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Hepatoprotective activity of ethanolic extract of *Diospyros virginiana* in CCl_4 induced hepatotoxicity in swiss albino rats

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Abstract: Liver is one of the largest organs in human body and health. Modern medicines have little to offer for the chief site for intense metabolism and excretion. Liver cell injury caused by various toxic chemicals (certain antibiotic, chemotherapeutic agents, carbon tetrachloride (CCL4), thioacetamide (TAA) etc excessive alcohol consumption and microbes is well studied. The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver. Hence, drugs from plants have become increasingly popular and their use is wide spread. The present study was to evaluate the hepatoprotective potential of ethanolic leaves and barks extract of *Diospyros virginiana* against experimentally induced hepatoxicity models in swiss albino rats. Silymarine was given as reference standard. The both leaves and barks ethanolic extract of *Diospyros virginiana* have shown very significant hepatoprotection against CCl_4 induced hepatotoxicity in swiss albino rats in reducing serum total bilirubin,total protein, ALP, SGPT and SGOT levels respectively.
Keywords : Hepatoprotective activity, carbon tetrachloride, ethanolic extract, *D.virginiana.*

Introduction

Liver is one of the largest organs in human body and health. Modern medicines have little to offer for the chief site for intense metabolism and excretion. So it alleviation of hepatic diseases and it is chiefly the plant has a surprising role in the maintenance, performance and based preparations which are employed for their treatment regulating homeostasis of the body[1]. It is involved with of liver disorders. But there are not much drug available almost all the biochemical pathways to growth, fight for the treatment of liver disorders. Therefore, many against disease, nutrient supply, energy provision and folk remedies from plant origin are tested for its potential reproduction[2].

Free radicals are found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. A free radical is a reactive molecule that contains one or more unpaired electrons. Such molecules are generally highly reactive[3]. They are unstable and posses extra energy, to reduce their energy they react with certain cells to function normally. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neuro degenerative disorders, inflammation, viral infection, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and gastric ulcer[4]. Studies have shown attraction in the antioxidant status following ulceration, indicating that free radicals seem to be associated.

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Diospyros is a genus of over 700 species of deciduous and ever-green trees, shrubs, and small bushes. *Diospyros virginiana* L. is a persimmon species commonly called the American Persimmon from Ebenaceae family. It is a deciduous tree native to China, and the southeastern portion of the United States. The tree grows wild but has been cultivated for its fruit and wood since prehistoric times by Native Americans[6].The peculiar characteristics of its fruit have made the tree well known. The fruit is a globular berry, with variation in the number of seeds, some-times with eight and sometimes without any. The fruit had high content of vitamin C and it contained anti-oxidant compounds like vitamin-A, beta-carotene, lycopene, lutein, and cryptoxanthin. Fresh fruits also contained healthy amounts of minerals like potassium, manganese, copper, and phosphorus. it has been beneficial in various forms of disease of the bowels, chronic dysentery and uterine haemorrhage. A wide range of medicinal plant parts extract is used as raw drugs and they had varied medicinal properties [7]. The different parts used include root, leaves, fruits, stems, flowers, and modified plant organs. Hence, the present study was undertaken to evaluate the possible This study revealed that ethanolic extract significantly reduced serum bilirubin, total protein, SGOT, SGPT, and ALP levels.

Material and Methods

Plant collection and preparation of the extract

D.virginiana belongs to the family *Ebenaceae* was collected from Coonoor, Nilgiris District, Tamil Nadu, India and identified by the special key given Cambell flora[8]. The leaf and bark of *D.virginiana* were washed with sterile distilled water. After, the leaves and bark were shade dried and powdered by using pestle and mortar. 25g of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. The ethanolic extracts of the plant was diluted with distilled water and was administered orally to mice.

Hepatoprotective Activity

The hepatoprotective activity of the *Diospyros virginiana* plant leaves and bark extracts was tested using CCl_4 model. Rats were divided into five groups (100-140g) of six animals in each group.

