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Hepatoprotective Effects of Various Solvent Extracted Samples of *Paeonia Emodi* (wall) in Mice with Paracetamol-Induced Hepatotoxicity

Liaqat Zeb (Corresponding Author)

Doctor of Philosophy Student, School of Life Science and Biotechnology,

Dalian University of Technology, Dalian, China. E-mail: Liaqatzeb@dlut.edu.cn

E-mail: Liaqatzeb135@gmail.com

Muhammad Shafiq

Doctor of Philosophy student, School of Life Science and Biotechnology,

Dalian University of Technology, Dalian, China. E-mail: mshafiq.biotech@gmail.com

Asaf Khan

Doctor of Philosophy student, School of Life Science,

Sun Yat-sen University, Guangzhou, China. E-mail: asafkhan07@gmail.com

Ali Akbar

Doctor of Philosophy student, School of Science,

Liaoning University, Shenyang, China. E-mail: aliswatian77@gmail.com

Ishfaq Ahmad

Doctor of Philosophy student, College of Life Sciences,

Capital Normal University, Beijing, China. E-mail: Ishnew147@yahoo.com

Abdul Majid

Doctor of Philosophy student, College of Life Sciences,

Capital Normal University, Beijing, China. E-mail: 4160838003@cnu.edu.cn

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Abstract

The goal of present study is to evaluate and recognize the most effective hepatoprotective solvent fractions of methanolic extract of *Paeonia emodi* by fractionating and assessing its fractions for hepatoprotective effect in paracetamol-induced liver damage in mice. In this study, some liver-related serum parameters such as AST, ALP, and ALT were studied. Maximum reduction was caused by aqueous extract (61.08 %) followed by a butanol extract (60.26 %), chloroform extract (48.76 %), crude extract (45.48 %) and drug (37.93 %), in case of ALT. During the study of AST, chloroform extract caused a maximum reduction (65.94 %), followed by a butanol extract (60.43 %), aqueous extract (57.13 %), crude extract (57.13 %) and drug (43.94 %). During the study of ALP aqueous fraction caused a maximum reduction (47.22 %), followed by a butanol fraction (44.44 %), chloroform (41.51 %), methanol extract (34.72 %) and drug (20.83 %). In all the treatment groups the serum levels of ALT, AST and ALP were significantly lower as compared to those of untreated controlled group ($P<0.05$). The present research showed that *Paeonia emodi* has the potential to reduce paracetamol-induced hepatotoxicity in mice and restore the normal liver function.

Keywords: Hepatoprotective, *Paeonia emodi*, extracts, ALP, AST, ALT

1. Introduction

The liver is the largest internal reddish-brown organ in the human body. A Typical human liver weighs between 1.1 and 1.7 kg. The liver is a highly vascular and dynamic organ. One of its main vascular functions is to act as a blood reservoir. The liver's unique vascular structure serves as a blood filter. Metabolically the liver is responsible for controlling the synthesis, utilization, and storage of carbohydrates, proteins, fats, and vitamins and for detoxifying metabolic waste products and toxins that enter the body (Burkitt et al., 1997). The liver is also responsible for the production and secretion of bile to aid in digestion (bile is salt that emulsifies fats so that they are easier to digest) (Guyton and Hall, 1996). The liver has the unique capability of regeneration; whether injured or a small piece removed, the liver seems capable of unlimited proliferation to meet the demands of its body (Brown, 1997). It has an important target of toxicity to xenobiotic, oxidative stress and toxic chemicals (Patel et al., 2009). Damage to the liver inflicted by hepatotoxic agents is of grave consequences. Liver damage occurs due to exposure to toxic chemicals, excessive use of alcohol, infections such as hepatitis A, B, C, D, E, fats accumulation, cancer, and drugs. Chemicals such as acetaminophen (PCM), tetracycline, ethanol and carbon tetrachloride induce lipid peroxidation, damage the membranes of liver cells and organelles, cause the swelling and necrosis of hepatocytes and result in the release of cytosolic enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) into the blood circulation (Shaaraway et al., 2009).

