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

A. Srinivasa Rao, Mohammed Fazil Ahmed and Mohammed Ibrahim

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 posses 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have been implicated in causing liver injury (Friedman et al., 2003) and it is the most common reason for a drug to be withdrawn from the market.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 (Ricaure 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 meliacarps, 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 (1991) and antifertility activity (Choudhary et al., 2006) and larvicidal activity (Wandscheer et al., 2004). The leaves 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 I: 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 31th 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 Oxidative Stress 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) (Nechele 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 by a bovine solution for 6 h. Then the livers were paraffin embedded and 5 μ thickness microtome 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 microscope 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 up to 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* on 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 3.31 g/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±0.35** | 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 III | 6.02±0.22** | 90.86±6.7** | 0.24±0.02** | 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.
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 (Pitchon 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 500 mg/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 500 mg/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.

ACKNOWLEDGMENTS

My sincere thanks to Dr. A Srinavasa Rao, Principal, Bhaskar Pharmacy College, Hyderabad and Dr. Mohammed Ibrahim, Principal, Nizam Institute of Pharmacy, Hyderabad, for rendering their suggestions and helping me in each and every step of completing this research work successfully.

REFERENCES


Kakate, C.K., Purohit, A.P. &Gokhale, S.B. Pharmacognosy, Nirali Prakashan, Pune, (1990) 120.


