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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *ADIANTUM LUNULATUM BURM.F*

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ABSTRACT

Aim: The present study is to evaluate the Hepatoprotective activity of ethanolic extract of *Adiantum lunulatum Burm.f* against simvastatin induced hepatotoxicity. **Method:** The whole plant was extracted using ethanol and hepatotoxicity in rats was induced by the administration of simvastatin (20 mg/kg p.o for 30 days), the protective effect of *Adiantum lunulatum Burm. f* (200 mg/kg p.o) and (400 mg/kg p.o) for 30 days, and standard silymarin (25mg/kg p.o) for 30 days. **Result:** The significant changes in Biochemical parameters such as increased Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Serum bilirubin and decrease in Total proteins during simvastatin induced toxicity were restored towards normalization in *Adiantum lunulatum Burm.f* (200 mg/kg and 400 mg/kg) treated animals. Oxidative stress markers GSH (Reduced glutathione) and SOD (Superoxide dismutase) showed decrease in simvastatin induced group than normal group, and *Adiantum lunulatum Burm.f* treated groups increases respectively. Histopathological studies also confirmed the protective effect of *Adiantum lunulatum Burm.f* **Conclusion:** Thus the present study ascertains that the ethanolic extract of *Adiantum lunulatum Burm.f* possesses significant hepatoprotective activity and it may due to the antioxidant activity of the plant.

KEYWORDS: *Adiantum lunulatum Burm.f,* Hepatotoxicity, Antioxidant activity, Simvastatin, Silymarin, Ethanol.

INTRODUCTION

Liver is the largest and almost complex organ in the body. It plays an important role in maintenance of internal environment through its multiple diverse functions. The liver is the key organ of regulating homeostasis in the body and it is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction. In recent years, liver problems are on the rise and Injury to liver and damage to the hepatic parenchyma cells were always proved to be associated with distortion of different metabolic functions of liver.[1]

Etiologically various infectious agents including viruses and different hepatotoxins along with environmental pollutants are thought to be responsible for causing different types of liver damage and hepatic injuries [2-3]. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.[4-5]

Since 1987, statins are among the most widely prescribed medications for primary and secondary prevention of cardiovascular disease around the world. Thus, simvastatin causes oxidative stress mediated hepatotoxicity by depleting antioxidant enzymes.[6] 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.[7]

Hamsapadi, Adiantum lunulatum Burm.f. (*A. Philippense Linn)* usually known as '*Walking Maiden hair fern'* is used as an ornamental plant and widely distributed in India. It is commonly found in wet, shaded areas and on moist mud walls during monsoon. *Adiantum lunulatum Burm. f* is a cosmopolitan fern belonging to the family Adiantaceae, and genus Adiantum. In India it is found very commonly in the South in plains and lower slopes of the hills and in the North along the foot of the Himalayas from East to West at an altitude of 1000-3000 feet.

Pteridophytes have been poorly studied and considered economically less important group of plants in the plant kingdom. Several phyto-constituents have been isolated and identified from different parts of the plant such as Carotenoids, Flavonoids, Nortriterpene-adiantone etc. The dried whole plant has been used as a medicine for bronchitis and cough. It is used in

bleeding diseases, burning sensation, epileptic fits, dysentery, and elephantiasis [8]. The present study was directed to investigate the hepatoprotective activities of *Adiantum lunulatum Burm.f.* against simvastatin induced hepatotoxicity.

MATERIALS AND METHOD

Plant material

Whole plant *Adiantum lunulatum Burm.f* was collected from cheruvandoor Kottayam district Kerala. The plant identified and authenticated by taxonomist Rogimon P Thomas, Assistant Professor Department of Botany CMS college ,Kottayam, Kerala. The plant was collected from the month of October to December

Preparation of ethanolic extract

The whole plant of *Adiantum lunulatum Burm.f* were collected and shadow dried. The shaded plant were subjected to pulverization to get coarse powder. The coarsely powderd plant were used for the extraction with ethanol. *Adiantum lunulatum Burm.f* 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 1% CMC solution at different concentration.