Alanine transaminase (ALT) is also called glutamate pyruvate transaminase (GPT). This enzyme catalyzes transamination reaction. Transamination is the major process for the removal of nitrogen from amino acids. In most instances the nitrogen is transferred as an amino group from the original amino acid to α-ketoglutarate, forming glutamate, while the original amino acid is converted to its corresponding α-keto acid. For most of these reactions, α-ketoglutarate and glutamate serve as one of the α-keto acid-amino acid pairs. Pyridoxal phosphate acts as a cofactor. Overall these reactions are involved both in the amino acid degradation and in amino acid synthesis.

Ammonia in the form of the amino acid alanine is transported to the liver. Alanine is synthesized in the muscle tissue by a transamination reaction between pyruvate and glutamate. Alanine is then transported through the bloodstream to the liver where it reacts with α-ketoglutarate to form pyruvate and glutamate. The reaction is catalyzed by alanine transaminase (ALT). The nitrogen originating from the glutamate is processed by the urea cycle. When the blood glucose concentration is low, the pyruvate resulting from alanine transamination is used to make glucose via gluconeogenesis pathway. The glucose can be returned to skeletal muscle to supply quick energy. ALT is produced and localized in the hepatic cells and their level is increased in the circulation when the hepatic cells are damaged. In tissues, serum ALT occurs in two locations, the cytosol, and mitochondria (Rej, 1978). Serum ALT is a more specific enzyme for liver damage and therefore a high level of this enzyme indicates liver damage (Himmerich et al., 2001).

Aspartate transaminase (AST) also called glutamic oxaloacetic transaminase (GOT). This is pyridoxal phosphate (PLP)-dependent transaminase enzyme which catalyzes the reversible transfer of an α-amino group between aspartate and glutamate, and interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate (Almo et al., 1994). In healthy human adults, this enzyme is mainly derived from liver, heart, skeletal muscle and in a lesser amount from kidneys, brain, and red blood cells. Aspartate transaminase is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health (Nalpas et al., 1986). Dramatic elevation in serum AST indicates liver damage. Serum AST level also increases due to damage to various other tissues and organs.

Alkaline phosphatases comprise a group of enzymes that catalyze the hydrolysis of a number of phosphate esters, in an alkaline environment, generating an organic radical and inorganic phosphate. Alkaline phosphatase is a membrane-bound metalloenzyme comprising a group of isoenzymes encoded by at least four different gene loci (Afonja and Baron, 1974). In healthy adults, this enzyme is mainly derived from the liver, bones and in a lesser amount from the intestine, placenta, kidneys, and leukocytes (Friedman, 1996). The two major and clinically most relevant isoenzymes in human serum are bone and liver alkaline phosphatases formed through post-translational modifications of the tissue non-specific gene product and they mainly circulate insoluble dimeric forms (Tillyer, 1994).

The primary clinical utility of alkaline phosphatase is in cases of suspected bone disorders and obstructive liver diseases. Raised serum levels are seen in different bone disorders including Paget's disease, osteomalacia, rickets, hyperparathyroidism, osteogenic sarcoma,

fractures and osteoblastic metastases. Increased serum levels are also seen in liver disease associated with extra or intrahepatic obstruction, obstructive jaundice, diabetes, infectious mononucleosis, biliary cirrhosis and cholestasis (Kechrid and Kenouz, 2003).

Many clinically useful drugs can cause cellular damage after metabolic activation to highly reactive compounds. One of the commonly used over-the-counter analgesics is paracetamol (PCM). Paracetamol is a drug of the para-aminophenol group, which is considered quite safe at recommended doses and is commonly used in humans to relieve mild to moderate pain, as well as to reduce fever (Meotti et al., 2006). The main problem with this medication is misuse through intentional or unintentional ingestion of supratherapeutic dosages, which usually lead to hepatic necrosis. When administered at normal doses, PCM is primarily metabolized by conjugation with sulfate and glucuronic acid. A minor pathway through CYP450 has also been reported to yield a highly reactive metabolite, *N*-acetyl-p-benzoquinone imine (NAPQI). This metabolite is generally stabilized through conjugation with glutathione (GSH) and eliminated *via* the kidney. However, when an overdose of PCM is administered, the production of NAPQI overloads the capacity of GSH to detoxify it. The excess NAPQI then causes liver damage associated with oxidative stress. Paracetamol overdose is also known to be associated with inflammation, marked by an increase in the inflammatory cytokines; tumor necrosis-*a* (TNF-*a*), interleukin-1*a* and interleukin-1*b*, as well as the upregulation of nitrogen oxide (NO) from serum, macrophages and hepatocytes (Jaeschke et al., 2003; Ghosh et al., 2010). Oxidative stress is reported to constitute a major mechanism in the pathogenesis of PCM-induced liver and renal damage in experimental animals (Ghosh et al., 2010).

