

EFFECTS OF FRACTIONS OF *MELIA AZEDARACH* (L.) FRUIT EXTRACTS ON SOME BIOCHEMICAL PARAMETERS IN RABBITS

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Abstract- This study aimed to investigate the effects of n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions of the methanolic extract of *Melia azedarach* fruits on serum glucose, lipid profile, GPT, ALP and creatinine of normal rabbits. Each fraction at a dose of 50 mg/kg body weight was orally administered to normal rabbits for 40 days and the serum biochemical parameters were estimated on days 20 and 40 after treatment. All of the extracts significantly decreased serum glucose, cholesterol, triglycerides and LDL concentrations, however, their administration caused elevation of serum HDL levels. All except the aqueous extract, caused a significant rise in the serum levels of GPT, ALP and creatinine. The present study demonstrates that all of the extracts possess hypoglycemic, hypolipidemic and HDL boosting properties. Of the tested extracts, only the aqueous fraction was found safe, as it caused no significant alterations in the serum levels of GPT, ALP and creatinine.

Key words: Glucose; lipid profile; GPT; ALP; creatinine

INTRODUCTION

Medicinal plants possess a great potential for the treatment of several human diseases. Interest in phytomedicine research has increased during the last decades and many medicinal plants have been screened for their pharmacological activities (Kunwar et al., 2009). An adequate standard of herbal preparations for the treatment of various ailments is highly desired. Therefore, various extracts of medicinal plants must be evaluated for their *in vivo* pharmacological and toxicological effects and active biochemicals.

A variety of wild plants is exploited in Pakistan for their medicinal and aromatic purposes. The characteristic properties and proper uses of many of these

medicinal plants are traditionally well known, however many others have yet to be explored for their therapeutic values (Ahmad and Husain, 2008). *Melia azedarach* Linn. is a medicinal plant commonly known as “Persian Lilac”, “akain” or “think”. It grows in the wild throughout the Sub-Himalayan belt, and is cultivated in India and Pakistan for both ornamental and medicinal purposes (Watt and Brandwijk, 1962). It is a perennial tree that belongs to the family Meliaceae. Its English name is “China berry” and it is locally known as “Tora Shandi” in the Swat and Malakand regions of Khyber Pukhtunkhwa, Pakistan.

Various preparations of *M. azedarach* are used for the treatment of several diseases (Watt and Brandwijk, 1962; Baquar, 1989). The powder of dried

fruits of this plant was shown to be effective in diabetes (Ahmad et al., 2009). There are also some reports about *M. azedarach* toxicity and poisoning. Ferreir et al. (2010) reported *M. azedarach* fruit poisoning in dog. Del Mendez et al. (2002) reported toxicity of the fruits of *M. azedarach* in calves and established its adverse effects on the kidney and liver. Other toxicological studies of *M. azedarach* fruits, leaves and bark revealed poisoning in human (Phua et al., 2008). The toxicity of this plant is a common problem because of its universal and perennial occurrence. More important still is that many plants, although quite harmless to man and animals under one set of conditions, can prove to be toxic and noxious under other circumstances (Kiat, 1969).

The residents in certain areas of the Malakand division, Khyber Pakhtunkhwa, Pakistan, use the powder of dry fruits of *M. azedarach* to cure stomach problems, fever and diabetes. The present study aimed to fractionate the extract of *M. azedarach* fruit using various solvents and to test the effects of administration of different fractions on several biochemical parameters in rabbits.

MATERIALS AND METHODS

Melia azedarach fruits were collected from the campus of Malakand University, Dir Lower, Khyber Pakhtunkhwa, Pakistan in August 2010, and were identified and authenticated by Mehboob-ur-Rahman, Assistant professor, Department of Botany, Government Postgraduate Jahanzeb College, Swat, Pakistan. The voucher specimen of the fruit (H.UOM.BG. 215) has been retained in the herbarium of the University of Malakand.

