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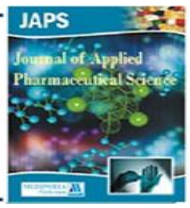

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Hepatoprotective activity of *Melia azedarach* leaf extract against simvastatin induced Hepatotoxicity in rats

A. Srinivasa Rao, Mohammed Fazil Ahmed and Mohammed Ibrahim

A. Srinivasa Rao
Bhaskar Pharmacy College, Yeknapally, Moinabad (Mandal), R.R (Dist), Hyderabad- 500075.

Mohammed Fazil Ahmed, Mohammed Ibrahim
Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Nalgonda, (AP), IND IA-508284.

For Correspondence
A. Srinivasa Rao
Bhaskar Pharmacy College, Yeknapally, Moinabad (Mandal), R.R (Dist), Hyderabad- 500075.

ABSTRACT

The aim of the study is to investigate the hepatoprotective activity of *Melia azedarach* L leaves extracts against simvastatin induced hepatotoxicity. The phytochemical screening was carried on the leaves extracts of *Melia azedarach* revealed the presence of some active ingredients such as Alkaloids, Tannins, Sponginess, Phenols, glycosides, steroids, terpenoids and flavonoids. Leaves of *Melia azedarach* was successively extracted with ethanol against simvastatin (20mg/kg.p.o) induced hepatotoxicity using Standard drug Silymarin (25 mg/kg). There was a significant changes in biochemical parameters (increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP),serum bilirubin and decrease the total proteins content.) in simvastatin treated rats, which were restored towards normalization in *Melia azedarach* (300 mg/kg and 500 mg/kg) treated animals. Thus the present study ascertains that the leaf extract of *Melia azedarach* possesses significant hepatoprotective activity.

Keywords: *Melia azedarach*, hepatoprotective activity, simvastatin, ethanol and Silymarin.

INTRODUCTION

In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward *et al.,* 1999). The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang *et al.,* 1992). Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders (Ross *et al.,* 1996). The disorders associated with the liver are also numerous and varied (Wolf P *et al.,* 1999). More than 900 drugs have been implicated in causing liver injury (Friedman *et al.,* 2003) and it is the most common reason for a drug to be withdrawn from the market. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Friedman *et al.,* 2006; Ostapowicz *et al.,* 2002).

Simvastatin hepatotoxicity is hypothesized to occur due to drug-drug interactions (Ricaurte *et al.,* 2006; Kanathur *et al.,* 2001). Simvastatin (Lipid Lowering Agent) competitively inhibits HMG-Co A (3-hydroxy-3 methylglutaryl coenzyme A) to mevalonate. Mevalonate is also a precursor of Coenzyme Q10 (CoQ10). Thus, treatment with statins could also lower its levels. CoQ10 acts as an antioxidant, has membrane stabilising effects, and is important for cellular mitochondrial respiration, which is essential for energy production in organs (Frei *et al.,* 1990; Stocker *et al*., 1991**).** Thus, simvastatin causes oxidative stress mediated hepatotoxicity by depleting antioxidant enzymes (Vaghasiya et *al.,* 2008). *Melia azedarach* linn (meliaceae; Neem) is an indigenous plant possessing several medicinal properties. Melia azedarach linn (synonym: Melia dubia Cav, Indian lilac, Persian lilac) belonging to the family Meliaceae is a tree found in India. It is popular as Indian lilac. Different phytochemicals present in leaf, root and stem, are meliacarpins, limonoids, sendanins, trichilins and azedarachins (Wealth of India Vol-IV (L.M) Page no.323).The plant is traditionally used for the treatment of leprosy, (inflammations, Analgesics and cardiac disorders. Its fruits extracts possess ovicidal Corpinella *et al*., 2006) and larvicidal activity (Wandscheer *et al.,* 2004).The leafs extracts also possess antiviral(Descalzo *et al.,*1989) and antifertility activity(Choudhary *et al*.,1990).The main objective of this study was to assess the hepatoprotective effect of *Melia azedarach linn,* in simvastatin induced hepatotoxicity

MATERIALS and METHOD

Plant materials

The basic plant material of *Melia azedarach Linn* used for the investigation was obtained from Mount Opera Garden, Near Ramoji Film City, and Nalgonda Dist. The plant can be identified authenticated by Department of Botany, research office (Botanist), Anwar-ul- loom College of Pharmacy, Hyderabad.