Many plants have been reported for their hepatoprotective activities. These include *Litchi chinensis* Sonn (Basu et al., 2012), *Ardisia solanacea* (Samal, 2013), *Cestrum parqui* (Linn) (Rashed et al., 2013), *Ocimum americanum* (Linn) (Aluko et al., 2012) ,*Urtica* dioica (Linn) (Kataki et al., 2012), *Mentha* arvensis (Linn), *Amorphophallus paeoniifolius* (Hurkadale et al., 2012), *Homalium zeylanicum*, *Vitis vinifera*, *Ficus carica* (Linn), *Nilgirianthus ciliates*, *Gymnosporiaemerginata* and *Marsedeniavolubillis* (Rubab et al., 2013). In Pakistan, there are a wide variety of wild plants which are being used for medicinal and aromatic purposes and many plants have still to be explored for their medicinal values. During the present study, various extracts of *Paeonia emodi* (Wall) were studied for their hepatoprotective effects in mice with paracetamol-induced hepatotoxicity.

Paeonia emodi is a highly medicinal plant that belongs to family Paeoniaceae, locally known as “Mamekh”.It is a perennial herb, 70cm in height, Stem is glabrous, the proximal leaves are two ternate; oblong-elliptic or oblong-lanceolate, both leaf surfaces are glabrous, base cuneate, decurrent and apex acuminate. Petals are generally white and obovate. Seeds are black in color and globose. The flowering time is May and June in the Himalayan range (Haq et al., 2012). It is widely distributed in Northern Himalayan region of Pakistan, India, Nepal, and China (Khan et al., 2005). *P. emodi* has been used for a long time in the traditional medical system. The roots and rhizomes are locally used as the best remedy for a 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.

Various valuable compounds have been isolated from this plant including oleanolic acid, ethyl gallate, methyl Greville, betulinic acid and 1, 5-dihydroxy-3-methylanthraquinone (Nawaz et al., 2000). Other constituents extracted from this plant are; monoterpene glycosides, wording, and benzoylwurdin along with lactoferrin, paeoniflorin, and oxy-paeoniflorin (Muhammad et al., 1999). Emodinol is an important constituent of this plant which is an oleane type triterpene, having substantial glucuronidase inhibitory action. Benzoic acid and 3-hydroxybenzoic acids are also reported in it. Paeonins A and B, monoterpene galactosides isolated from the rhizome are potent chemical compounds with significant lipoxygenase inhibitory activity has also been reported (Riaz et al., 2003).

2. Materials and Methods

2.1 Materials Collection

The rhizome part of the plant *Paeonia emodi* (Wall) was collected in Wari valley of District Upper Dir, Khyber Pukhtoon Khwa Pakistan. Grime and dust were removed, washed with distilled water and shade dried at room temperature, then trailed by crunching to coarse powder exhausting an electronic chopper.

2.2 Extract preparation

During preparation of extract, 450gram of powder was soaked in 2000 mL f 100% methanol. The soaking was done in seven days with occasional shaking. The soaked plant material was filtered through Whatman No. 1 filter paper and placed in a beaker, and the further filtrate was then evaporated through rotary evaporator 44℃. The extract of plant material was obtained in the form of thick paste yielding approximately 170 grams (37.77 w/w) by weight. The extracts were divided into two portions; one portion was used as a crude sample, and other was fractioned with chloroform, n-butanol, n-hexane and ethyl acetate.

2.3 Fractionation

About 80 g of crude extract was dissolved 180 mL distilled water in a beaker. This mixture was introduced into a separating funnel and shaken well. Then 180 ml of n-hexane was added to the separating funnel. The upper layer of n-hexane was separated and concentrated in a rotary evaporator to obtain the n-hexane fraction. This procedure was repeated 3 times by adding new n-hexane to the aqueous phase and n-hexane layer was collected. The sample was collected under condensed pressure using rotary evaporator at 44℃. The similar method was followed for n-butanol, chloroform, and ethyl acetate. The amount and percentage yield (w/w) of these fractions has been shown in Table 1the fractions were stored at 4℃.

