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Evaluation of Phytochemical, Antioxidant and Hepatoprotective Activity of Tuber of *Geodorum laxiflorum* Griff

R. Chandra Besra, L. Majhee and J. Sharma
Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Jharkhand, India

Corresponding Author: R. Chandra Besra, Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Bariatu, Ranchi-834009 Jharkhand, India Tel: +91-9006471746

ABSTRACT

Liver diseases are one of the major causes of mortality and morbidity in humans. Incidence and prevalence of hepatotoxicity due to drugs are increasing and is one of the main contributing factor of death. Herbs of medicinal value are being used since ancient times for the treatment of liver diseases. Orchid plants have ornamental importance but few are known for medicinal use. So, the present study was designed to investigate phytochemical, anti-oxidant and hepatoprotective property of methanolic extract of tuber of *Geodorum laxiflorum*. Tubers were powdered and extracted with methanol in Soxhlet apparatus to get residue (yield, 25 g, 10%). Phytochemical tests confirmed the presence of flavonoid, polyphenols, sterols and carbohydrates in methanolic extract of the tuber. Chromatographic separation was performed and two major flavonoid were isolated (yield, 25.6% of fraction IV and 31.2% of fraction V). The 50-250 μg mL^{-1} of extract were evaluated for antioxidant activity in DPPH model. IC_{50} value of extract was 213 μg mL^{-1}. In acute oral toxicity study, extract was found safe and non-toxic even in higher dose of 2000 mg kg^{-1} b.wt. Thirty-six healthy adult Wistar albino rats (150-200 g) of either sex were used for the investigation of *in vivo* hepatoprotective activity of methanolic extract. About 50, 100 and 200 mg kg^{-1} per oral of extract were used for hepatoprotective activity and compared with 100 mg kg^{-1} silymarin as standard. Methanolic extract showed prominent reduction in elevated hepatic marker enzymes and significant ($p<0.001$). In histopathological examination it is observed that extract has significant hepatoprotective activity. In *in vitro* antioxidant study, extract has shown significant free- radical inhibition activity. Phytochemical analysis of the extract has proved to have flavonoids and polyphenols. In conclusion, the MeOH extract of *Geodorum laxiflorum* has antioxidant and hepatoprotective activity and contain potent phytochemicals.

Key words: Chromatography, polyphenols, flavonoids, carbon tetrachloride, 1,1-diphenyl-2-picryl-hydrazil, methanolic extract

INTRODUCTION

Liver is a vital organ that functions as metabolic centre for various nutrients such as carbohydrates, proteins and lipids (Barrett *et al*., 2010).

Liver is a largest internal organ as well as gland of the human body and function as a metabolic centre. It has specialized tissue and is capable to regulate many biochemical reactions of valuable nutrients such as carbohydrates, proteins and lipids are controlled by liver. It also takes part in

metabolism of drugs, xenobiotics and excretion of their waste metabolites from the body and protects the organs against various toxicants (Frank and Tukey, 2006).

It is well established that liver injury is caused by various toxicants (Ramachandran and Kakar, 2009) such as certain chemotherapeutic agents (anti-tubercular drugs, anti-HIV drugs and some antibiotics), carbon tetrachloride, thioacetamide, chronic alcohol consumption (e.g., liver cirrhosis) and microbes.

As herbal based therapeutic drugs has been popularised worldwide for the treatment of liver disorders by leading pharmaceutical industries and is worthwhile to search safe hepatoprotective agents (Agarwal, 2001). Most of the liver protective plants may contain various biologically active phytochemicals in it. Recently, investigators have reported about hepatoprotective activity of alkaloids (Gong *et al*., 2010), polyphenols (Kumarappan *et al*., 2011), glycosides (Jaishree and Shrishailappa, 2010; Ma *et al*., 2011), carotenoids (Sindhu *et al*., 2010; Firdous *et al*., 2011), coumarins (Bahadir *et al*., 2011) and flavonoids (Yue-Tao *et al*., 2011).