Preparation of ethanolic extract

The leaves were collected and shadow dried. The shade leaves were subjected to pulverization to get coarse powder. The coarsely powder leaves of *Melia azedarach* were used for extraction. The shade dry coarsely leaves of *Melia azedarach* were used for extraction with ethanol. *Melia azedarach* leaf powder (250 g) was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.

Phytochemical investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in ethanol extracts (Trease *et al.,* 2002; Kokate *et al*., 1990; Khandelwal *et al*., 2006)

Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of NIZAM INSTITUTE OF PHARMACY, Deshmukhi, Ramoji film city, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

Acute toxicity study

Melia azedarach in the dose range of 110 mg-630 mg/kg were administered orally to different group of mice comprising of ten mice in each group. Mortality was observed after 72 h. Acute toxicity was determined according to the method of (Litchfield and Wilcoxon 1949).

Experimental design for hepatoprotective activity (Vaghasiya *et al.,* 2009)

Animals are divided into 5 groups, each comprising 6 rats.

Group I : Normal control (saline)
Group II: simvastatin (20mg/kg.p.o)
Group III: Simvastatin (20mg/kg.p.o) + *Melia azedarach* leaf extract (300mg/kg, p.o)
Group IV: Simvastatin (20mg/kg.p.o)+*Melia azedarach* leaf extract (500mg/kg, p.o)
Group V: Simvastatin (20mg/kg.p.o) +Silymarin (25mg/kg, p.o)

Animals were divided into five different groups, each having 6 rats and treated accordingly. Group 1: rats fed with a normal standard diet for 30 days. Group II rats receives Simvastatin (SMT) (20mg/kg.p.o alone for 30 days). Group III and IV rats receive SMT along with *Melia azedarach* leaf extracts(300mg/kg and 500mg/kg.p.o respectively for 30days) and Group V rats receive SMT along with silymarin (20 mg/kg/p.o.for 30 days). On the 31st day, all the animals were sacrificed by mild ether anesthesia.

Blood biochemistry

Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT (Reitman *et al.,* 1957), ALP (Walter *et al.,* 1974.) and bilirubin (Malloy *et al.,* 1937) by standard method. Serum total protein was measured according to the method of Lowry *et al*., 1951.

Estimation of OxidativeStress Markers

All the animals were euthanized after blood collection with the spinal dislocation method under light ether anesthesia and

the liver was removed for study of oxidative stress markers like Superoxide dismutase (SOD)(Moron *et al.*, 1979) Catalase (CAT)(Takahara *et al.*, 1960) , Glutathione peroxidase (GPX)(Necheles *et al.*, 1968) and Glutathione S transferase (GST)(Habig *et al.*, 1974) were assayed.

Histopathology

Histopathology of liver was carried out by a modified Luna (Luna 1999). In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 h. Then the livers were paraffin embedded and 5 µ thickness microtone sections were made (Krajian 1963).The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light micro-scope for any histological damage/protection.

Statistical analysis

The data are represented as mean ± S.E.M. Students't-test is used for statistical analysis of blood serum parameters and for statistical analysis of liver enzymes

RESULTS

The acute oral toxicity study of *Melia azedarach* showed no mortality upto 610 mg/kg. The phytochemical screening of *Melia azedarach* shows the presence of Alkaloids, Carbohydrates, Steroids, Tannins, Flavonoids and Glycosides (Table 1).The effect of ethanol extract of *Melia azedarach* an serum transaminases, alkaline phosphates, bilirubin and total protein level in Simvastatin intoxicated rats are summarized in Table 2. There was a significant increase in bilirubin levels, SGOT, SGPT and ALP, in Simvastatin intoxicated group compared to the normal control group. The total protein levels were significantly decreased to 3.31g/dl in Simvastatin intoxicated rats from the level of 6.46 g/dl in normal group. On the other hand the groups with received both *Melia azedarach* leaf extract (300mg/kg, and 500mg/kg,) + Simvastatin (20mg/kg.p.o) (Group III & IV) and Simvastatin (20mg/kg.p.o) +Silymarin (25mg/kg, p.o) (Group V) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 2).