Phytochemical investigation

The ethanolic extracts of *Adiantum lunulatum Burm.f* were subjected to preliminary phytochemical screening to identify the chemical constituents present in the plant by the following methods.[9-11]

Experimental animals

Wistar albino rats (150-200g) were procured from small animals breeding station, Kerala Veterinary and animal sciences university, Thrissur and housed in standard animal house according to the CPCSEA guidelines with access to food and water *ad libitum*. The animals were kept for acclimatization for 1 week. Animal model consists of five groups comprising of 6 animals per cage at 27±2°C with constant 55% humidity on a 12 h light/dark cycle. 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

Adiantum lunulatum Burm.f in the dose of 2000 mg/kg were administered orally to different group of rats, Mortality was observed after 72 hours. Acute toxicity was determined according to the method of Litchfield and Wilcoxon (1949).[12]

Experimental design for hepatoprotective activity[13]

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

Group I: Normal control (CMC)

Group II: simvastatin (20mg/ kg.p.o)

Group III: Simvastatin (20mg/ kg.p.o) + *Adiantum lunulatum Burm.f* extract (200mg/ kg, p.o)

Group IV: Simvastatin (20mg/ kg.p.o) + *Adiantum lunulatum Burm.f* extract (400mg/ kg, p.o)

Group V: Simvastatin (20mg/ kg.p.o) + Silymarin (25mg/kg, p.o)

Animals were divided into five different groups, each group having 6 rats and treated accordingly. Group I rats fed with a normal standard diet for 30 days. Group II rats receives Simvastatin (20mg/ kg.p.o alone for 30 days). Group III and IV rats receive simvastatin along with *Adiantum lunulatum burm.f* extracts (200mg/ kg and 400mg/ kg.p.o) respectively for 30days) and Group V rats receive simvastatin along with silymarin (25 mg/kg/p.o.for 30 days). On the 31st day, all the animals were fasted 24 hrs. Blood was collected by retro orbital puncture and serum was used for the estimation of biochemical parameters and animals were sacrificed by cervical dislocation, Liver was used for the study of oxidative stress parameters and histopathology.

Blood biochemistry

Blood samples were collected in glass tube by retro orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT according to the method of Reitman 1957,[14] ALP,[15] and bilirubin (Malloy 1937) by standard method.[16] Serum total protein was measured according to the method of Lowry 1951.[17]

Estimation of Oxidative Stress Markers

All the animals were euthanized after blood collection with the cervical dislocation method under light ether anesthesia and the liver was removed for study of oxidative stress markers

such as Superoxide dismutase (SOD) by Moron 1979 [18] and Reduced glutathione (GSH) by Bonye and Ellman, 1972.[19]

Histopathology

Histopathology of liver was carried out by a modified Luna 1999.[20] 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.[21] 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 obtained were analyzed by one-way-analysis of variance (ANOVA), followed by Tukey's multiple comparison test, using Graph pad prism software. p-value < 0.05 was taken as the criterion of significance.

RESULTS

Acute toxicity of *Adiantum lunulatum Burm.f* in the dose of 2000mg/kg were administered orally, shows no signs of toxicity up to 2000mg/kg. The phytochemical screening of *Adiantum lunulatum Burm.f* shows the presence of Carbohydrates, Steroids, Terpenoids, Flavonoids, phenols and Glycosides (Table 1).The effect of ethanolic extract of *Adiantum lunulatum Burm.f* on SGPT, SGOT, ALP, bilirubin and total protein level in Simvastatin intoxicated rats are summarized in Table 2. There was a significant increase in SGOT, SGPT and ALP, and bilirubin levels in Simvastatin intoxicated group compared to the normal control group. And the total protein levels were significantly decreased in simvastatin (20mg/kg, p.o) treated rats compared to normal group. The effect of *Adiantum lunulatum Burm.f* on GSH, and SOD is shown in Table 3. were significantly decrease in Simvastatin - intoxicated rats when compared with those animals in normal control group.

Table 1: Preliminary Phytochemical Screening.

