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PHYTOCHEMICAL EVALUATION AND BIOLOGICAL SCREENING OF *DENDROBIUM CHRYSANTHEMUM* WALL. EX. LINDL.

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Abstract

A large number of populations are suffering due to various reasons from hepatic disorders of unknown origin. The development of anti-hepatotoxic drugs has drawn the attention of scientists in the field of natural product research because synthetic drugs may cause serious side effects. The present work was aimed to study the *in-vivo* hepatoprotective activity of *Dendrobium chrysanthemum* Wall. ex Lindl (Orchidaceae). The leaves of *D .chrysanthemum* were used traditionally in rural districts of Andhra Pradesh State. To give a scientific support, the leaf extract was studied on carbon tetrachloride (CCl_4) induced hepatotoxicity on animal model. The elevated levels of SGOT, SGPT, ALP and total bilirubin were prevented by *D. chrysanthemum* leaf extract. The present study confirms that the methanolic extract of *D. chrysanthemum* has significant hepatoprotective activity against CCl_4 induced hepatotoxicity and further supports the folklore claims. The histological investigations also evidenced activity against hepatotoxicity and the activity may be attributed to the presence of bioactive compounds like flavonoids, alkaloids, triterpenoids, glycosides and steroids.

Key words: *Dendrobium chrysanthemum* Wall. ex. Lindl., Total Phenolic contents, Total Flavonoid Content, Total Alkaloid Content, Hepatoprotective activity, Histopathology.

Introduction

Since the origin of the herbal medicines, people were impelled to consider the importance of herbs for treating several physiological disorders. It is no wonder, during the past decade there has been an exponential rise in the application of herbal remedies. However, several herbal products are not really standardized in terms of its effectiveness and safety (Shikha *et al*., 2012). In the absence of reliable liver protective drugs in modern medicine, in India, a number of medical plants and their formulations are used to cure hepatic disorders (Felix *et al*., 2005).

Dendrobium (family: Orchidaceae) contains approximately 1100 species which are mainly distributed in the subtropical and tropical regions of Asia. Since ancient times, many *Dendrobium* plants have been used as ingredients for nutraceutical beverages and food products. *Dendrobium* plants have been used by traditional Chinese medical practitioners to alleviate diabetes, obesity, rheumatoid arthritis, and liver disorders (Raheleh *et al*., 2017). Among all the species of *Dendrobium*, *D. chrysanthemum* is considered to be the most precious (Jessica *et al*., 2010). *Dendrobium* is a type of orchid that is generally yellow or pink in color. The plant has a long thin stem that is used for various herbal and medicinal treatments. *D. chrysanthemum* has been used in traditional Chinese medicine for centuries now. The Chinese believe that the tonic extracted from the *Dendrobium* plant is 'yin' in nature and can be used to treat ailments ranging from stomach pain, heatstroke, dry mouth and sores in the mouth (Chung *et al*., 2005). It is considered a safe herb when consumed in the recommended dosages. Too much of *D. chrysanthemum* in any form can cause convulsions and may even affect the heart and lungs. Research is still being conducted on the interactions of *D. chrysanthemum* with other drugs or medications. Among its many uses, the Chinese use *D. chrysanthemum* tonic for longevity. It is believed that *D. chrysanthemum* when mixed with *licorice* roots and made into a tea transmits healing energy to all parts of

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the body. It helps moisten and nourish the skin and prevents dryness and flaky skin. When air pollution and smoke dry out the lungs and air passages and increase thirst, *D. chrysanthemum* can be consumed for quick relief and to moisten the passageways (Arditti and Ernst, 1993). It is used as an effective tonic for the treatment of tuberculosis, flatulence, night sweats, anorexia, fever, and dyspepsia (Manorama *et al*., 1984). *D. chrysanthemum* tonic improves the functioning of the lungs, kidneys, and stomach. It can reduce stomach pain and cramping and reduce vomiting. It is believed that regular consumption of *D. chrysanthemum* can also treat sexual impotency. Pain in the feet and hands, lumbago, and arthralgia can be treated with *D. chrysanthemum* extract (Chung *et al*., 2007). It can boost the immune system and help the body fight infections. It has long since been used to replenish lost fluids from the body and reduce severe thirst. Natives of the Eastern Himalayas use this plant to heal problems with the eyes. Along with their pretty colors and decorative qualities, growing *D. chrysanthemum* at home can eradicate pollutants and toxins from the air and create a clean environment. Its blossoms and canes are edible (Nayak *et al*., 1997). Countries like Thailand and Singapore, deep fry these delicacies and eat them as snacks. In Europe, *D. chrysanthemum* blossoms are used as edible cake and as garnishes. Pickle is made from the flowers in Nepal (Pillon and Chase, 2007). *D. chrysanthemum* possesses phenolics and also flavonoids. Phenolic acids and flavonoids possess diverse biological activities including antioxidant and hepatoprotective properties. Recently it has been considered that polyphenolic compounds are great antioxidants and proved to be more effective than vitamin C, E and carotenoids (Girish *et al*., 2009). In view of the above, the authors have made an attempt to screen the leaf extracts of *D. chrysanthemum* which is being used by folklore in the north coastal districts of Andhra Pradesh State for treating liver disorders.

