ORIGINAL ARTICLE

Evaluation of Hepatoprotective and Antioxidant Activity Of Salvadora Persica

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ABSTRACT

Objective: To investigate hepatoprotective activity of aqueous extract of *Salvadora persica* against paracetamol intoxication in rats and its correlation with antioxidant activity. **Methods:** In vitro lipid peroxidation was determined by Ohkawa H et al., 1979 and hydroxyl radical scavenging activity was determined by deoxyribose degradation method in comparison to standard ascorbic acid. Albino Wister rats of either sex weighing between 150-250 g were divided into five groups. Group 1 received normal saline. Groups 2 to 5 received 2 g/kg paracetamol for 3 days and then groups 3 and 4 received 200 mg/kg and 400 mg/kg of aqueous extract of *Salvadora persica* whereas group 5 received silymarin 100 mg/kg from 4th day to 10 day. On 0th day (one day before the dosing) and 11th day blood was collected by retro orbital puncture. Serum was separated and biochemical parameters measured. **Results:** IC_{50} value of the aqueous extract of *Salvadora persica* was found to be329.67µg and 351.57 µg and 191.63 µg and 184.96 µg for ascorbic acid respectively for lipid peroxidation and hydroxyl radical activity. Administration of aqueous extract of *Salvadora persica* (200 and 400 mg/kg) markedly reduced the biochemical parameters. Average percentage change in these parameters was significantly reduced on 11th day. **Conclusion:**Aqueous extract of *Salvadora persica* possessed significant hepatoprotective activity, in accordance with its in vitro antioxidant activity. The in vitro antioxidant activity further provides support to the hepatoprotective activity and provides insight in understanding the probable mechanism of action of the *Salvadora persica* as hepatoprotective.

Key words: Antioxidant, hepatoprotection, *S. persica*

INTRODUCTION

The liver is the heaviest gland regulating homeostasis within the body. The liver is unceasingly subjected to environmental toxins and harmed by bad drug practices for prescribed and overthecounter drugs which can ultimately lead to various liver disorders like hepatitis and cirrhosis.[1] Treatment choices for common hepatic diseases such as cirrhosis, fatty liver, and chronic hepatitis are easier said than done. The usefulness of treatment such as colchicine, interferon, penicillamine, and corticosteroid are erratic and the occurrences of side effects arenumerous.[2]

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Recent findings show that oxidative stress might be a vital deriving factor in the pathogenesis of liver diseases along with theinadequacy of glutathione. Due to many side effects of allopathic medicine, nowadays the world is returning toward the natural options for diseases treatment.[3]

Salvadora persica ***belonging to the family*** Salvadoraceae, in Arabic language refer to tooth-cleaning stick (Arak or Miswak). For religious and cultural motivations, **Salvadora persica** ***(SP)*** use is strongly established and well-known in Saudi Arabia and most other Muslim countries as an oral hygiene tool.[4]

The **Salvadora persica** is a sizeable much-branched, evergreen shrub or a tree rarely more than one foot in diameter reaching utmost height of three meters.[5] Chemical analysis of **S. persica**has revealed the presence of flavonoids, including kaempferol, quercetin, rutin, and a quercetinglucoside as well as, moderate concentrations of silica, sulfur, and vitamin C; and small quantities of tannins, saponins, and sterols. High amounts of sodium chloride and potassium chloride were also noted.[6] There are various pharmacological activities methodically confirmed on ***Salvadora persica like***antihyperlipidemic activity,[7] effects on fertility,[8] anticancer and antiplatelet activity,[9] antioxidant properties[10] and antibacterial properties.[11] As there is no scientific report about the possible hepatoprotective effect of **Salvadora persica**, even though it possessed antioxidant activity, the present work is undertaken to evaluate the antioxidant and hepatoprotective effect of *Salvadora persica*.

MATERIALS AND METHODS

Plant material

The *Salvadora persica* was purchased from the local market in Abha. *Salvadora persica* was shade dried and grounded in grinder. The aqueous extract is prepared in Soxhlet extractor. The extract was dried in film evaporator and stored in anhydrous condition for further study.

Animals

Albino Wister rats (150–250 g) and mice (25–35 g) were housed under standard conditions of constant temperature and lighting (12 h light/dark cycles). They had free access to standard pellet diet and water *ad libitum*.

