanol extract and its fractions and then assess the *in vitro* antioxidant and hepatoprotective activities of fractions rich in phenolic compounds. Maximum quantity of total phenolic content was observed in butanol, chloroform and aqueous fractions. Total phenolic content of plant extracts have been reported (Stankovic, 2011). Plant phenolic compounds are diverse secondary metabolites that occur ubiquitously in plants (Naczk and Shahidi, 2004) and possess antioxidant activity (Nabavi *et al*., 2009a). They are used in food industry to prevent oxidative damage of lipids and maintain or improve the quality of food (Kahkonen *et al*., 1999).

The methanol extract of *P. emodi* rhizome and its phenolic compounds rich fractions i.e. butanol, chloroform and aqueous fractions were assessed for their *in vitro* antioxidant potential on the basis of DPPH radical scavenging assay. DPPH is a stable free radical having violet color which changes to yellow when this radicle is reduced by accepting electron or hydrogen donated by antioxidants. Antioxidants have the potential to donate electron or hydrogen to the free radicals and reduce them and are therefore called radical scavengers (Dehpour *et al.*, 2009). During this research, the methanol extract and its fractions showed strong antioxidant activity. Total phenolic content rich extracts i.e. butanol and chloroform fractions showed remarkable antioxidant activity against DPPH radicle. The free radical scavenging property of medicinal plants is attributed to their possession of phenolic compounds (Nahak and Sahu, 2010). During the current research, there was found significantly strong correlation (R square=0.95) between total phenolic content and IC_{50} values for scavenging DPPH radical (fig. 1). Significantly higher amount ($P<0.05$) of total phenolic content was noted in butanol and chloroform fraction and the same fractions showed significantly higher radical scavenging activity. This indicated that phenolic compounds were the main contributors in scavenging DPPH radical. Phenolic compounds possess antioxidant property due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge

free radicals (Kefalas *et al*., 2003). Plant phenolic compounds are natural antioxidants and have the potential to donate hydrogen atoms to the radical and make them stable (Goupy *et al*., 2003). It has been reported that the antioxidant activity of medicinal plants is positively correlated with total phenolic content (Maizura *et al*., 2011). Khan *et al*. (2005) studied the total phenolic content and radical scavenging activity of *P. emodi* aerial parts ethanol extract and its various solvent fractions. They also reported a strong correlation between total phenolic content and antioxidant activity. The current study differs from the study of Khan *et al*. (2005). They studied *P.* emodi leaves ethanol extract and its fractions but during the present study, *P. emodi* rhizome methanol extract and its fractions were studied.

During the present study, *P. emodi* rhizome methanol extract and its butanol, chloroform and aqueous fractions which were rich in total phenolic content were also screened for hepatoprotective effects in mice with paracetamol induced hepatotoxicity (table 2). The enzymatic activities of serum alkaline phosphatase (ALP) and transaminases such as alanine transaminase (ALT) and aspartate transaminase (AST) were used as biochemical markers of hepatotoxicity. Paracetamol intoxication caused significant (<0.05) increase in the serum levels of AST (>200 U/L), ALT (>60 U/L) and ALP (>200 U/L) when compared with the healthy control. ALT and AST are enzymes, synthesized and localized in hepatic cells that are responsible for catalyzing transamination reactions in the liver. High level of these enzymes in circulation indicates liver damage (Himmerich *et al.,* 2001). ALP is an important group of enzymes, responsible for catalyzing the hydrolysis of phosphate ester (Reichling, 1988). It is mainly synthesized and secreted by liver and bones and in small amount it is also derived from several other tissues including placenta, intestine, kidneys, leukocytes and placenta (Friedman *et al*., 1996). Elevated serum ALP level is frequently associated with liver injury (Wiwanitkit, 2001). Raised levels of liver enzymes such as ALT, AST and ALP in serum of animals with acute paracetamol intoxication have been reported (Thapa and Walia, 2007). Paracetamol intoxication causes oxidative stress that result in the production and building up of lipid hydro peroxides (Kanbur *et al*., 2009). Lipid hydro peroxides damage membranes of hepatocytes that result in the bulk release of ALT, AST and ALP (Yousef *et al*., 2010).

During the present study, the paracetamol intoxicated mice were treated with *P. emodi* rhizome extracts for 5 days. Silymarin was used as a standard hepatoprotective drug during this study. The levels of liver enzymes such as ALT, AST and ALP in serum of paracetamol intoxicated mice treated with extracts and Silymarin were significantly lower as compared to paracetamol intoxicated control group (P<0.05). The extracts showed hepatoprotective activity as evident from the remarkable decrease in the serum levels of ALT, AST and ALP of paracetamol intoxicated mice treated with plant extracts. Silymarin, a standard antioxidant and hepatoprotective drug, decreased the levels of ALT, AST and ALP but not as effectively as the plant extracts in attenuation of paracetamol induced hepatotoxicity. Treatment with plant extracts resulted into the suppression of the leakage of ALT, AST and ALP into blood circulation, suggesting the role of extracts in repairing the hepatic injury and restoring the cellular permeability. The hepatoprotective effects of plants are attributed to the presence of antioxidant constituents including phenolic compounds which have the potential to prevent oxidative degradation of cellular components (Kähkönen *et al*., 1999).

CONCLUSION

From the findings of the present research it was concluded that the chloroform, butanol and aqueous extracts of *P. emodi* rhizome are rich in total phenolic content and possess high potential for scavenging DPPH radical and attenuate hepatic injury in paracetamol intoxicated mice.

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