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Phytochemical evaluation and hepatoprotective activity of *Catharanthus rosea* against simvastatin-induced hepatotoxicity in rats

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ABSTRACT

Background: The main aim of the present study is to evaluate the hepatoprotective activity of ethanolic extract of *Catharanthus rosea* L. leaves against simvastatin-induced hepatotoxicity in rats.
Material and methods: The phytochemical study was also carried out on the ethanolic extract of *Catharanthus rosea* leaves. Hepatotoxicity was induced by simvastatin (20 mg/kg p.o. for 30 days) in Wistar rats. The hepatoprotective effect was evaluated by extract of *Catharanthus rosea* leaves (300 mg/kg and 500 mg/kg for 30 days).
Results: The observation of the phytochemical investigations includes the presence of alkaloids, tannins, saponins, glycosides, phenols, and flavonoids. There was significant changes in biochemical parameters e.g. increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP), serum bilirubin and decrease the total protein contents in *Catharanthus rosea* (300 mg/kg and 500 mg/kg) treated animals. The results were comparable to standard hepatoprotective drug Silymarin (25 mg/kg).
Conclusion: Thus the present study ascertains that the ethanolic extract of *Catharanthus rosea* leaves possesses significant hepatoprotective activity.

INTRODUCTION

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). Within the human body, millions of chemical reactions are occurring constantly which require oxygen. Reactive oxygen spices (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O_2^-) and hydroxyl radicals (OH^-), as well as non-free-radicals species such as hydrogen peroxide (H_2O_2) (Halliwell et al., 1995; Squadriato et al., 1998). They tend to attack the healthy cells, DNA as well as proteins and fats, causing them to deteriorate. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen specious, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has linked to cancer, ageing, atherosclerosis, and ischemia injury, inflammation and neurodegenerative diseases like parkinsonism and alzheimer (Donald et al., 1987).

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market (Friedman et al., 2003). Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Friedman et al., 2003; Ostapowicz et al., 2002). Simvastatin causes oxidative stress mediated hepatotoxicity by depleting antioxidant enzymes (Vaghasiya et al., 2008). 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 stabilizing effects, and is important for cellular mitochondrial respiration, which is essential for energy production in organs (Frei et al., 1990; Stocker et al., 1991).

Catharanthus rosea, which is commonly known as 'periwinkle' or '*Vinca rosea*' belongs to family Apocynaceae and is an important source of indole alkaloids, which are present in all plant parts. The physiologically important and antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine, and reserpine are reported to be present in the roots (Mishra et al., 2001). Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia (Farnsworth et al., 1968; Svoboda et al., 1975). Crude decoction of (200 mg and 1 g herb/ml water) *Catharanthus* showed a moderate anti-angiogenesis effect in vitro (Wang *et al* 2004). Numerous animal studies have shown that ethanolic extracts of leaves and flowers of *Catharanthus* lower blood glucose levels (Ghosh and Gupta, 1980; Chattopadhyay et al., 1991, 1992). The main objective of this study was to assess the hepatoprotective effect of *Catharanthus rosea* in simvastatin-induced hepatotoxicity.

MATERIAL AND METHODS

Plant collection and identification

The plant material *Catharanthus rosea or Vinca rosea* L. obtained from Mount Opera Garden, Near Ramoji Film City, Nalgonda Dist., AP, India. The plant was identified and authenticated by Research Office (Botanist), Department of Botany, Anwar-ul-uloom College of Pharmacy, Hyderabad, India.

Extraction

The leaves were dried under shade and powdered in a mechanical grinder. The powdered material (250 gms) was extracted successively in ethanol using Soxhlet apparatus at 55 °C for 18 h. The extracts was concentrated in vacuo and kept in a vacuum dessicator for complete removal of solvent and weighed.

Phytochemical investigation

The phytochemical studies of *Catharanthus rosea* or *Vinca rosea* L. leaves were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in extracts (Trease et al., 1978; 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, India. 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 experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional Animal Ethical Committee; 1330/ac/10/CPCSEA).

Experimental design for hepatoprotective activity

Animals were divided into five groups, each comprising 6 rats.

Group I :	Normal control (saline, p.o.)
Group II:	Simvastatin (20 mg/kg, p.o.)
Group III:	Simvastatin (20 mg/kg, p.o.) + *Catharanthus rosea* extract (300 mg/kg, p.o.)
Group IV:	Simvastatin (20 mg/kg, p.o.) + *Catharanthus rosea* extract (500 mg/kg, p.o.)
Group V:	Simvastatin (20 mg/kg p.o.) + Silymarin (25 mg/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 received Simvastatin (SMT) (20 mg/kg, p.o. alone for 30 days). Group III and IV rats received SMT along with *Catharanthus rosea* extracts (300 mg/kg and 500 mg/kg, p.o. respectively for 30 days) and Group V rats received SMT along with silymarin (20 mg/kg/p.o. for 30 days) (Vaghasiya et al., 2009). 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) and other biochemical investigations. Serum total protein was measured according to the method of Lowry et al., 1951.