SI. NO.	Phytochemical constituents	Inference
1	Alkaloids	_
2	Carbohydrates	+
3	Flavonoids	+
4	Steroids	+
5	Tannins	_
6	Glycosides	+

7	Saponins	_
8	Terpenoids	+
9	Phenols	+
10	Proteins and amino acids	_

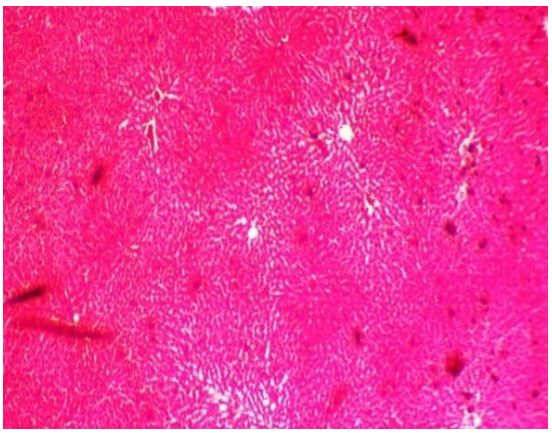
Table 2: Effect of ethanolic extracts of *Adiantum lunulatum Burm.f* on biochemical parameters.

Groups	Treatment	SGPT	SGOT	ALP	Bilirubin	Total protein
Normal	CMC	35.721± 5.9863***	38.496± 1.049***	123.625± 3.4513***	0.446± 0.0332***	7.3583± 0.3706***
Simvastatin	20mg/kg	120.345± 10.1935	134.825± 5.158	186.146± 5.6906	1.488± 0.0611	3.09± 0.22199
EEAL	200mg/kg	99.001± 1.8429***	96.09± 2.968***	170.386± 3.3719***	1.248± 0.1423***	4.5483± 0.3179***
EEAL	400mg/kg	84.861± 2.6520***	89.153± 4.335***	150.728± 2.8777***	0.653± 0.0273***	5.631± 0.31044***
Standard	25mg/kg	40.13± 5.1432***	58.186± 4.774***	132.865± 3.2970***	0.528± 0.0372***	6.455± 0.31156***

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

Groups	Treatment	SOD	GSH
Normal	CMC	6.76±0.0045***	19.67±0.02811***
Simvastatin	20mg/kg	5.23±0.0024	10.98±0.009772
EEAL	200mg/kg	5.56±0.0036 **	12.67±0.02145**
EEAL	400mg/kg	5.87±0.0056***	16.876±0.03405 ***
Standard	25mg/kg	6.34±0.0039 ***	18.45±0.01075 ***

Values are Mean ± SD, followed by Tukey's multiple comparison test 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 toxic group.



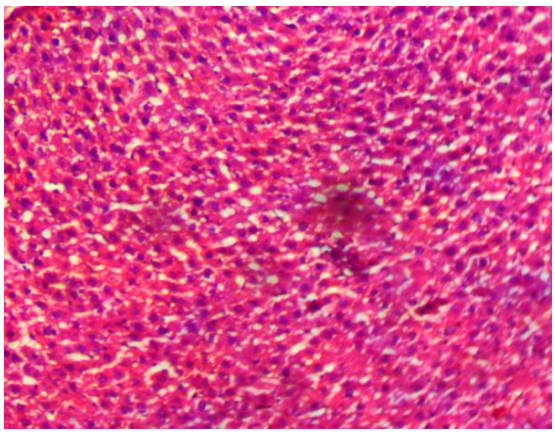


Fig. 1: Section of Liver of Control Group. Fig 2: Section of Liver of Simvastatin Treated Group.