In vitro antioxidant activity

i. Determination of lipid peroxidation inhibiting activity by Fe^{2+} /ascorbate system.

Ohkawa H *et al.*[12] method was followed. Naive rat liver tissue weighing 10 g was homogenizedwith a polytron homogenizer in ice-cold Tris-HCl buffer to produce a 25% w/v homogenate. Then it was centrifuged at 4000 rpm for 10 min. An aliquot of supernatant 0.1 ml was mixed with 0.1 ml of the aqueous extract of *S. persica* (Aq. Ext. of SP) at different concentrations and ascorbic acid (AA) followed by addition of 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06 mM) and 0.1 ml of ammonium ferrous sulphate (0.16 mM) and were incubated for one hour at 37 °C. The reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20% acetic acid (pH 3.5). The total volume was then made up to 4 ml by adding distilled water and kept in an oil bath at 100 °C for 1 hour. After the mixture had been cooled, 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substance (TBARS) was measured at 532 nm. A control was prepared using 0.1 ml of respective vehicle in the place of Aq. Ext. of SP/ ascorbic acid.

ii. Determination of hydroxyl radical scavenging activity bydeoxyribose degradation method[13]

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the plants material for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (fenton reaction). Fenton reaction mixture consisting of 200 µl of 10 mM ferrous sulphate ($FeSO_4.7H_2O$), 200 µl of 10 mM EDTA and 200 µl of 10 mM 2-deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200 ml of Aq. Ext. of SP. Thereafter, 200 µl of 10 mM H_2O_2 was added before the incubation at 37 °C for 4 hour. Then 1 ml of this Fenton reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20% acetic acid.

The total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100 °C for 1 hour. After the mixture had been cooled, 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and then

absorbance of the organic layer containing the thiobarbituric acid reactive substance was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in place of Aq. Ext. of SP/ ascorbic acid.

Calculation of percentage inhibition

The percentage inhibition was calculated using the formula

$$Percent\ inhibition = \frac{Average\ of\ the\ control\ OD - Average\ of\ test\ sample\ OD}{Average\ of\ the\ Control\ OD} x100$$

Calculation of 50% inhibition concentration

The optical density (OD) obtained with each concentration of the plants material and ascorbic acid was plotted on a graph taking concentration on X-axis and the percentage of inhibition on Y-axis. The graph was extrapolated to find the concentration needed for 50% inhibition.

Acute toxicity studies

According to Organization for Economic Co-operation and Development (OECD) drafted guideline No. 423, the study was conducted on three female albino mice weighing between 25-35 g. They were fasted overnight and maintained with water *ad libitum*. The Aq. Ext. of SP was administered at a dose level of 2000 mg/kg body weight.The animals were observed for 24 h for any sign of toxicity and lethality.

Hepatoprotective activity of aqueous extract of *Salvadora Persica* on paracetamol induced hepatotoxicity in rats[14]

Wister rats of either sex weighing between 150-250 g were used for the study. The rats were housed under standard conditions of constant temperature and lighting (12 hours light/dark cycle). They had access to standard pellet diet and water *ad libitum*. The rats were selected and divided into 5 groups each containing six rats. Paracetamol (PCM), Aq. Ext. of SP and silymarin were dissolved in 2% gum acacia suspension. The treatment protocol is summarized and given below.

Group 1: Normal control, 2% w/v gum acacia suspension orally, 1 ml/kg once daily for 10 days

Group 2: Paracetamol as toxicant 2 g/kg orally once daily for 3 days followed by 1 ml/kg 2% gum acacia suspension from 4th day to 10th day.

Group 3: PCM 2 g/kg orally for 3 days followed by Aq. Ext. of SP 200 mg/kg orally from 4thday to 10th day

Group 4: PCM 2 g/kg orally for 3 days followed by Aq. Ext. of SP 400 mg/kg orally from 4th day to 10th day

Group 5: PCM 2 g/kg orally for 3 days followed by silymarin 100 mg/kg orally from 4th day to 10th day

On 0th day (one day before the dosing) and 11th day blood was collected by retro orbital puncture from all the animals. Serum was separated by centrifugation (3000 rpm for 15 min.) and subjected for estimation of biochemical parameters like SGPT, SGOT, ALP, Total Bilirubin and Direct Bilirubin.