Antioxidants are molecules of synthetic or natural origin and have ability to stabilize or deactivate free radicals. Highly reactive free radicals are major causative agents for different cellular injury including liver damage (Halliwell, 1994). Many dietary constituents and phytochemicals of medicinal plants are being investigated as antioxidants for its protective effects (Maria *et al*., 2010; Nakasa *et al*., 2011; Gupta *et al*., 2011; De Oliviera *et al*., 2011). Phytochemicals having antioxidant activity are mainly flavonoids (Zhou *et al*., 2011; Till *et al*., 2011) and polyphenols (Jaggadeo and Brody, 2011).

Natural products are a rich source of active phytochemicals and have been extensively used for biological activity. Thus, phytochemical analyses play a vital role in search of new and novel molecules (Shu, 1998). So, further need of paradigm shift for the standardization and evaluation of herbal remedies for liver diseases is required.

Geodorum laxiflorum is a rare endangered terrestrial orchid plant (Genus: *Geodorum*; Family: Orchidaceae). It can be seen only during the rainy season and is widely distributed in humid tropical forest of India and exclusively found in Chotanagpur reason of the state Jharkhand (Kumar *et al*., 2007). It is known for traditional medicine in the treatment of malignant tumor, stomach ache and joint pain and is marketed by the local practitioners of Jharkhand. We are documenting first time the ethno botanical property of *Geodorum laxiflorum*.

Hence, aim of this study was to evaluate phytochemical, antioxidant and hepatoprotective property of methanolic extract of tuber of *Geodorum laxiflorum*.

MATERIALS AND METHODS

Study was conducted in the Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences (RIMS), Jharkhand, India. Research work was of three years duration and started in the year 2009.

Chemicals: Carbon tetrachloride (CCl_4) was purchased from Merck India Ltd., Mumbai. Silymarin, DPPH (1,1-diphenyl-2-picryl-hydrazil) and ascorbic acid were purchased from Sigma Aldrich, India. Silica gel 60-120 and silica gel for thin layer chromatography were purchased from Loba Chemie, India. Commercially, available diagnostic kits (SGOT, SGPT, Alkaline Phosphatase, Total Protein, Albumin and Bilirubin) were procured from chemical store, of the institute. The reagent kits used were made of Crest Biosystem, Accurex and Beacon Ltd., India.

Plant materials: The plant *Geodorum laxiflorum* was collected from the forest at Kalamati, 18 km from Ranchi in the month of June and July. The plant was authenticated in the department of Botany, Ranchi College, Ranchi, Ranchi University, Jharkhand. The voucher specimen was submitted in the departmental herbarium of the college. Tubers of the plant were crushed, dried in the shade and powdered.

Preparation of plant extract: Two hundred and fifty gram powdered tuber of the plant was extracted with 500 mL volume of methanol for 12 h in a Soxhlet apparatus. The extract was evaporated by thermostatically controlled water bath and then dried in laboratory vacuum pump. A sticky residue obtained (yield was 25 g, 10%).

Animals: Thirty-nine adult Wistar albino rats were procured from the central animal house, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand. The animals were acclimatized to in-house conditions. They were allowed to feed a commercial pellet diet (Hindustan Liver Limited, Bangalore, India) and water *ad libitum*. All the experimental protocol was undertaken in accordance with ethical guidelines. All the studies were approved by the Institutional Animal Ethics Committee (IAEC).

Preliminary phytochemical screening: It involves testing of methanolic extracts of *Geodorum laxiflorum* for their contents of different classes of compounds. The common qualitative chemical tests were used for the screening of various phytochemical to give the general idea regarding the nature of constituents present in extract. The qualitative chemical tests for various phytoconstituents were carried in extract and fraction IV and V of *Geodorum laxiflorum* (Silva *et al*., 1998).