Table 1. The yield of Fractions from 80 gram of crude methanol extract (100%) of *P. emodi* rhizomes

Yield	n-Hexane	Chloroform	Ethyl acetate	Butanol	Aqueous
Actual yield (w/w)	6.2 g	23.3 g	10 g	9.3 g	31.6 g
Percentage (%)	7.44	28.2	12.16	11.24	38.08

2.4. Animal used

A total of 28 Swiss albino adult healthy male mice weighing 24-28 grams were purchased from the National Institute of Health, Islamabad. Seven cages were separately arranged, four mice in each cage (5"×9"×11" made of steel mesh). Standard rodent food and water access were all time available to mice. In 12:12 light/dark cycle of photoperiod, mice were maintained. Two weeks before starting experiment they were acclimatized.

2.4.1. Grouping of the used animal

All mice were randomly allocated into seven groups, 4 mice in each group. For the identity of different mice groups, various color tags were used. The detail of the grouping is described as under:

Group 1: served as healthy control and was orally administered with normal saline for 14 days.

Group 2: served as negative control and received PCM (600 mg/kg body weight) three times on day zero followed by normal saline for 14 consecutive days.

Group 3: served as a positive control group received PCM (600 mg/kg b.w, p.o. in 80 uL distilled water) three times on day 0 followed by Silymarin (100 mg/kg b.w, p.o. in 50 uL distilled water) for 13 consecutive days.

Group 4: received PCM (600 mg/kg b.w, p.o. in 80 uL distilled water) three times on day 0 followed by methanol extract (300 mg/kg b.w, p.o. suspended in 70 uL distilled water) for 13 consecutive days.

Group 5: received PCM (600 mg/kg b.w, p.o. in 80 uL distilled water) three times on day 0 followed by chloroform fraction (300 mg/kg b.w, p.o. suspended in 70 uL distilled water) for 13 consecutive days.

Group 6: received PCM (600 mg/kg b.w, p.o. in 80 uL distilled water) three times on day 0 followed by butanol fraction (300 mg/kg b.w, p.o. suspended in 70 uL distilled water) for 13 consecutive days.

Group 7: received PCM (600 mg/kg b.w, p.o. in 80 uL distilled water) three times on day 0

followed by aqueous fraction (300 mg/kg b.w, p.o. suspended in 70 uL distilled water) for 13 consecutive days.

2.4.2. Collection of Blood

On day 13 approximately 1.5 ml of blood samples were obtained from each mice. All mice were starved for 12 hours, reweighed, and anesthetized with sodium pentobarbital 60 mg/kg b.w. Then blood samples from each animal were taken by cervical dislocation.

2.4.3. Isolation of Serum

Blood was allowed to stand at room temperature for 1 hour; serum was separated by centrifugation at 3000 rpm for 10 min (Eppendorf 5702R, Germany). Serum was stored at –20˚C until assayed.

2.5. Assessment of Serum Marker Enzymes

The enzymatic activities of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were used as biochemical markers for hepatotoxicity. The ALT, AST and ALP activities in serum supernatants were determined using commercially available kits (AMP Diagnostics, Austria) on a UV-visible light spectrophotometer (Agilent 8453).

2.5.1. Alanine Transaminase (ALT)

Reagent composition

Reagent R1 contained tris buffer, pH 7.50 (125mmol/L), L-Alanine (680 mmol/L), and LDH (≥ 2000 U/L), while reagent R2 containedα-ketoglutarate (97 mmol/L) and NADH (1.1 mmol/L).

Procedure

Four volumes of R1 were mixed with one volume of R2 to make the working reagent. 20 µl of serum sample was mixed with 200 µl of working reagent, incubated at for 1 min at 37 ℃ in a water bath and the optical density per minute (ΔOD/min) was read at 340 nm. The final concentration was determined by the following formula:

$$\text{Activity (U/L)} = \Delta\text{OD/min} \times 1746$$

2.5.2. Aspartate Transaminase (AST)

Reagent composition

Reagent 1 (R1) contains Tris buffer (pH 7.6) and L.aspartate, Reagent 2 (R2) contains malate dehydrogenase (MDH), Lactate dehydrogenase (LDH), a-ketoglutarate and NADH. The same procedure was followed as mentioned in 2.5.1.