RESULTS

Inhibition of lipid peroxidation

The Aq. Ext. of SP and ascorbic acid at different concentrations (12.5-400 µg) inhibited the lipid peroxidation in a dose dependent manner. The amount needed for 50% inhibition (IC_{50}) of lipid peroxide was found to be 329.57 µg and 191.63 µg, respectively.

Hydroxyl radical scavenging activity

The Aq. Ext. of SP and ascorbic acid at different concentrations (12.5-400 µg) scavenged the hydroxyl radical in a dose dependent manner. The amount needed for 50% inhibition(IC_{50}) of hydroxyl radicals was found to be 351.57µg and 184.96 µg, respectively.

Acute toxicity studies

Oral administration of the Aq. Ext. of SP at a dose of 2000 mg/kg body weight in the form of suspension did not show any toxic signs during the observation period of 24 hr. in all the tested mice. The Aq. Ext. of SP was safe at 2000 mg/kg body weight and 1/10th and 1/5th (200 mg/kg and 400 mg/kg) of this cut off dose have been selected for further *in vivo* studies.

Hepatoprotective activity of aqueous extract of *Salvadora persica* on paracetamol- induced hepatotoxicity in rats

The dose of 2g/kg body weight of paracetamol induced significant increase in serum glutamate pyruvate transaminase (SGPT) levels with an increase of 269.91% compared to normal control where the increase was2.06 %. The decrease in SGPT levels in animals treated with Aq. Ext. of SP at a dose of 200 and 400 mg/kg showed 190.23%

decrease and 131.13% decrease, respectively. Whereas silymarin 100 mg/kg showed 19.96% decrease. The dose of 2g/kg body weight of paracetamol induced significant increase inserum glutamate oxaloacetate transaminase(SGOT) levels with an increase of 458.01% compared to normal control where the increase was 1.14%. The decrease in SGOT levels in animals treated with Aq. Ext. of SP at a dose of 200 and 400 mg/kg showed 315.11% decrease and 124.45% decrease, respectively. Whereas silymarin 100 mg/kg showed 15.69% decrease.The dose of 2g/kg body weight of paracetamol induced significant increase in serum alkaline phosphatase(ALP)levels with an increase of 228.46% compared to normal control where the increase was 0.29%. The decrease in serum ALP levels in animals treated with Aq. Ext. of SP at a dose of 200 and 400 mg/kg showed 153.72% decrease and 81.15% decrease, respectively. Whereas silymarin 100 mg/kg showed 11.85% decrease. The dose of 2g/kg body weight of paracetamol induced significant increase in serum total bilirubin levels with an increase of 185.71% comparedto normal control where the increase was 1.16%. The decrease in total bilirubin levels in animals treated with Aq. Ext. of SP at a dose of 200 and 400 mg/kg showed 166.25% (non-significant compared to group 2) and 114.28% decrease, respectively. Whereas silymarin 100 mg/kg showed 21.42% decrease. The dose of 2g/kg body weight of paracetamol induced significant increase in serum direct bilirubin levels with an increase of 205.55% compared to normal control where the increase was 0.0%. The rise in direct bilirubin levels in animals treated with Aq. Ext. of SP at a dose of 200 and 400 mg/kg showed 70.00% decrease and 22.22% decrease, respectively. Whereas silymarin 100 mg/kg showed 5.26% decrease.

Table 1: Basel levels of selected biochemical parameters in rats for PCM induced hepatotoxicity on 0th day[a]

Groups	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
1	2% Gum acacia (1 ml/kg; p.o.)	38.24 ± 3.60	88.18 ± 3.66	206.5 ± 4.696	0.86 ± 0.07	0.18 ± 0.018
2	PCM (2 g/kg; p.o.)	39.55 ± 2.97	88.17 ± 4.02	208.3 ± 11.14	0.70 ± 0.03	0.18 ± 0.016
3	PCM + Aq. Ext. of SP (2 g/kg; p.o. + 200 mg/kg; p.o)	43.09 ± 2.53	89.18 ± 5.53	201.2 ± 6.892	0.80 ± 0.05	0.20 ± 0.019
4	PCM + Aq. Ext. of SP (2 g/kg; p.o. + 400 mg/kg; p.o)	39.38 ± 4.66	89.29 ± 5.77	204.3 ± 11.36	0.70 ± 0.05	0.18 ± 0.018
5	PCM + Silymarin (2 g/kg; p.o. + 100 mg/kg; p.o)	50.15 ± 4.67	94.39 ± 5.80	207.5 ± 11.68	0.70 ± 0.05	0.19 ± 0.013