Extraction and fractionation

The fruits were cleaned, dried in the shade and coarsely ground. The powdered material (2.3 kg) was soaked in 7.5 l of 50% methanol for 3 days with occasional shaking at 28°C. The soaked material was filtered through Whatman No.1 filter paper. The solvent was evaporated with the help of a rotary evaporator (Heidolph Laborta 4000) under reduced pressure,

and a partially dried gummy extract was removed and fully dried in a water bath at 40°C; finally, a reddish-brown crude extract was obtained. The weight of the extract was 503 g (21.86 % w/w). The methanolic extract was fractionated into n-hexane, ethyl acetate, chloroform, n-butanol and soluble residual aqueous fractions using a separating funnel. Each fraction was concentrated using a rotary evaporator at 40° C under reduced pressure. Finally, dry masses of n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions were obtained and stored at 4°C until future use. The weight of the dry mass of the *M. azedarach* n-hexane fraction was 18.6 g (7.4%), chloroform 70.5 g (28.2%), ethyl acetate 30.4 g (12.16 %), n-butanol 28.1 g (11.24%) and aqueous fraction was 95.2 g (38.08 %).

Animals

Healthy domestic 5-6 month-old male rabbits (*Oryctolagus cuniculus*), weighing 1 200-1 350 g and were purchased from a local market. They were housed for acclimation in wide and well-ventilated chambers for three months in the Animal House at the University of Malakand, Khyber Pakhtunkhwa, Pakistan. The rabbits were fed on green vegetables and chow pellets and allowed tap water *ad libitum*.

Animal grouping and experiments

The experiments were conducted on 48 rabbits divided into six groups, with eight rabbits in each group. These groups included a control group that was treated with 2 ml of normal saline, an n-hexane group treated with n-hexane fraction, a chloroform group treated with the chloroform fraction, an ethyl acetate group treated with the ethyl acetate fraction, a butanol group treated with the butanol fraction, and an aqueous group that was treated with the aqueous fraction. Each day, each fraction (50 mg/kg rabbit body weight) was suspended in 2 ml normal saline and administered orally using an esophageal catheter. The duration of dosing was 40 days. Four rabbits from each group were killed on day 20, and 4 on day 40 after 16 h of fasting (Naito et al., 1986). During fasting, the animals had free access to water. The ani-

mals were killed with a sharp blade after anesthetizing with ether, and blood samples were collected. An animal ethical committee clearance certificate was issued by the University of Malakand Animal Ethics Committee.

Isolation of serum and biochemical analysis

Blood from each animal was collected in a centrifuge tube and allowed to clot. Serum samples were obtained by centrifugation of clotted blood samples at 3 000 rpm for 5 min. Serum biochemical parameters such as glucose, cholesterol, triglycerides, low density lipoproteins, high density lipoproteins, GPT, ALP and creatinine were analyzed using commercially available kits using the Blood Chemistry Analyzer 4000 Italy. The data were compared using Duncan's Multiple Range Test and Co-Stat V.64 software.

RESULTS

Serum glucose

The effects of 20 and 40 days of oral administration of different solvent fractions of *M. azedarach* fruit extract on the serum glucose of rabbits are shown in Tables 1-2. After 20 days, all fractions except the ethyl acetate fraction caused a significant reduction in the serum glucose level as compared to the control group ($P < 0.05$). The percent decreases in serum glucose level caused by the n-hexane fraction was 17.26%, 26.98% by the chloroform fraction, 2.063% by the ethyl acetate fraction, 10.88% by the butanol fraction, and 8.95 % by the aqueous fraction. The chloroform fraction was found to be more hypoglycemic compared to the other fractions ($P < 0.05$).

After 40 days of treatment, all extracts significantly decreased the serum glucose concentration as compared to the control ($P < 0.05$). Uniform hypoglycemic activities were observed. Decreasing percentage decrease in serum glucose concentration was as follows: ethyl acetate fraction (40%), aqueous fraction (39.40%), butanol fraction (38.82%), n-hexane fraction (37.25%), chloroform fraction (33.52%).

Serum lipid profile

The effects of 20 and 40 days of oral administration of different solvent fractions of the extract of *M. azedarach* fruit on serum cholesterol, TG, LDL and HDL in rabbits are shown in Tables 1-2.