2.5.3. Alkaline Phosphatase (ALP)

Reagent composition

Reagent R1 contained diethanolamine buffer, pH 10.2 (1.25 mol/L) and magnesium chloride (0.625 mmol/L), while reagent R2 contained p-Triphenylphosphate (50 mmol/L). The similar method was employed as mentioned in 2.5.1.

2.6. Statistical Analysis

Results are presented as means + Standard deviation of means (SEM), the means were compared by using Tukey Test of Post Hoc Multiple comparisons in One Way ANOVA. For these analysis computers software, SPSS 16.0 was used.

3. Results

During the present study, the hepatoprotective effects of methanol extract and chloroform, butanol and aqueous fractions of the rhizome of *Paeonia emodi* (Wall) were investigated in mice with Paracetamol-induced hepatotoxicity. The enzymatic activities of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were used as biochemical markers for hepatotoxicity.

3.1. ALT

During the study of alanine transaminase, the drug (Silymarin) and the plant extracts caused a reduction in its serum level. Maximum reduction was caused by the aqueous fraction (61.08 %) followed by butanol fraction (60.26 %), chloroform fraction (48.76 %), crude extract (45.48 %) and drug (37.93 %) (Table 3.1) The effects of *P.emodi* methanol extract and its various solvent fractions on serum ALT were compared statistically. In all the treatment groups the serum level of ALT was significantly lower as compared to untreated controlled group ($P<0.05$). Among the treatment groups, the serum level in aqueous, butanol and chloroform fractions groups was significantly lower as compared to standard group (drug-treated group)($P<0.05$). The standard and crude extract treated groups showed homogeneity in serum ALT level ($P>0.05$). Similarly, chloroform and crude extract, and aqueous and butanol fractions treated groups showed homogeneity in serum ALT level ($P>0.05$) (Table 3.4).

3.2. ALP

During the study of alkaline phosphatase, the drug (Silymarin) and the plant extracts caused a reduction in its serum level. Aqueous fraction caused maximum reduction (47.22 %), followed by butanol fraction (44.44 %), chloroform (41.51 %), methanol extract (34.72 %) and drug (20.83 %) (Table, 3.2) The effects of *P. emodi* methanol extract and its various solvent fractions on serum ALP were compared statistically. In all the treatment groups the serum level of ALP was significantly lower as compared to untreated controlled group ($P<0.05$). Among the treatment groups, the serum ALP level in extracts treated groups was significantly lower as compared to standard group (drug-treated group)($P<0.05$). Methanol extract and chloroform fraction treated groups showed homogeneity in serum ALP level

(P>0.05). Similarly chloroform, butanol and aqueous fractions treated groups showed homogeneity in serum ALP level (P>0.05) (Table 3.4).

3.3. AST

During the study of aspartate transaminase, the drug (Silymarin) and the plant extracts caused a reduction in its serum level. Chloroform fraction caused maximum reduction (65.94 %), followed by butanol fraction (60.43 %), aqueous fraction (57.13 %), crude extract (57.13 %) and drug (43.94 %) (Table 3.3). The effects of *P.emodi* methanol extract and its various solvent fractions on serum AST were compared statistically. In all the treatment groups the serum level of AST was significantly lower as compared to untreated controlled group (P<0.05). Compared to standard group (drug-treated group), other treatment groups chloroform, butanol, aqueous and crude fractions groups significantly lower serum level (P<0.05). The standard and various extract treated groups did not show homogeneity in serum AST level (P<0.05). Chloroform, crude, aqueous and butanol fractions treated groups showed homogeneity in serum AST level (P>0.05) (Table 3.4).