Histopathology

Histopathology of liver was carried out by a modified Luna method (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 $\pm$ S.E.M. Students' '*t*'-test is used for statistical analysis of blood serum parameters and for statistical analysis of liver enzymes.

RESULTS

The phytochemical evaluation of *Catharanthus rosea* shows the presence of tannins, saponins, glycosides, phenols, flavonoids and while steroids and terpenes were absent in *Catharanthus rosea* (Table 1).

The effect of ethanolic extract of *Catharanthus rosea* leaves 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 in simvastatin intoxicated rats.

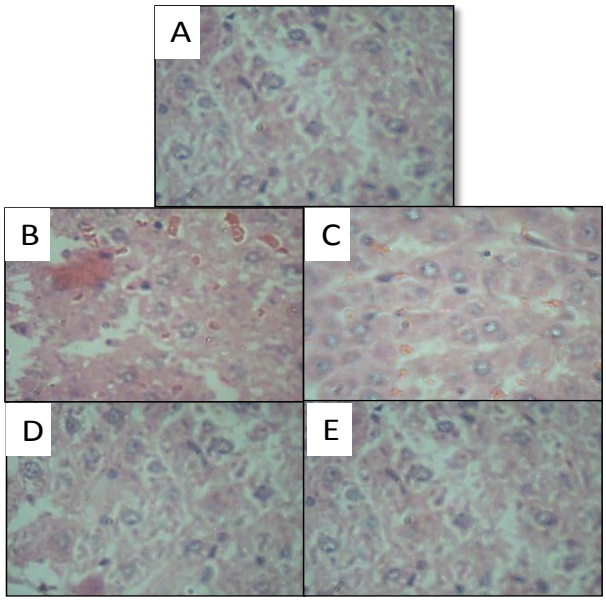

Figure 1.
(A) Section of liver of (Normal control) (B) Section of liver of (Negative control; Simvastatin); (C) Section of the liver of Test-low dose; *Catharanthus rosea* + Simvastatin; (D) Test-high dose; *Catharanthus rosea* + Simvastatin); (E) Section of liver of Positive control (Simvastatin + Silymarin).

Table 1. Phytochemical analysis of ethanolic extracts of *Catharanthus rosea* leaves

S. No.	Phytochemical Tests	*Catharanthus rosea*
1	Alkaloids	+
2	Steroids	-
3	Tannins	+
4	Phenols	+
5	Flavonoids	+
6	Glycosides	+
7	Saponins	+
8	Terpenes	-

+ = Present - = Absent

Table 2. Effect of ethanolic extracts of *Catharanthus rosea* leaves on biochemical 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 (Normal control)	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 (Negative control; Simvastatin)	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 (Test-low dose; *Catharanthus rosea* + Simvastatin)	112.2 ± 0.60*	168.01 ± 2.75*	162.2 ± 2.42*	0.86 ± 0.04 *	1.42 ± 0.03*	3.98 ± 0.06*
Group IV (Test-high dose; *Catharanthus rosea* + Simvastatin)	98.6 ± 2.38 **	142.58 ± 2.53**	118.24 ± 1.88 **	0.69 ± 0.01**	0.99 ± 0.05**	4.41 ± 0.02**
Group V Positive control (Simvastatin + Silymarin)	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, *P <0.05, **P < 0.01, ***P < 0.001. All values are compared with group II negative control.

On the other hand the groups which received both *Catharanthus rosea* extract (300 mg/kg, and 500 mg/kg,) + simvastatin (20 mg/kg, p.o.) (Group III & IV) and simvastatin (20 mg/kg, p.o.) + silymarin (25 mg/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 and Fig 1(A-E)].

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). 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 studies, 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).

In simvastatin group histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed. While in ethanolic extracts of *Catharanthus rosea*

(300 mg/kg and 500mg/kg, p.o) prevented these histological changes, further indicating their hepatoprotective activity.

CONCLUSION

The results of present study demonstrate that *Catharanthus rosea* leaves extract has potent hepatoprotective activity against simvastatin induced liver damage in rats. The results also imply that the hepatoprotective effects of *Catharanthus rosea* may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituents responsible for hepataprotective effect.

CONFLICT OF INTEREST

None declared.

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