Cholesterol

The level of serum cholesterol was significantly reduced ($P < 0.05$) in each fraction-treated group after 20 days. The order of percentage decrease in serum cholesterol was: ethyl acetate fraction (45.72%), n-hexane fraction (45.3%), chloroform fraction (36.32%), butanol fraction (17.95%), aqueous fraction (17.52%). Similarly, a significant decrease in serum cholesterol levels was observed in all groups on day 40. The maximum percentage decrease was obtained after treatment with the n-hexane fraction (27.09%), followed by the aqueous fraction (25.09%), butanol fraction (24.30%), ethyl acetate fraction (22.70%) and chloroform fraction (18.32%).

Triglycerides (TG)

All the fractions caused a significant decrease in serum triglyceride concentration on days 20 and 40. After 20 days of administration, the maximum percentage decrease (58.58%) was observed in the ethyl acetate fraction group, while the minimum decrease (31.90%) was recorded in the n-hexane fraction-treated group. After 40 days of exposure, the maximum percentage decrease (37.56%) in serum triglyceride level was observed in the chloroform fraction group, and a minimal decrease (22.65%) in the aqueous fraction group.

Low-density lipoproteins (LDL)

Serum LDL levels were significantly reduced in all of the treatment groups ($P < 0.05$) on days 20 and 40 of administration. The percentage reduction in serum LDL level after 20 days was in the following order: butanol fraction (58.62%), aqueous fraction (57.05%), ethyl acetate fraction (51.72%), chloroform fraction (49.84%), n-hexane fraction (34.48%). After

Table 1. Serum glucose and lipid profile of rabbits after 20 days exposure to various fractions of *M. azedarach* fruit methanol extract.

Groups	Serum Biochemical Parameters (mg/dl)				
	Glucose	Cholesterol	Triglyceride	Low density lipoproteins	High density lipoproteins
Normal Control	133.3±5.3 ^a	58.5±1.9 ^a	81.5±5.7 ^a	79.7 ±3.2 ^a	15.0 ± 0.8 ^c
n-Hexane fraction	110.3±1.1 ^b (-17.26%)	32.0±1.1 ^d (-45.3%)	55.5±1.3 ^b (-31.9%)	52.3±2.7 ^b (-34.48%)	24.3±0.8 ^a (+61.6%)
Chloroform fraction	97.3±2.2 ^c (-26.98%)	37.25±0.8 ^c (-36.32%)	40.75±2.3 ^{cd} (-50%)	40.0±1.8 ^c (-49.84%)	26.0±1.6 ^a (+73.33%)
Ethyl acetate fraction	130.5±2.7 ^a (-2.063%)	31.75±0.8 ^d (-45.72%)	33.75±1.7 ^d (-58.58%)	38.50±2.1 ^c (-51.72%)	20.50±1.3 ^b (+36.66%)
Butanol fraction	118.75±1.7 ^b (-10.88%)	48.0±2.3 ^b (-17.95%)	47.75±2.4 ^{bc} (-41.41%)	33.0±2.2 ^c (-58.62%)	17.75±1.3 ^{bc} (+18.33%)
Aqueous fraction	108±4.8 ^b (-18.97%)	48.3±2.2 ^b (-17.52%)	36.0±5.8 ^d (-55.83%)	34.3±2.8 ^c (-57.05%)	14.75±1.1 ^c (-1.66%)

The values are means ± SD (n=4). The alphabetical order is according to decreasing mean values. Means sharing no letter are significantly different at P < 0.05. Any two means sharing a letter are not significantly different at P < 0.05. Values in parentheses indicate percent increase (+%) or decrease (-%) from the normal control group.

Table 2. Serum glucose and lipid profile of rabbits after 40 days exposure to various fractions of *M. azedarach* fruit methanol extract.