Table 3.1: Effects of two weeks oral administration of various solvent extracts of rhizome part of *P. emodi* on serum ALT level of mice with PCM induced hepatotoxicity

Groups	Mean	SD	95% Confidence interval	
			Lower Bound	Upper Bound
Normal control	15.25	3.3	11.133	19.97
Negative control	203.00	10.000	178.16	227.84
Drug	126.00(-37.93 %)	5.000	113.58	138.42
Crude	110.67(-45.48 %)	5.508	96.99	124.35
Chloroform	104.00(-48.76 %)	5.000	91.58	116.42
Butanol	80.67(-60.26 %)	6.506	64.50	96.83
Aqueous	79.00(-61.08 %)	5.000	66.58	91.42

PCM, Paracetamol; SD, standard deviation

Table 3.2: Effects of two weeks oral administration of various solvent extracts of rhizome part of *P. emodi* on serum ALP level of mice with PCM induced hepatotoxicity

Groups	Mean	SD	95% Confidence interval	
			Lower Bound	Upper Bound
Normal control	64.75	5.7	56.12	74.4
Negative control	216.00	10.000	191.16	240.84
Standard	171.00 (-20.83 %)	6.000	190.10	219.90
Crude	141.00 (-34.72%)	7.000	123.61	158.39
Chloroform	126.33 (-41.51%)	5.132	113.59	139.08
Butanol	120.00 (-44.44%)	7.211	102.09	137.91
Aqueous	114.00 (-47.22%)	6.000	99.10	128.90

LB, Lower bound; UB, Upper bound

Table 3.3: Effects of two weeks of oral administration of various solvent extracts of rhizome part of *P. emodi* on serum AST level of mice with PCM induced hepatotoxicity

Groups	Mean	SD	95% Confidence interval	
			Lower Bound	Upper Bound
Negative control	8.0	2.58	4.91	11.64
Negative control	30.33	4.509	19.13	41.53
Standard	17.00 (-43.94%)	5.000	4.58	29.42
Crude	13.00 (-57.13%)	5.000	.58	25.42
Chloroform	10.33 (-65.94%)	2.082	5.16	15.50
Butanol	12.00 (-60.43%)	.000	12.00	12.00
Aqueous	13.00 (-57.13%)	2.646	6.43	19.57

M, Mean; SED, Standard deviation of means

Table 3.4: Comparison of the hepatoprotective effects of various solvent extracts of rhizome part of *P.emodi* in mice with PCM induced hepatotoxicity

Groups	Liver Enzymes		
	ALT	AST	ALP
Normal control	$15.25^{e}\pm 3.3$	$8.0^{bcd}\pm 2.58$	$64.75^{f}\pm 5.7$
Negative control	$203^{a}\pm 10$	$30.33\pm^{a} 4.509$	$216.00\pm^{a} 10.00$
Standard	$126.0\pm^{b}5$	$17.00\pm^{b}5.00$	$171.00\pm^{b}6.00$
Methanol	$110.67\pm^{bc}5.51$	$13.00\pm^{bc}5.00$	$141.00\pm^{c}7.00$
Chloroform	$104.0\pm^{c}5$	$10.33\pm^{bcd}2.082$	$126.33\pm^{cd}5.132$
Butanol	$80.67\pm^{d}6.50$	$12.00\pm^{bc}0.00$	$120.00\pm^{de}7.211$
Aqueous	$79.0\pm^{d}5.00$	$13.00\pm^{bc}2.646$	$114.00\pm^{de}6.00$

PCM, Paracetamol

4. Discussion

During the present study, hepatotoxicity was induced through oral administration of high dose of paracetamol. Paracetamol intoxicated mice revealed significantly increased AST, ALT and ALP activities (<0.05) when compared with the healthy controls. The sharp increase in serum ALT level is considered to be a significant indicator of PCM-induced acute liver damage (Thapa and Walia, 2007). Alanine transaminase (ALT) is an enzyme specific to liver damage. The present study demonstrated a significant increase in the serum ALT levels of PCM-intoxicated mice, which signifies PCM-induced acute liver damage to have developed. During the present study PCM-intoxication also resulted in increased levels of serum AST and ALP. Serum AST and ALP levels also increase due to damage to various other tissues and organs. Acute PCM intoxication in experimental animals results in elevated levels of serum AST and ALP in addition to ALT levels (Thapa and Walia, 2007).

Paracetamol is a drug of the para-aminophenol group, which is considered quite safe at recommended doses and is commonly used in humans to relieve mild to moderate pain, as well as to reduce fever (Meotti *et al*., 2006). The main problem with this medication is misuse through intentional or unintentional ingestion of supratherapeutic dosages, which usually lead to hepatic necrosis. When administered at normal doses, PCM is primarily metabolized by conjugation with sulfate and glucuronic acid. A minor pathway through CYP450 has been also reported to yield a highly reactive metabolite, *N*-acetyl-p-benzoquinone imine (NAPQI). This metabolite is generally stabilized through conjugation with glutathione (GSH) and eliminated *via* the kidney. However, when an overdose of PCM is administered, the production of NAPQI overloads the capacity of GSH to detoxify it. The excess NAPQI then causes liver damage associated with oxidative stress (Ojo et al., 2006).