Table. 1: Preliminary Phytochemical Screening.

Sl. No	Constituents	Ethanol Extract
1	Alkaloids	+
2	Steroids	+
3	Tannins	+
4	Phenols	+
5	Flavonoids	+
6	Glycosides	+
7	Saponins	+
8	Terpenes	+
9	Reducing Sugar	-
10	Anthraquinone	+

The effect of *Melia azedarach* on GPx, GST, SOD and Catalase activity is shown in Table 3. It showed that GPx, GST, SOD and Catalase activity were significantly decrease in Simvastatin -intoxicated rats when compared with those animals in normal control group On the other hand the groups with received both *Melia azedarach* leaf extracts (300mg/kg, and 500mg/kg,) and Simvastatin(20mg/kg.p.o) (Group III & IV)**,** the values of above enzymatic parameters were near normal compared to Group I animals and were significantly different from their Simvastatin(20mg/kg.p.o) treated control group(Group II). The results are well compared with Silymarin standard drug treated group (Group V).

Table 2. Effect of various groups on some serum chemical parameters.

Groups	SGPT levels(U/L)	SGOT levels(U/L)	ALP levels(U/L)	Direct bilirubin levels(mg/dl)	Total bilirubin(mg/dl)	Total protein(g/dl)
Group I	34.32±0.75	36.89±2.30	71.45 ± 0.22	0.19±0.09	0.40±0.02	6.46±0.02
Group II	126.9±1.50	179.95 ±1.350	172.68±0.64	0.93±0.08	1.96±0.12	3.31±0.08
Group III	83.2±0.27*	123.56±0.750*	133.0±1.63*	0.70±0.01*	0.68±0.08*	4.08±0.15*
Group IV	46.6±0.55**	68.63±0.82**	98.0±1.24**	0.32±0.20**	0.56±0.01**	6.12±0.15**
Group V	36.98±2.74***	38.67±1.25***	72.6±1.04***	0.20±0.02***	0.42±0.02***	6.50±0.12***

Values are mean ± SEM (n=6).
Where, * represents significant at <0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001. All values are compared with toxicant

Table 3. Effect of various groups on antioxidant enzymes in liver.

Groups	SOD	CAT	GST	GPX
Group I	9.24± 0.23	135.34 ± 10.2	0.36 ± 0.039	7.345± 0.56
Group II	5.32± 0.38	82.72 ± 8.8	0.22 ± 0.028	4.824± 0.2
Group I11	6.02± 0.22**	90.86± 6.7**	0.24 ± 0.024**	5.262± 0.4**
Group IV	7.86± 0.26***	122.4± 2.4***	0.28± 0.052***	6.84± 0.34***
Group V	8.98± 0.68 ***	132.12 ± 11.2 ***	0.348 ± 0.036 ***	7.28± 0.37***

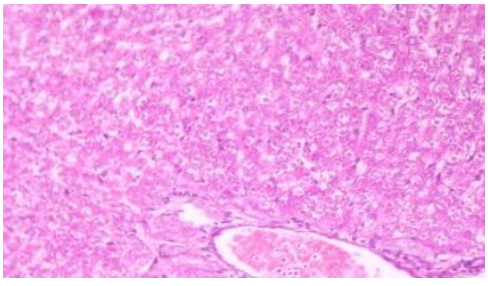

Fig. 1: Section of Liver Of Control Group.