Isolation and chromatographic separation: Residue was dissolved in methanol and extracted with each of 250 mL hexane until the yellow colour of hexane layer become colourless in separating funnel. Methanol layer was evaporated and adsorbed in silica gel, dried and subjected to chromatographic separation. Chromatographic separation was carried out in glass column (20 mm×45 mm bore size). The glass was packed with 25 g of silica gel 60-120 as stationary phase and equilibrated with hexane before fractionation. Two hundred and fifty milligram of adsorbed extract was packed on top of the column and eluted with gradient solvent system as mobile phase. Phytochemicals were separated by column fractionation, primarily by hexane-ethyl acetate and then with ethyl acetate-methanol solvent system. Percentage of methanol in ethyl acetate were selected for chromatographic fractionation as shown in parenthesis to get fraction I (4-10%), fraction II (16%), fraction III (20%), fraction IV (30-40%) and fraction V (50-80%). Each eluted fractions were collected and assessed for the separation of each components by thin layer chromatography.

Thin Layer Chromatography (TLC) was prepared in the laboratory on a glass plate (76×26 mm size, 1.15×1.35 mm thickness). Plates were coated with slurry of thin layer of adsorbent material, silica gel G (known as stationary phase). Coated plates were air dried and activated by heating in oven at 110°C. All Fractionated samples were applied on the activated chromatographic plates and drawn up with a solvent or solvent mixture (known as mobile phase). In brief, all samples of the separated fractions were dissolved in methanol and applied as a single spot in a row along one side of chromo plate, about 2 cm from the edge, by capillary tubes. To make a choice of suitable solvent system, elutropic series of different solvents were tried first by running on the TLC

plate. The TLC plate was placed at 45 degree angle in the development chamber covering the bottom of the plate by the solvent up to nearly 1 cm. The solvent front was marked in the plate and finally allowed to dry. The colored substances were visualized on the chromatogram in ultra violet cabinet at 254 and 236 nm. Colourless components were detected by visualizing agent, iodine vapours in iodine chamber. The qualitative evaluation of the plate was done by the determination of R_f value of each separated substances (Salituro and Dufresne, 1998). R_f stands for retention value of each separated components in chromatography and is determined by:

$$R_f \text{ value} = \frac{\text{Distance travelled by each separated components from baseline}}{\text{Distance travelled by solvents from baseline}}$$

Study design

Free-radical scavenging activity: In this study, DPPH radical scavenging activity model was performed (Bhujbal *et al*., 2009).

Acute oral toxicity study: Acute oral toxicity study of the extract was performed according to OECD 423 guidelines. Adult Wistar female rats were used in this study (Ecobichon, 1997).

***In-vivo* hepatoprotective activity:** The crude methanolic extract of tubers of *Geodorum laxiflorum* was investigated for the hepatoprotective activity. All the animals were maintained under standard conditions, allowed to feed with commercially available food and water throughout the experiment. Silymarin was used as reference hepatoprotective agent. Liver damaged was induced by administration of 30% CCl_4 suspended in olive oil (1 mL kg^{-1} body weight i.p.). Adult Wistar rats (150-200 g) were used for this study. The animals were divided into six groups of six rats each and subjected to the following treatments (Raj *et al*., 2010).

Group I received the vehicle (Sodium CMC 0.3%, p.o.) only and served as normal control. Group II served as toxicant control and treated with CCl_4. Group III received Silymarin (standard) at the dose of 100 mg kg^{-1} b.wt. Group IV, Group V and Group VI were received the extract at the dose of 50, 100 and 200 mg kg^{-1} b.wt., respectively. All the animals received these treatments by oral route for seven days. On the seventh day except group 1, the entire remaining groups were received 30% CCl_4 suspended in olive oil (1 mL kg^{-1} b.wt. i.p.). After 24 h of CCl_4 intoxication, on the 8th day animals were anesthetized under light ether anaesthesia. Blood was collected by cardiac puncture in a centrifuge tubes and separated serum was used for the assay of hepatic marker enzymes. Aspartate aminotransferase (ASAT) (Bergmeyer *et al*., 1986a), alanine Aminotransferase (ALAT) (Bergmeyer *et al*., 1986b), Alkaline phosphatase (ALP) (McComb and Bowers, 1972), along with albumin (Doumas *et al*., 1971), total protein (Strickland *et al*., 1961), total bilirubin and cholesterol (Richmond, 1973) were estimated using diagnostic kits in clinical autoanalyser. All animals of the experimental groups were sacrificed under anaesthesia. Liver of all animals were incised out and preserved in 10% formalin solution for histopathological examination.