[a]Values are the mean ± S.E.M. of six rats/treatment

Table 2: Influence of Aq. Ext. SP on biochemical parameters in rats for PCM induced hepatotoxicity on 11th day[a]

Groups	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
1	2% Gum acacia (1 ml/kg; p.o.)	39.03 ± 4.57	89.19±5.97	207.1±9.81	0.87 ± 0.03	0.18 ± 0.02
2	PCM (2 g/kg; p.o.)	146.3±16.70	492.0± 43.97	684.2±35.27	2.00 ± 0.05	0.55 ± 0.04
3	PCM + Aq. Ext. of SP (2 g/kg; p.o. + 200 mg/kg; p.o)	125.1±14.51^{NS}	370.2± 22.51**	510.5±32.46***	2.13 ± 0.13^{NS}	0.34 ± 0.08*
4	PCM + Aq. Ext. of SP (2 g/kg; p.o. + 400 mg/kg; p.o)	91.02±5.91**	200.5±5.27***	370.1±26.38***	1.50 ± 0.16**	0.22 ±0.03***
5	PCM + Silymarin (2 g/kg; p.o. + 100 mg/kg; p.o)	60.16±4.92***	109.2±3.27***	232.1±16.09***	0.85±0.08***	0.20 ±0.02***

[a] Values are the mean ± S.E.M. of six rats/treatment; compared to PCM treated group

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

Table 3: Average % change in selected biochemical parameters in PCM induced hepatotoxicity from day 0 to day 11

Groups	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
1	2% Gum acacia (1 ml/kg; p.o.)	2.06	1.14	0.29	1.162	00.00
2	PCM (2 g/kg; p.o.)	269.91	458.01	228.46	185.71	205.55
3	PCM + Aq. Ext. SP (2 g/kg; p.o. + 200 mg/kg; p.o)	190.32	315.11	153.72	166.25	70.00
4	PCM + Aq. Ext. SP (2 mg/kg; p.o. + 400 mg/kg; p.o)	131.13	124.54	81.15	114.28	22.22
5	PCM + Silymarin (2 g/kg; p.o. + 100 mg/kg; p.o)	19.96	15.69	11.85	21.42	5.26

DISCUSSION

Oxidative stress in the liver leads to hepatocellular injury, stellate cell activation and on-going fibrosis in Hepatitis C Virus (HCV) infection, non-alcoholic fatty liver disease (NAFLD), drug- induced liver disease and genetic hemochromatosis.[15] Although human antioxidant defense is equipped with enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase, the exogenous hydrophilic scavengers like urate, ascorbate, glutathione and flavonoids and the lipophilic radical scavengers such as tocopherols, carotenoids and ubiquinol are crucial for full protection. The defence also encompasses enzymes involved in the reduction of oxidized forms of molecular antioxidants like glutathione reductase and dehydroascorbate reductase. Some of these agents synthesized by cell itself; however, the majority including ascorbic acid, lipoic acid, polyphenols and carotenoids are derived from dietary sources. In disease conditions, the defence against reactive oxygen species (ROS) is weakened or damaged and the oxidant load increases. In such circumstances, external source of antioxidants is indispensable to countervail the detrimental consequences of oxidative stress.[16]

In recent years, antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases.[17]

In the present study the standard ascorbic acid showed 50% inhibitory concentration (IC_{50}) at 191.63µg for lipid peroxidation whereas the Aq. Ext. of SP showed IC_{50} values at 329.67µg for lipid peroxidation. The standard ascorbic acid showed 184.96µg IC_{50} value for hydroxyl radical scavenging activity whereas the Aq. Ext. of SP showed IC_{50} values 351.57 µgfor hydroxyl radical scavenging activity.

The observed antioxidant activity of the Aq. Ext. of SP might be due to the presence of active constituents like flavonoids (like quercetin and rutin), vitamin C and tannins.

Since reactive oxygen species are involved in stress and stress related disorders, *Salvadora Persica*may be beneficial in preventing the initiation or progression of such disorders. Liver disorders like cirrhosis, jaundice etc. being stress related disorders,[18] *Salvadora Persica* by virtue of their antioxidant effect might prevent initiation and progression of such disorders. Hepatotoxicity produced by paracetamol is due to the free radical generation; hence *Salvadora Persica*with antioxidant activity can be used for their hepatoprotective activity.