In the present study, the hepatic cellular injury might be due to increasing oxidative stress that leads to lipid peroxidation. Lipid hydroperoxides are byproducts of lipid peroxidation and increased levels of lipid peroxidation products are associated with a variety of chemical-induced toxicities including PCM (Choi et al., 2010; Yousef et al., 2010). Lipid peroxidation products are known to cause cellular injury by inactivation of membrane enzymes and receptors, depolymerization of polysaccharide, as well as protein cross-linking and fragmentation (Luqman and Rizvi, 2006). It has been reported that lipid peroxidation products increase in the liver tissues of paracetamol intoxicated mice (Kanbur et al., 2009).

Damage to the membranes of liver cells and organelles, cause the swelling and necrosis of hepatocytes and result in the release of cytosolic enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) into the blood circulation (Madhumitha et al., 2010).

During the present research, the mice with paracetamol-induced hepatotoxicity were treated with a standard antioxidant drug, Silymarin, and various solvent extracts of the rhizome of *P. emodi* for two weeks. During the two week treatment period the drug and plant extracts showed ameliorative activities as evident from the significant reduction in the serum levels of ALT, AST and ALP in drug and extracts treated mice when compared with mice that received PCM 600 mg/kg body weight but not treated with drug or extracts. Silymarin decreased the levels of ALT, AST, and ALP but not as effectively as the plant extracts in attenuation of hepatotoxicity caused by PCM. The significant decrease in the serum levels of ALT, AST and ALP in drug and plant extracts treated animals might be due to decreased leakage from the liver cells. The observed hepatoprotective effect might be a consequence of the amelioration of oxidative stress and maintenance of the antioxidant capacity conferred by the drug and plant extracts. Treatment with drug and extract resulted in the suppression of the leakage of ALT, AST, and ALP into blood circulation. This suggests that the plant extracts possessed the potential of repairing the hepatic injury and restoring the cellular permeability. The extracts were able to reduce the toxic effect of paracetamol in the liver tissues.

The hepatoprotective effects of plants are attributed to the presence of flavonoids, ascorbic acid, carotenoids, phenolic compounds (Hu et al, 1965), Tannins and lignins (Greger and

Hofer, 1980) among the plant constituents. The flavonoids are known to be antioxidant (Faure et al., 1990), free radical scavengers (Bors and Saran, 1987) and anti-lipo per oxidant leading to hepatoprotection (Handa et al., 1986). Similarly ascorbic acid serves as an anti-oxidant (Bus and Gibson, 1984) and consequently can minimize the toxic effect to the liver (Harman, 1985). Moreover, carotenoids are also reputed to be antioxidant and thus showing antihepatotoxic activity. Phenolic compounds are reported to have antioxidant and free radical scavenging potential (Zhou and Zheng, 1991) and can prevent peroxidative damage to biomembranes in hepatocytes (Liu et al., 1992). Furthermore, the hepatoprotective activity of tannins as well as lignans (Faure et al., 1990) is also well documented.

5. Conclusion

Plants and their extracts are well known for a number of chemicals belongs to different classes. The chemicals such as flavonoids, steroids, triterpenoids and their glycosides, alkaloids etc, are well known for their pharmacological importance. The results of the study revealed that the present research concluded that methanol, chloroform, butanol and aqueous extracts of the rhizome part of *Paeonia emodi* have the potential to reduce paracetamol-induced hepatotoxicity in mice and have potential to restore the normal liver function. Current findings of the study authenticated the ethnomedicinal value of the plant *Paeonia emodi* used in the treatment of liver diseases like hepatitis etc. Furthermore, a broad analysis of bioactive components of the fractions is needed to justify the active principle(s), to assess the efficacy and toxicity in various mechanisms of action for producing it as a protective and effective herbal hepatoprotective drug.

Conflict of Interests

The authors have not declared any conflict of interests.

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