Statistical analysis: The results were expressed as Mean±SEM. Statistical significant was determined by one-way Analysis of Variance (ANOVA) and subjected to Tukey's multiple comparison tests. Significant difference between the mean was accepted when $p<0.05$. SPSS 17 software was used for statistical analysis.

RESULTS

Chromatographic separation: All the isolated fractions were almost inactive in short and long wave ultraviolet light. Separated components were made visible in Thin Layer Chromatography (TLC) by passing iodine vapour through the developed chromatogram. Isolated yield of separated fractions were 15(6%), 8 (3.2%), 13 (5.2%), 64 (25.6%) and 78 mg (31.2%) for fraction I, II, III, IV and V. R_f value of each fractions were determined as follows 0.44, 0.68, 0.86, 0.16 for fraction I, II, IV and V. Two components were identified in fraction III having R_f value of 0.32 and 0.50. It was confirmed in the qualitative tests, that fraction IV (yellowish oily liquid) is chalcones and aurones while fraction V (solid) is flavones and flavonols type of flavonoids. Fraction V was isolated as solid substance in cold condition but melts down at room temperature (Table 1).

Preliminary phytochemical screening: Table 2 describes the various qualitative tests for methanolic extract, isolated chromatographic fraction IV and V. Various tests confirmed the presence of potent polyphenols particularly flavonoids in tubers along with other phytochemical.

Free-radical scavenging activity: Table 3 represents the various concentrations (50-250 μg mL^{-1}) of methanolic extract (test) and ascorbic acid (standard) which were used for free-radical scavenging activity. All concentrations had showed the significant inhibitory activity. IC_{50} value of extract and ascorbic acid are 213 and 143 μg mL^{-1}.

Acute oral toxicity study: All the doses 5, 50, 300 and 2000 mg kg^{-1} b.wt. of methanolic extract used for acute oral toxicity study were found non-toxic and safe. No mortality and behavioural changes were observed even in highest dose (2000 mg kg^{-1}) employed to animals. So, doses of 50, 100 and 200 mg kg^{-1} body weight of the extract were selected for the pharmacological investigation of hepatoprotective activity.

Table 1: Isolated chromatographic fractions of methanolic extract of *Geodorum laxiflorum*

Fractions	Rf value	Solvent system for TLC	Isolated yield (%)
I	0.44	Acetone: chloroform (1:9)	6.0
II	0.68	ethyl acetate: methanol (9.9:1)	3.2
III	0.32, 0.50	acetone: chloroform (2:8)	5.2
IV	0.86	chloroform: methanol (1:9)	25.6
V	0.16	acetone: methanol (1:9)	31.2

Rf: Retention value; %: Percentage; TLC: Thin layer chromatography

Table 2: Phytochemical tests for methanolic extract and fraction IV and V

Phytochemicals	Qualitative tests	Methanolic extracts	*Fraction IV	**Fraction V	References
Alkaloids	Wagner's test	-	-	-	De *et al.* (2010)
Polyphenols	Ferric chloride test	+++	-	-	Farnsworth (1966)
Flavonoids	Shinoda test	+++	-	-	Farnsworth (1966)
	Sulphuric acid test	+++	+++	+++	Markham (1982)
Sterols	Salkowski reaction	+++	-	-	Bairi *et al.* (2011)
Saponins	Production of foam	+++	-	-	Bairi *et al.* (2011)
Carbohydrate	Molisch's reaction	+++	-	-	Bairi *et al.* (2011)

+++: Present; -: Absent, *: Deep yellow colour, **: Red colour

Table 3: Effect of methanolic extract on DPPH radical scavenging activity

	Ascorbic acid		*Methanolic extract	
Concentration (μg mL^{-1})	% Scavenging	IC_{50} value	% Scavenging	IC_{50} value
50	28±0.57	143	16±0.33	213
100	39±0.57		27±0.33	
150	51±0.33		38±0.33	
200	64±0.57		47±0.33	
250	72±0.33		60±0.57	