Group I: Normal control
Group II: Toxic control, treated with CCl_4 (CCl_4 and Olive oil in 1:3 ratio).
Group III: CCl_4 + standard Silymarin (70 mg/kg, p.o)
Group IV: CCl_4 + *Diospyros virginiana* bark (500mg/kg)
Group V: CCl_4 + *Diospyros virginiana* leaves (500mg/kg)

The *Diospyros virginiana* was given simultaneously with carbon tetrachloride. Treatment duration was 7 days. Dosage of carbon tetrachloride was administered as 30% solution in olive oil for every 72 h. Animals were sacrificed 48 hrs after the last injection. At the end of the experimental period, the animals were killed cervical dislocation after an overnight fasting. The blood sample was collected. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10 minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for biochemical analysis.

The separated serum was used for the estimation of some biochemical parameters. The present studies had been attempted to Aspartate aminotransferase (AST/SGOT), SGPT, ALP and demonstrate the role of hepatoprotective activity of bilirubin and total protein. The results thus obtained were subjected to statistical analysis using analysis of variance.

Results and Discussion

The present study had been attempted to demonstrate the role of hepatoprotective activity of crude ethanol extracts of plant materials of *Diospyros virginiana in* CCl_4 induced hepatotoxicity. The results of hepatoprotective activities of crude ethanol extracts of these plant at a dose of 500mg/kg b.wt. on rats intoxicated with carbon tetrachloride were illustrated in the Table 1. The level of serum marker enzymes SGOT, SGPT, ALP TB and TP were found to be significantly increased in CCl_4 induced liver damaged rats when compared with the normal group (p<0.001). Whereas treatment with ethanolic extract of leaf and bark of *Diospyros virginiana* at the dose of 500mg/kg/p.o. showed decrease in the elevated serum enzyme levels in CCl_4 induced liver damage in rats compared to that of control groups (p<0.001). Silymarin (70mg/kg/ p.o.) also significantly decreased the levels of serum enzymes and bilirubin content in CCl_4 treated groups as compared with the respective control.

CCl_4 induced liver toxicity that is frequently used as model to study hepatoprotective activity of drugs. Silymarin was used as standard drug, Silymarin, a standardized extract of *Diospyros virginiana* is also a potent hepatoprotective agent [9,10]. It reverses hepatotoxin induced alterations of biochemical parameters and has so far been the most thoroughly investigated of all the plant substances in preventing liver damage induced by carbon tetra chloride, D-galN and paracetamol in rat models[11,12].

The ALT, AST, GGT and ALP activity and serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury. Administration of acetaminophen caused a significant elevation of enzymes level such as AST, ALT, GGT, ALP and bilirubin level has been attributed to the damage structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity[13]. The co administrations of all examined plant extract have prevented the increased serum marker enzymes AST, ALT, ALP, GGT level and bilirubin level [14]. This is in agreement with the commonly accepted view that serum levels of AST, ALT, GGT and ALP return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.

It can be concluded that the hepatoprotective activity of *Diospyros virginiana* may be due to its ability to normalize the levels of marker enzymes and antioxidants which may be due to the presence of secondary metabolites in *Diospyros virginiana.*

Table 1. Hepatoprotective activity of *D.virginiana* ethanolic leaf and bark extracts in experimental animals

Groups	SGOT	SGPT	ALP	TB	TP
Group I	155.6±3.82	66.56±11.05	198.25±0.52	0.75±0.14	14.17±0.00
Group II	316.40±12.50	179.22±4.26	495.50±9.27	1.22±0.17	7.81±0.05
Group III	172.10±8.33***	73.47±2.39***	220.08±6.36***	0.75±0.02***	10.65±0.06***
Group IV	213.50±1.62*	103.45±2.45*	276.25±2.54*	0.84±0.22*	7.21±0.081*
Group V	198.02±5.78**	87.05±2.61***	210.30±7.01***	0.75±0.22***	6.07±0.012***

Values are expressed as S.E.M; *P<0.01 Vs control; **P< 0.001 Vs control;
6 Number of animals were used in each group.

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