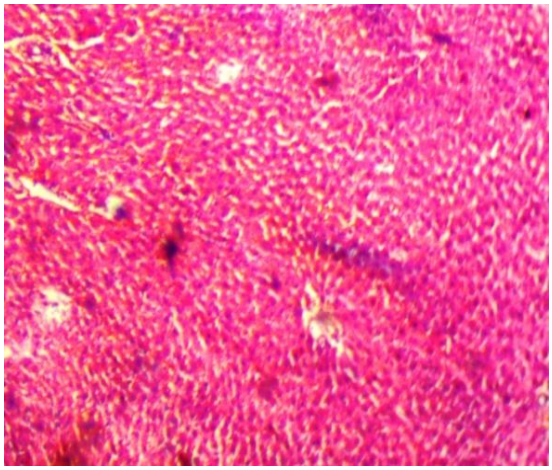


Fig. 3: Section of Liver of Simvastatin and Extract (200mg/kg) Treated Group

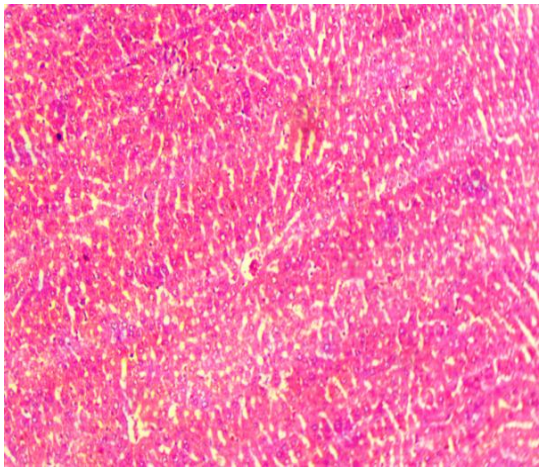


Fig. 4: Section of Liver of Simvastatin and Extract (400mg/Kg) Treated Group

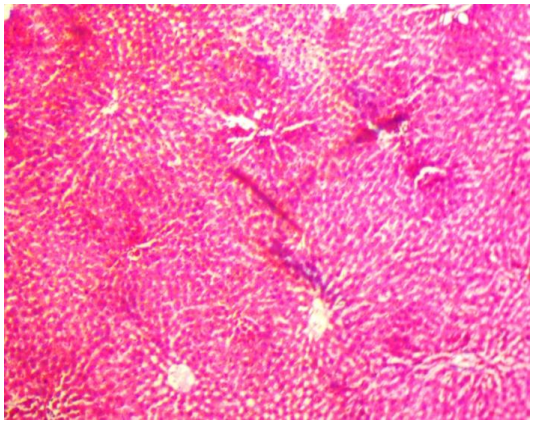


Fig. 5: Section of Liver of Simvastatin and Silymarin Group.

DISCUSSION

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 .The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. Simvastatin hepatotoxicity is

hypothesized to occur due to drug-drug interactions. 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). The hepatotoxicity model in wistar albino rats was successfully produced by the oral administration of simvastatin (20mg/kg) daily for 30 days. On 31St day all the animals were sacrificed, Biochemical and histopathological studies were conducted. Phytochemical screening showed the presence of most of the chemical constituents. Administration of simvastatin for 30 days significantly increased serum enzymes like SGPT SGOT, ALP, Bilirubin, and decreases total protein. Simvastatin increased oxidative stress mediated hepatotoxicity leads to elevation in the serum enzymes level. It was observed that EEAL decreases the elevated enzyme level and increases the decreased total protein level. The effect of *Adiantum lunulatum Burm.f* on GSH, and SOD were significantly decrease in Simvastatin -intoxicated rats when compared with those animals in normal control group. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant.[23] They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals.[24-25] Phenolic compounds are considered to be the most important antioxidative components of the plants. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in simvastatin groups. Ethanolic extracts of *Adiantum lunulatum Burm.f* (200 mg/kg and 400mg/kg, p.o) prevented steatosis and perivenular fibrosis.

CONCLUSION

The results of present study demonstrate that *Adiantum lunulatum Burm.f* extracts (200 mg/kg and 400 mg/kg) has potent hepatoprotective activity against simvastatin induced model. From the phytochemical, biochemical, oxidative stress markers and histopathological studies it is confirmed that the *Adiantum lunulatum Burm.f* posseses significant hepatoprotective activity. The results also imply that the hepatoprotective effects of *Adiantum lunulatum Burm.f* may be due to its antioxidant property and Further investigation are necessary to determine the exact mechanism.

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