Data represent the mean±SEM (n = 3), *p<0.05, by student's t-test for values between the methanolic extract and ascorbic acid. %: Percentage; IC: Inhibitory concentration; DPPH: 1, 1-diphenyl-2-picryl-hydrazil

Table 4: Effect of treatment with the methanolic extract of *Geodorum laxiflorum* on the biochemical parameters of CCl_4 intoxicated rats

Treatment	dose	ALAT (U L^{-1})	ASAT (U L^{-1})	ALP (U L^{-1})	Albumin (mg dL^{-1})	Total protein (g dL^{-1})	Total bilirubin (mg dL^{-1})	Cholesterol (mg dL^{-1})
Normal	-	25.33±0.882	73.00±2.266	7.83±0.601	3.23±0.187	7.68±0.291	0.45±0.042	66.17±1.424
CCl_4	1 mL kg^{-1} body wt.	78.83±1.537 *	210.17±4.086 *	38.00±0.816 *	1.01±0.162 *	5.90±0.073 *	1.61±0.094 *	92.17±1.195*
Silymarin (standard) methanolic	100 mg kg^{-1} body wt.	35.17±0.946**	78.17±1.797 **	8.67±0.882 **	3.36±0.212 **	7.56±0.236 **	0.53±0.084 **	68.17±1.167**
Extract	50 mg kg^{-1} body wt.	77.17±1.778	204.17±2.315	37.00±0.730	0. .90±0.036	5.75±0.076	1.48±0.094	90.33±1.256
	100 mg kg^{-1} body wt.	74.83±1.922	190.50±2.277**	34.00±0.730***	1.46±0.172	5.93±0.084	1.00±0.089 **	85.00±1.390**
	200 mg kg^{-1} body wt.	49.00±1.483 **	160.50±2.320**	30.67±0.955**	2.35±0.176 **	6.58±0.130	0. 767±0.088 **	77.83±1.19**

No. of animals (n = 6). *p<0.001, when compared to the normal group. **p<0.001 when compared to the CCl_4 group. ***p<0.01, when compared to the CCl_4 group. CCl_4: Carbon tetrachloride; wt.: Weight; ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; ALP: Alkaline phosphatase; U L^{-1}: International unit per litre; mg dL^{-1}: Milligram per decilitre; g dL^{-1}: Gram per decilitre

Biochemical estimations: Table 4 shows the results of hepatoprotective activity of extract on CCl_4 intoxicated rats. The liver enzymes ALAT, ASAT, ALP along with albumin, total bilirubin and cholesterol in serum are elevated significantly (p<0.001) in CCl_4 intoxicated animals, when compared to normal control. Serum albumin and total protein decreased significantly (p<0.001) in CCl_4 treated rats, when compared to normal. Treatment with 50 mg kg^{-1} b.wt. dose did not show any significant effect on liver enzymes, albumin, total bilirubin, total protein and cholesterol.

Animals treated with 100 mg kg^{-1} reversed their increased serum levels of ASAT, total bilirubin, cholesterol significantly (p<0.001), ALP (p<0.01) and altered in serum ALAT, albumin and total protein were observed but not significant, when compared to CCl_4 treated animals. Animals treated with 200 mg kg^{-1} dose has shown highly significant (p<0.001) lowering in serum levels of ALAT, ASAT, ALP and albumin, total bilirubin, cholesterol except total protein, when compared to CCl_4 treated animals. Silymarin (100 mg kg^{-1}) treated animals reversed their serum levels significantly (p<0.001), when compared to CCl_4 treated animals.