Materials and Methods

The whole plant of *D. chrysanthemum* was collected from Araku and surrounding villages of Andhra Pradesh, India and authenticated by Dr. M. Venkayya, Taxonomist, Dept of Botany, Andhra University, Visakhapatnam, India (Voucher specimen No.PS-SG-02), Ascorbic acid (Sigma Aldrich Chemie, Germany), Riboflavin (S.D chemicals, India), Silymarin, Gallic acid, and Catechin (Nature remedies, Bangalore, Karnataka, India). CCl_4 (Poona Chemical Laboratory, Pune, India) and SGOT, SGPT, ALP, total bilirubin estimation kits (Span Diagnostics, Surat, India). All other solvents and chemicals used were of analytical grade purchased from local source.

Preparation of extract

The collected plant material (1kg) of *D.*

Table 1: Standardization and qualitative-quantitative analysis of whole pant of *D. chrysanthemum*.

S. No.	Parameter		*Dendrobium chrysanthemum*
1.	Organoleptic characters		
	Colour		Pale greenish white
	Odour		Characteristic
	Taste		Characteristic
	Physical appearance		Free flowing powder
2.	Physiochemical characters		
	Water soluble extractive		72.45%
	Alcohol soluble extractive		80.00%
	pH 1% w/v solution		6.14
	Loss on drying		4.50%
	Ash content		6.32%
	Acid insoluble ash		0.63%
	Moisture content by K.F		1.21%
	Foreign organic matter		3.73%
3.	Heavy metals		
	Lead		5.00 ppm
	Arsenic		1 ppm
	Cadmium		0.2 ppm
	Mercury		1 ppm
4.	Microbiological analysis		
	Total aerobic count		410 CFU/g
	Yeast & Mould		30 CFU/g
5.	Pathogen analysis		
	E. Coli		Absent
	Salmonella		Absent
	Pseudomonas aeruginosa		Absent
	Staphylococcus aureus		Absent
6.	Qualitative preliminary phytochemical analysis		
	Alkaloids		+
	Carbohydrates		+
	Flavonoids		+
	Glycosides		+
	Phytosterols		+
	Proteins & amino acids		-
	Saponins		-
	Tannins		-
	Triterpenoids		+
7.	Quantitative phytochemical analysis		
	Phenolic content (g GAE/100 g dw)	Flavonoid content (g CE/100 g dw)	Alkaloid content (mg/100 g plant material)
	4.51±0.4*	5.34±0.93*	48.16±0.46*

(+) Present; (-) Absent *Values are means of triplicate determination ± Standard deviation.

chrysanthemum was subjected to standardization as per WHO guidelines for organoleptic, physiochemical, heavy metal, microbiological analysis (Anonymous, 2007). The plant materials were shade dried, powdered (40 mesh size) to get a coarse powder and then subjected to Soxhlet extraction (4 hrs) using methanol as a solvent. The extract was filtered and concentrated at reduced temperature to a small volume on a rotary evaporator (yield: 30%w/w). The extract was subjected to preliminary qualitative (Harborne, 1984 and Mondal *et al*., 2017) and quantitative (for phenolics, flavonoids and alkaloids) and phytochemical analysis table 1.

Determination of total phenolic content

The total phenolic content was estimated using the modified Folin-Ciocalteu's photometric saturated sodium carbonate (Vabkova *et al*., 2012). The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 nm after 90 minutes of incubation at room temperature against the blank gallic acid was used as standard. The total phenolic content is here expressed as gallic acid equivalents (GAE) per 100 g of dry weight (dw) table 1.

Determination of total flavonoid content

The total flavonoid content was measured using a modified colorimetric method (Vabkova *et al*., 2012). The appropriate amount of extract was added to a test-tube together with distilled water. Then was added 5% $NaNO_2$, after 5 minutes 10% $AlCl_3$ and after another 5 minutes 1M NaOH followed by the addition of distilled water. The absorbance was measured against the blank at 510 nm after 15 minutes. The standard curve was prepared using different concentration of catechin. The flavonoid content was expressed as gram catechin equivalents (CE) per 100 g of dry weight (dw) table 1.