Drug-induced injury is the second main cause of acute liver failure and predominates in much of the developed world.[19] More than 56,000 emergency visits and nearly 500 deaths in the United States each year result from paracetamol toxicity, owing to either deliberate or unintentional overdoses.[20] In the USA, paracetamol is the commonest cause of acute liver failure and incidences seem to be increasing[21] and in other countries also the scenario is not different.

Paracetamol (N-acetyl-p-aminophenol) is a commonly used analgesic and antipyretic drug and is

innocuous when used in therapeutic doses. At lower doses, about 80% of ingested paracetamol is eliminated mainly as sulfate and glucuronide conjugates before oxidation and only 5% is oxidized by hepatic cytochrome P450 (CYP2E1) to a highly reactive and toxic electrophile i.e. N-acetyl-p-benzoquineimine (NAPQI), which is detoxified by glutathione (GSH) but higher doses of paracetamol result in the liver GSH pool depletion and the reactive intermediate reacts with other nucleophilic centres of vital molecules in liver cells leading consequently to hepatotoxicity.[22]

An evident sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbances caused in the transport functions of hepatocytes. When liver cell plasma is damaged, a variety of enzymes like SGOT, SGPT, ALP, and Bilirubin located normally in cytosol are released into the blood, thereby causing increased enzymes levels in the serum. The estimation of enzymes in the serum is a valuable measureable marker of the magnitude and type of hepatocellular damage.[23]

The main aim of any medication in the treatment of liver disorders is to block degeneration of hepatocytes and accompanying metabolic abnormalities and stimulate the regeneration of hepatic cells. In the present study the hepatoprotective activity of the aqueous extract of *Salvadora persica* isevaluated in paracetamol-induced liver toxicity by estimating the above mentioned biochemical parameters. Acute administration of paracetamol caused marked elevation of the serum levels of the selected parameters in treated rats (Group 2) compared to that of the control group (Group 1). Treatment with the *Salvadora Persica*at 200 mg/kg and 400 mg/kg doses produced dose dependent reduction in PCM-induced rise of the parameters. Silymarin 100 mg/kg bd. wt. significantly prevented such rise.

The cellular necrosis or membrane damage releases the enzymes into circulation and hence can be measured in serum. Elevated levels of serum enzymes are indicative of cellular leakage and loss of operational integrity of cell membrane in liver.[24] SGPT is more specific to the liver damage and hence a better parameter for determining the liver injury. High levels of SGOT indicate liver damage, cardiac infarction and muscle injury. The serum ALPis related to liver and bone while bilirubin on the other hand is related to liver and blood. Increase in the serum levels of ALP is due to increased synthesis in the presence of increasing biliary pressure.[25] The rises in bilirubin indicate liver damage or increased haemolysis.[26] The rise in SGPT indicated that paracetamol treatment increased the specific toxicity on hepatocytes in the study. The greater rise in SGOT indicated that it increased the toxicity due to nonhepatic damage also. Additionally the rise in ALP and bilirubin also indicate the damage of liver and other tissues.

The above changes due to PCM were reduced due to the treatment with the aqueous extract of *Salvadora Persica*in a dose dependent manner while silymarin at a dose of 100 mg/kg bd. wt. as a standard drug produced maximum response. The protective effect of silymarin was well established in several models of hepatotoxicity and was reported to be due to its antioxidant and membrane stabilizing activities.[27]

CONCLUSION

Salvadora persica is daily used in Kingdom of Saudi Arabia as a tooth brush. Along with the saliva the extract which came out during brushing is generally swallowed or spitted, hence in our study we have selected only the aqueous extract of*Salvadora Persica*. The combined activity of inhibiting lipid peroxidation and free radical scavenging activity of *Salvadora Persica*might be responsible for the hepatoprotective activity against paracetamol-induced hepatotoxicity.
The presence of active constituents in *Salvadora persica* like flavonoids (quercetin and rutin), Vit. C, tannins etc. might be responsible for their antioxidant activity and the same might be the reason for their hepatoprotective activity against PCM- induced hepatotoxicity in rats.

CONFLICT OF INTEREST

There is no conflict of interests

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