Significant (p<0.001), results were obtained at a dose of 100 mg kg^{-1} b.wt. in reduction of elevated serum levels of ASAT, total bilirubin, cholesterol only. Their mean values are 190.50, 1.00 and 85.00, respectively. About 200 mg kg^{-1} b.wt. of extract showed most significant results

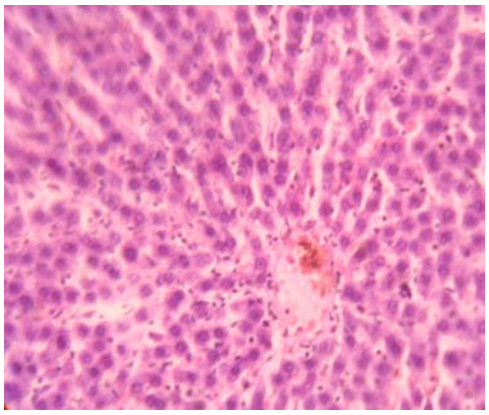

Fig. 1a: Group I (Control) - section of liver with normal cell structure. 40X

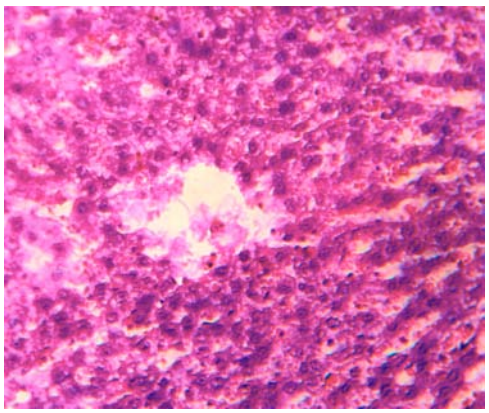

Fig. 1b: Group II (CCl_4)-section of liver showing complete fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities. 40X

($p<0.001$), in reduction of elevated serum levels of ALAT, ASAT, ALP, albumin, total bilirubin and cholesterol. Their mean values were 49.00, 160.50, 30.67, 2.35, 0.767 and 77.83, respectively.

Histopathological examination: In histopathological examination of the liver tissues also revealed the dose-dependent protection effect of extract in treatment groups from liver damage, when compared with CCl_4^- toxicated group. Change in liver histology, such as fatty liver change, degeneration of central hepatic vein, hepatocyte proliferation, necrosis, inflammation, lymphatic infiltration and in sinusoidal irregularities were reduced by the treatment of extract. Maximum protection from liver damage in animals was observed at a dose of 200 mg kg^{-1} b.wt. All biochemical findings of liver functions were supported by the positive results of histopathological study (Fig. 1a-f).

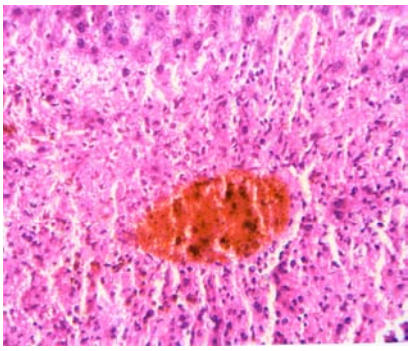

Fig. 1c: Group III (Silymarin 100 mg kg^{-1}, body weight) - section of liver showing reduced fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities. 40X

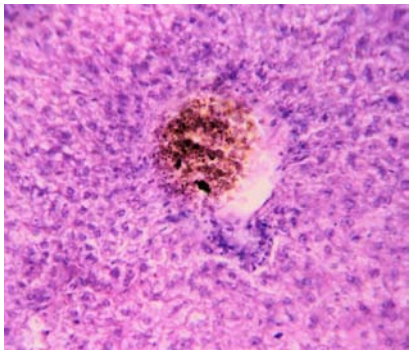

Fig. 1d: Group IV (50 mg kg^{-1}, body weight) - section of liver showing less reduction in fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities. 40X

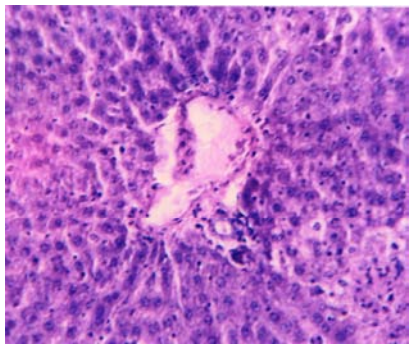

Fig. 1e: Group V (100 mg kg^{-1}, body weight) - section of liver showing comparatively reduced fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities. 40X