Groups	Serum Biochemical Parameters (mg/dl) on day 40				
	Glucose	Cholesterol	Triglyceride	Low density lipoproteins	High density lipoproteins
Normal Control	127.50±2.1 ^a	62.75±2.1 ^a	92.50±1.8 ^a	90.50±2.5 ^a	20.50±0.6 ^d
n-Hexane fraction	80.0±1.9 ^b (-37.25%)	45.75±2.1 ^b (-27.09%)	59.75±1.8 ^c (-35.41%)	47.0±2.2 ^b (-48.06%)	22.75±1.3 ^{cd} (+10.97%)
Chloroform fraction	84.75±2.4 ^b (-33.52%)	51.25±2.4 ^b (-18.32%)	58.50±1.3 ^c (-37.56%)	53.0±1.9 ^b (-41.43%)	31.50±1.7 ^{ab} (+53.65%)
Ethyl acetate fraction	76.50±2.6 ^b (-40%)	48.50±2.5 ^b (-22.70%)	58.0±1.7 ^c (-35.91%)	51.25±1.7 ^b (-43.37%)	35.0±1.6 ^a (+70.73%)
Butanol fraction	78.0±4 ^b (-38.82%)	47.50±1.2 ^b (-24.30%)	59.25±1.5 ^c (-34.53%)	47.75±2.1 ^b (-47.23%)	29.0±2.6 ^b (+41.46%)
Aqueous fraction	77.0±2.4 ^b (-39.60%)	47.0±2.6 ^b (-25.09%)	70.0±2.4 ^b (-22.65%)	48.25±1.1 ^b (-46.68%)	27.0±1.1 ^{bc} (+31.70%)

The values are means ± SD (n=4). The alphabetical order is according to decreasing mean values. Means sharing no letter are significantly different at P < 0.05. Any two means sharing a letter are not significantly different at P < 0.05. Values in parentheses indicate percent increase (+%) or decrease (-%) from the normal control group.

40 days, the maximum reduction in serum LDL level was caused by the n-hexane fraction (48%), followed by the butanol (47.32%), aqueous (46.68%), ethyl acetate fractions (43.37%) and chloroform fractions (41.43%).

High-density lipoproteins (HDL)

On day 20, the HDL level was significantly elevated in the groups that received the chloroform (73.33%), n-hexane (61.66%) and ethyl acetate fractions

Table 3. Serum GPT and ALP levels after 20 and 40 days exposure to various fractions of *M. azedarach* fruit methanol extract.

Groups	Serum GPT and ALP (U/L)			
	GPT on day 20	GPT on day 40	ALP on day 20	ALP on day 40
Normal Control	29.7±1.03 ^c	32.5 ± 1.5 ^d	47.5±1.3 ^d	45.7±1.7 ^d
n-Hexane fraction	164.5±1.5 ^a (+452.94%)	169.0±5.4 ^a (+420%)	219.0±5.08 ^a (+361.05%)	223.3±0.6 ^a (+387.98%)
Chloroform fraction	93.3±2.1 ^c (+213.44%)	79.7±2.5 ^c (+145.38%)	146.7±3.5 ^b (+208.94%)	67.0±3.7 ^c (+46.45%)
Ethyl acetate fraction	119.5±2.5 ^b (+301.68%)	97.7±3.7 ^c (+200.77%)	123.0±3.2 ^c (+158.94%)	75.0±1.7 ^c (+63.93%)
Butanol fraction	74.5±2.7 ^d (+150.42%)	121.5±2.3 ^b (+273.84%)	113.3±1.9 ^c (+138.42%)	86.5±2.2 ^b (+89.07%)
Aqueous fraction	34.7±1.7 ^e (+16.80%)	39.2±1.3 ^d (+20.74%)	50.7±3.3 ^d (+6.84%)	45.5±2.5 ^d (+0.54%)

The values are means ± SD (n=4). The alphabetical order is according to decreasing mean values. Means sharing no letter are significantly different at $P < 0.05$. Any two means sharing a letter are not significantly different at $P < 0.05$. Values in parentheses indicate percent increase (+%) from the normal control group.

(36.66%). After 40 days, all the fractions except the n-hexane fraction showed a significant elevation in serum HDL level when compared to the control ($P < 0.05$).

Atherogenic indices (LDL/HDL ratios)

The atherogenic indices (LDL/HDL ratios) of rabbits calculated on days 20 and 40 of fraction administration are shown in Fig 1. On day 20, all of the treated groups showed a significant decrease in the LDL/HDL ratio as compared to the control group ($P > 0.05$). The maximum decrease was observed in the chloroform-treated group (70.77%). The significant decrease in LDL/HDL ratios of the fraction-treated groups was observed on day 40 of administration. At this time, the maximum decrease in LDL/HDL ratio was observed in the ethyl acetate fraction-treated group (66.90%).