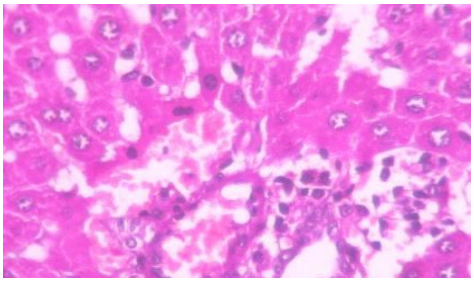

Fig 2: Section of The Liver of Simvastatin Treated Group.

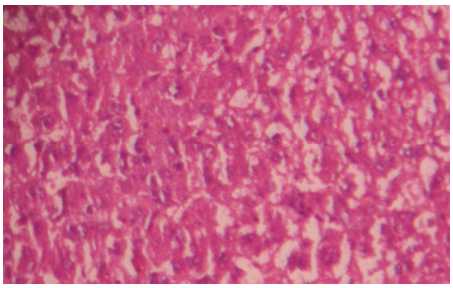

Fig. 3: Section of Liver of Simvastatin and Extract (300mg/Kg) Treated Group.

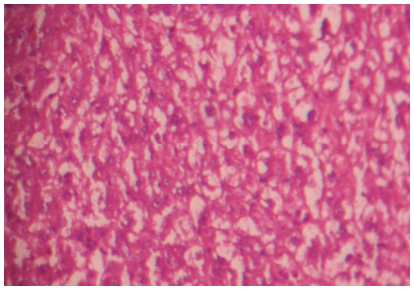

Fig. 4: Section of Liver of Simvastatin and Extract (500mg/Kg) Treated.

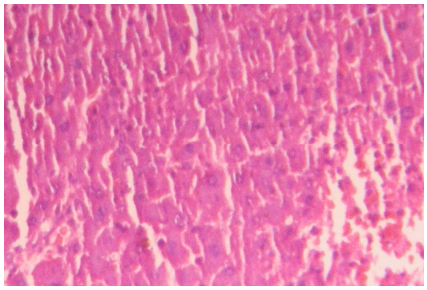

Fig. 5: Section of Liver of Simvastatin and Silymarin Group.

DISCUSSION

The liver can be injured by many chemicals and drugs (Leo *et al.,* 1982). During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration (Deb 1998).This decrease in elevated serum levels followed by simvastatin-treated animals in part may be due to the protective effect of *Melia azedarach* leaf extracts on liver cells following the restoration of liver cell membrane permeability (Kalab *et al.,* 1997). This protective effect indicates a reduction in enzymes present in the extra cellular milieu of the liver cell. The protective effect of the component of PHF has also been observed in several experimental studies (Sandhir *et al.,* 1999; Mathur *et al.,* 1994).

In the previous study, it was reported that simvastatin caused oxidative stress mediated hepatotoxicity (Vaghasiya *et al.,* 2008,). The protection of liver cells against toxic materials including drugs, lipid peroxidation, and free radical injury may decrease inflammation (Yang *et al.,* 2000). It is reported that phenols are responsible for the variation in the antioxidant activity of the plant (Cai *et al.,* 2004). They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pitchaon *et al.,* 2007; Pokorny *et al.,* 2001). Phenolic compounds are considered to be the most important antioxidative components of herbs and other plant materials, and a good correlation between the concentrations of plant phenolic and the total antioxidant capacities has been reported(Madsen *et al.,* 1998; Pellegrini *et al.,* 2000) .

Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in simvastatin control group. Ethanolic extracts of *Melia azedarach* (300 mg/kg and 500mg/kg, p.o) prevented these histological changes, further indicating their hepatoprotective activity. Although there is insufficient information to establish the mechanism of action of *Melia azedarach* protection, this could be due to its anti-oxidative of phenols.

CONCLUSION

In conclusion, the results of present study demonstrate that *Melia azedarach* leaf extracts (300 mg/kg and 500mg/kg,) has potent hepatoprotective activity against simvastatin induced liver damage in rats. The results also imply that the hepatoprotective effects of *Melia azedarach* may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituents responsible for hepataprotective effect.

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