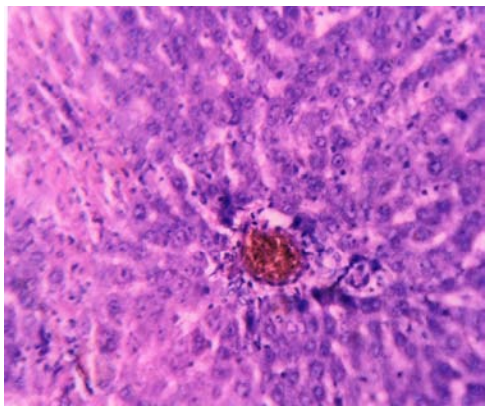

Fig. 1f: Group VI (200 mg kg^{-1}, body weight) - section of liver showing significantly reduced fatty liver change, centriolobular necrosis, hepatocytes degeneration, sinusoidal deformities. 40X

Figure 1a represents the section of liver of normal groups with normal cell structure and was not treated with toxicant and extract. Figure 1b is the histology of group II animals and was treated with CCl_4 toxicant and represents toxicant control. The histopathological change can be observed in liver histology showing complete fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities. Figure 1c indicates the group III animals; they were treated with silymarin 100 mg kg^{-1}, b.wt. and served as standard control. Histopathological examination of liver tissue of standard groups reveals the reduced fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities. Figure 1d is the histopathological examination of group IV animals and was treated with extract at a dose of 50 mg kg^{-1}, b.wt. Less reduction in fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities was observed in this dose. Figure 1e represents the histopathological examination of group V animals, treated with the extract at a dose of 100 mg kg^{-1}, b.wt. Microscopic evaluation of liver of this group showing comparatively reduced fatty liver change, centriolobular necrosis, hepatocytes degeneration and sinusoidal deformities. Figure 1f represents the liver histology of group VI animals; they were treated with extract at a dose of 200 mg kg^{-1}, b.wt. Liver histopathology of this group showing significantly reduced fatty liver change, centriolobular necrosis, hepatocytes degeneration, sinusoidal deformities. In comparative liver histopathological evaluation, it is observed that the effect of extract at a dose of 200 mg kg^{-1} is comparable with the results of silymarin (standard) at a dose of 100 mg kg^{-1}.

DISCUSSION

Plants from various genus of Orchidaceae family have been investigated for different biological activities. But, only species of the genus *Geodorum* is explored for antioxidant, cytotoxic and antibacterial activity (Razibul *et al.*, 2011) in *in vitro* model. However, no any study was conducted in *Geodorum laxiflorum* previously. Here, we reported first time the detail study of hepatoprotective activity of *Geodorum laxiflorum* in animal model and isolation of flavonoids.

Carbon tetrachloride a hepatotoxin is one of the most commonly used experimental model in animals for evaluation of liver diseases (Johnston and Kroening, 1998; Samudram *et al*., 2008; Kumar *et al*., 2008; Krishna *et al*., 2009; Subash *et al*., 2011; Solanki and Jain, 2011).

Carbon tetrachloride induces hepatotoxicity selectively by metabolic activation in liver cells. Activation takes place by cytochrome P450 in the endoplasmic reticulum and change into highly reactive trichloromethyl free radicals ($\bullet CCl_3$). It combines with cellular lipids and proteins in the presence of oxygen, thus induces lipid peroxidation. So, it causes the changes mainly in endoplasmic reticulum integrity, other membranes and loss of metabolic enzyme activation, reduction in protein synthesis and elevation of serum transaminase leading to liver damage (Lim *et al*., 2000). Aminotransaminases are liver specific enzymes considered as sensitive, reliable indices for hepatotoxicity and hepatoprotective activity of any agents.