Serum GPT

The effects of 20 and 40 days of oral administration of different solvent fractions on serum GPT are shown in Table 3. After 20 days, all fractions except the aqueous fraction, caused a significant increase

in the serum GPT level. The serum GPT level of the aqueous fraction-treated group was also elevated but the difference compared to the control group was insignificant ($P > 0.05$). The same effects were observed when the serum GPT concentration was estimated on day 40 of exposure. The percent increase in serum GPT level after 20 days was in the following order: n-hexane fraction (420%), butanol fraction (273.84%), ethyl acetate fraction (200.77%), chloroform fraction (145.38%), aqueous fraction (20.74%).

Serum ALP

The effects of 20 and 40 days of oral administration of various solvent fractions of fruits of *M. azedarach* on serum ALP are shown in Table 3. After 20 days, the levels of serum ALP were significantly elevated ($P < 0.05$) in all experimental groups except in the aqueous fraction-treated group. Among all of the tested fractions, the n-hexane fraction caused a remarkable elevation in serum ALP level (219.52 U/L). There was no significant change in the level of ALP in the aqueous group (50.47±3.3 U/L) when compared to the control group (47.50±1.3 U/L). After 40 days, the n-hexane fraction caused further elevation in

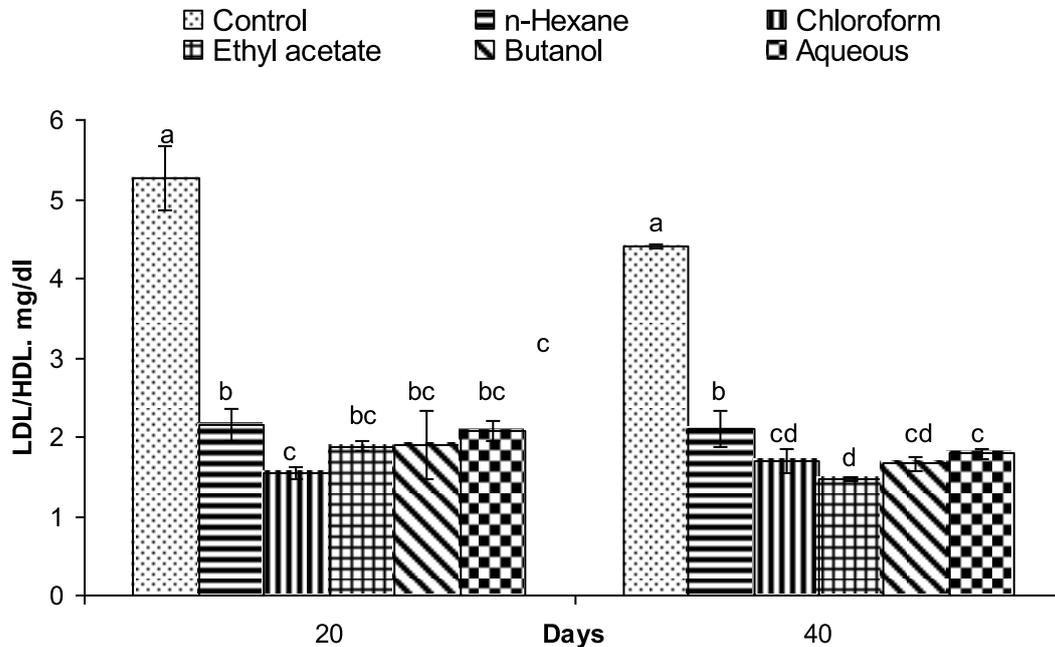


Fig. 1. Serum atherogenic indices (LDL/HDL ratios) of normal rabbits calculated on day 20 and 40 of exposure to various fractions of *M. azedarach* fruit methanol extract. The alphabetical order is according to decreasing mean values. Means sharing no letter are significantly different at $P < 0.05$. Any two means sharing a letter are not significantly different at $P < 0.05$.