The assessment of liver function is made by estimating serum levels of liver enzymes (ASAT, ALAT and ALP), albumin, total bilirubin, total protein and cholesterol. The altered serum levels of these biochemical's in CCl_4 treated animals in the present study corresponds to the extent of liver damage induced by toxin. It is demonstrated in the present study that the methanolic extract particularly (100 and 200 mg kg^{-1}) exhibited significant dose-dependent hepatoprotective activity against CCl_4 liver toxicity in rats.

Therefore, altered in serum levels of marker hepatic enzymes corresponds to the extent of hepatotoxicity in CCl_4 treated rats when compared to the normal, extract and standard treated rats.

Presence of flavonoids and polyphenols in the methanolic extract of *Geodorum laxiflorum* and its free-radical scavenging capacity in DPPH model is significantly comparable and supported by observation of *G. densiflorum* (Razibul *et al*., 2011) and *Itrifal kishneezi* (Koneru *et al*., 2011).

In chromatographic isolation study, we have identified and isolated five fractions. Here, we are again disclosing the isolation process of chalcones and aurones as well as flavones and flavonols type of flavonoids from the plant *Geodorum laxiflorum*. Maximum isolation was obtained with fraction IV (25.6%) and V (31.2%). Chromatographic isolation study was conducted first time in the *Geodorum laxiflorum* only, among the species of genus *Geodorum* of the family Orchidaceae.

In this study, 100 and 200 mg kg^{-1} body weight of methanolic extract of *G. laxiflorum* showed significant protection against CCl_4 induced liver toxicity by reduction in elevated marker liver enzymes. Hepatoprotective potential of the methanolic extract is significantly ($p<0.001$) comparable in the concentration of 200 mg kg^{-1} b.wt. with 300 mg kg^{-1} b.wt. (Krishna *et al*., 2010). Histopathological examination of the liver tissue revealed the significant protection in dose-dependent manner. This observation is also supported by recently reported hepatoprotective activity in CCl_4 induced hepatotoxicity in animal model of different species of orchid (Du *et al*., 2000, 2003; Shih *et al*., 2005; Wu *et al*., 2007) and other (Janakat and Nassar, 2010; Chavda *et al*., 2010; Adinarayana *et al*., 2011) plants. But, none of the authors have reported the hepatoprotective activity of *Geodorum laxiflorum*.

Evidence revealed that many phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of hepatotoxin or by inhibiting lipid peroxidation (Mehta *et al*., 1999). Flavonoids (Baek *et al*., 1996; Pandit *et al*., 2004; Di Carlo *et al*., 1999), triterpenes (Xiong *et al*., 2003), saponins and alkaloids (Tran *et al*., 2001) have been well established as hepatoprotective agents. It is reported about protective effects of naturally occurring compounds on liver and other organs (Dhiman and Chawla, 2005; Muriel and Rivera-Espinoza, 2008).

In our preliminary phytochemical investigation of methanolic extract has shown the presence of many potent phytoconstituents particularly flavonoids and phenolic components. It is evidence based that antioxidant therapy is a promising strategy to ameliorate liver injury (Glantzounis *et al*., 2005). Flavonoids in polyphenols have already been proved as potent antioxidants of plant origin (Pietta, 2000). There is a possibility that these natural active principles alone or in combination are responsible for hepatoprotective activity. This study proves that tuber extract has ability to ameliorate CCl_4 induced liver toxicity and it must have some indirect or direct effect on liver.

All the above mentioned reviews with respects to the results obtained in the experiments are supporting our study and not contradicting any observation.

CONCLUSION

Flavonoids and polyphenols are well known for their anti oxidant and hepatoprotective activity. Orchid plants are good sources of various phytochemicals. But very few species of orchids have been explored for their medicinal use. Genus *Geodorum* is least explored for their biological activities including species *G. laxiflorum*. In conclusion, the results of our study shows that methanolic extract of tubers of *Geodorum laxiflorum* has promising antioxidant and hepatoprotective activity and have potent phytochemicals. Therefore, further investigation is required to isolate and identify other active constituents responsible for this activity.

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