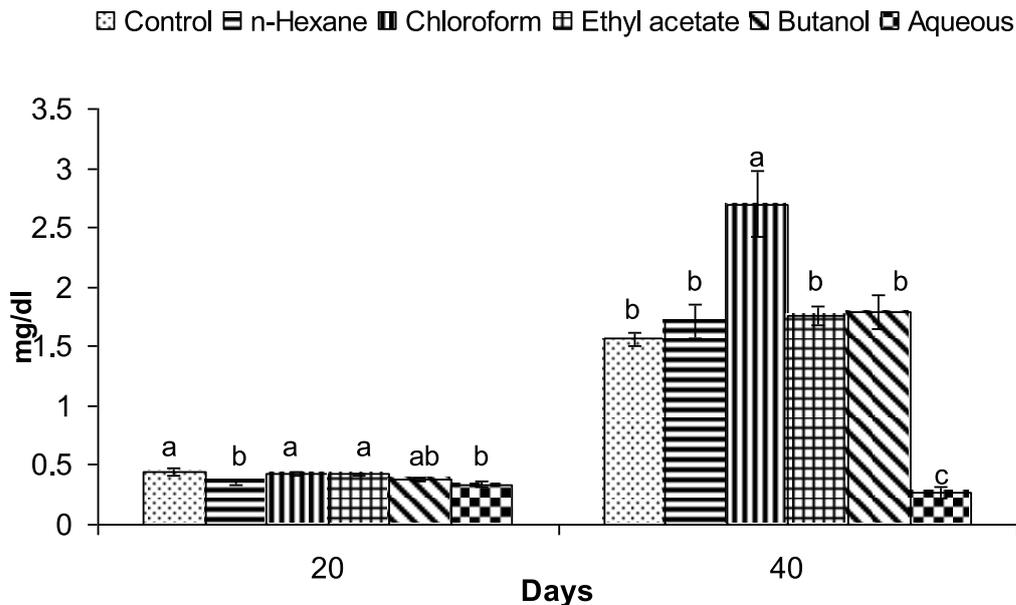


Fig. 2. Serum creatinine levels of normal rabbits after 20 and 40 days exposure to various fractions of *M. azedarach* fruit methanol extract. The alphabetical order is according to decreasing mean values. Means sharing no letter are significantly different at $P < 0.05$. Any two means sharing a letter are not significantly different at $P < 0.05$.

ALP activity. In the chloroform, ethyl acetate and butanol fraction-treated groups, the levels of ALP decreased but were still higher when compared to the control group ($P < 0.05$). The aqueous fraction caused no significant change in the level of serum ALP with respect to the control group ($P < 0.05$).

Serum creatinine

Fig. 2 shows the effects of the administration of different fractions of the methanol extract on the serum creatinine level in rabbits. After 20 days of administration, the serum creatinine level was significantly reduced in animals that received aqueous and n-hexane fractions as compared to the control group ($P < 0.05$). The remaining fractions caused no significant change in serum creatinine level. After 40 days, the aqueous fraction caused a significant reduction (37.74%; $P < 0.05$) in serum creatinine level as compared to the control group. At this time, the remaining fractions caused an increase in the serum creatinine level.

DISCUSSION

The present study demonstrated that most of the fractions of methanol extract of *M. azedarach* fruits caused significant reduction in serum glucose concentration. Previous studies revealed that sulphonylureas increase the release of insulin that results in the reduction of blood glucose level (Miura et al., 2001; Okine et al., 2005). The hypoglycemic effects induced by fractions after continuous oral administration in normal rabbits may be due to the increased release of insulin (Andrew, 2000). The hypoglycemic activity of plant extracts through insulin-release stimulatory effects have been reported (Gupta et al., 2005).

During the study of the lipid profile, all the fractions caused significant reduction in the serum levels of cholesterol, triglyceride and LDL of rabbits. Plant extracts possess various therapeutic ingredients, including tannins, saponins, flavonoids and alkaloids. Flavonoids have been reported for their effects on blood LDL and HDL levels in humans (Weggemans and Trautwein, 2003). Saponin has a resin-like ac-

tion, thereby reducing the enterohepatic circulation of bile acids (Topping et al., 1980); therefore, the conversion of cholesterol to bile acid is enhanced, resulting in low blood cholesterol levels (Potter et al., 1979). Phytosterols reduce cholesterol absorption from the intestine (Ikeda and Sugano, 1998). Saponins are known for lowering the blood triglyceride level by inhibiting pancreatic lipase activity (Han et al., 2002). Elevated levels of serum triglycerides have been correlated with the development of atherosclerosis and coronary heart diseases (Gotto Jr., 1998). In the present study, all the fractions exhibited a significant reduction in the serum level of LDL. Phyto-components are believed to be involved in enhancing the hepatic LDL-receptor levels, increasing hepatic uptake of LDL-cholesterol and facilitating its catabolism to bile acids (Fukushima et al., 2001; Venkatesan et al., 2003). A high level of blood low-density lipoproteins (LDL) is highly associated with an increased risk of cardiovascular diseases (Castro et al., 2009).

The examined fractions caused a concomitant increase in serum HDL concentration in rabbits. A significant decrease occurred in the atherogenic indices (LDL/HDL ratios) of the rabbits when calculated on days 20 and 40 of fraction administration. The LDL/HDL ratio has been directly correlated with the incidence of cardiovascular diseases. Lahoz et al. (2003) suggested that the increase in HDL levels after treatment might be due to the induction of apoA1 production. The major apolipoprotein of almost all HDLs is apoA1, which promotes cholesterol efflux from tissues to the liver for excretion (Dastani et al., 2006). Almost 30% of blood cholesterol is carried in the form of HDL and is responsible for transporting cholesterol from peripheral tissues to the liver for its metabolism and excretion (Kwiterovich, 2000). There are reports about the involvement of apoA1 in the removal of cholesterol from the sites of atherosclerotic lesions and in the prevention of plaque formation on the arterial lining (Von Eckardstein et al., 2001; Chiesa and Sirtori, 2003). HDL has also been reported to be involved in the preservation of vascular endothelial function, inhibition of platelet activation, anticoagulation, profibrinolytic activities, and

in the protection of LDL from oxidation (Nofer et al., 2002).

The effects of fractions of the fruit extract of *M. azedarach* on the level of serum glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) in rabbits were also evaluated. All of the fractions, except the aqueous fraction, caused a significant increase in the levels of serum GPT and ALP as compared to the control group. Glutamate pyruvate transaminase (GPT), which catalyzes transamination reaction, is produced and localized in the hepatic cells and its level is increased in the circulation when the hepatic cells are damaged (Himmerich et al., 2001). Alkaline phosphatase (ALP) comprises a group of enzymes that catalyze the hydrolysis of phosphate ester in an alkaline environment, generating an organic radical and inorganic phosphate (Reichling and Kaplan, 1988). In healthy adults, this enzyme is mainly derived from the liver, bones and in lesser amount from the intestine, placenta, kidneys and leukocytes (Friedman et al., 1996). An increase in serum ALP level is frequently associated with a variety of diseases such as extrahepatic biliary obstruction, intrahepatic cholestasis, infiltrative liver disease and hepatitis (Wiwanitkit, 2001). Elevated levels of serum GPT and ALP in the rabbit groups that received organic solvent fractions indicates liver damage. Low activities of these enzymes in the aqueous fraction-treated group suggest an intact liver.

The aqueous fraction caused a significant reduction in serum creatinine concentration. All other fractions caused an increase in the serum level of creatinine on day 40 of administration. A significant increase was due to the chloroform fraction. The increase caused by the other fractions was small and insignificant when compared to the control group. Our study showed that all the fractions, except the aqueous fraction, could be potentially nephrotoxic, especially when the duration of use is extended. Urea and creatinine are the waste products of protein metabolism that need to be excreted by the kidneys; therefore, a marked increase in serum urea and creatinine levels are indications of functional damage to the kidney (Panda, 1999). The urea level can be

increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney; thus, kidney damage is the significant factor that increases the serum creatinine level (Cheesbrough, 1988). Del Mendez et al. (2002) also reported the adverse effects of *M. azedarach* fruits on the kidney. In the present study, the normal or decreased level of serum creatinine in the group that received the aqueous fraction do not indicate the likelihood of the kidney being under extreme stress, suggesting positive or no adverse effects on the kidney.

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