Determination of total alkaloid content

The total alkaloid content was determined according to UV- Spectrophotometer method (Manjunath *et al*., 2012). This method is based on the reaction between alkaloid and bromocresol green. The part of the plant extract was dissolved in 2N HCl and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform, and then the pH of phosphate buffer solution was adjusted to neutral with 0.1N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of bromocresol solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of mean ± S.E.M, in table 1. The appropriate amount of filtered methanol extracts were oxidized with Folin-Ciocalteu's reagents and after 5 minutes was the reaction neutralized with saturated sodium carbonate. The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 nm after 90 minutes of incubation at room temperature against the blank. As the standard was used gallic acid. The total phenolic content is here expressed as gallic acid equivalents (GAE) per 100 g of dry weight table 1.

Acute toxicity study

The acute toxicity studies were conducted for selected plant methanolic extracts as per the OECD guidelines 423 (Anonymous, 2000), with slight modifications (Ganapaty *et al*., 2002), using female albino rats. The selected three extracts showed neither visible sign of toxicity nor mortality. The results clearly indicated non-toxicity of the extracts at a dose of 2000 mg/kg p.o. From this, 1/20th 1/10th, and 1/5th and doses were selected for the experimental study. Hence there is no LD_{50} and all the extracts tested are considered safe and nontoxic.

***In-vivo* screening for hepatoprotective activity**

Animals used

Wistar albino rats of either sex weighing between 200-250 g were obtained from Mahaveer Enterprises,

Table 2: Effect of methanolic extract of whole plant of *D. chrysanthemum* against CCl_4 induced hepatotoxicity in albino rats.

S. No.	Treatment group	Serum biochemical parameters			
		SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	T.BILI.(mg/dl)
1	Control (5% gum acacia 1ml/kg p.o.)	77.81±0.42	63.75±0.49	134.08±0.80	0.49±0.01
2	Hepatotoxin - CCl_4(1ml/kg p.o.)	508.47±0.78***	398.87±0.62***	785.74±0.59***	4.78±0.57***
3	Standard- Silymarin(50 mg/kg p.o.)	133.11±0.53***	102.96±0.67***	222.41±1.17***	1.44±0.05
4	Methanolic extract of*D. chrysanthemum*(100mg/kg p.o.)	318.11±0.83***	265.94±0.46***	548.07±0.53***	2.92±0.03**
5	Methanolic extract of*D. chrysanthemum*(200mg/kg p.o.)	221.06±0.48***	182.27±1.02***	390.33±0.88***	1.40±0.01
6	Methanolic extract of*D. chrysanthemum*(400mg/kg p.o.)	146.06±0.39***	108.55±0.96***	246.88±0.66***	1.73±0.04

Values are mean ± SEM, n=6, Significance: *P<0.05, **P<0.01, ***P<0.001

Hyderabad. The animals were housed under standard environmental conditions (temperature of 22 ± 1^{0}C with an alternating 12 hrs. light- dark cycle and relative humidity of 60 ± 5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Ethical Committee and by animal regulatory body of the Government (Regd: No: 516/01/ CPCSEA). They were fed with standard pellet laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad and water *ad libitum*.

Experimental procedure

In this screening (Janbaz *et al*., 2002) albino rats of either sex (200-250 g) were used. The animals were fed with standard diet and water *ad libitum* two weeks before and during the experimental period. The *Dendrobium chrysanthemum* methanolic extract (DOME) was tested at 100, 200 and 400 mg/kg, p.o., dose level. The animals were divided in to 6 groups (I-VI), each group consisting of 6 animals. Group I received 5% gum acacia suspension and acts as a normal control and Group II received CCl_4 at a dose of 1 ml/kg orally (p.o.) acts as negative control. Groups III-VI were treated with selected drugs (silymarin and plant extract) for 5 days before the commencement of experiment and on day 6th of the experiment, blood samples were collected (6th day) at 0 hr in all groups and CCl_4 was administered to all groups except group I (normal control) one hour after the administration of drugs. On 7th day blood samples were collected from all groups by retro orbital puncture, serum was separated by centrifugation and used for the estimation of blood serum parameters (SGOT, SGPT, ALP and total bilirubin) according to the standard procedures. The liver sections also dissected out subjected to histopathology studies and results are shown tables 2 and Fig, 1 and 2.

Histopathological studies

All the animals were anesthetized with Sodium pentobarbital (50 mg/kg, IP) (Phifer *et al*., 1986) and livers were dissected out quickly by cutting on the ventral side. The isolated liver specimen was trimmed to small pieces and preserved in neutral buffered formalin (10% formaldehyde in phosphate buffered saline) solution for 24 hrs. The liver specimen was subjected to dehydration with acetone of strength 70, 80, 100 % respectively, each for one hour. The infiltration and impregnation was done by treatment with paraffin wax twice each time for one hour. Specimens were cut into sections of 3-5 µm thickness using microtome and were stained with hematoxylin and eosin and later the microscopic slides of the liver were photographed at 40X magnification (Vabkova *et al*., 2012 and Manjunath *et al*., 2012) Fig. 2.

Statistical analysis

The data obtained in the studies were subjected to

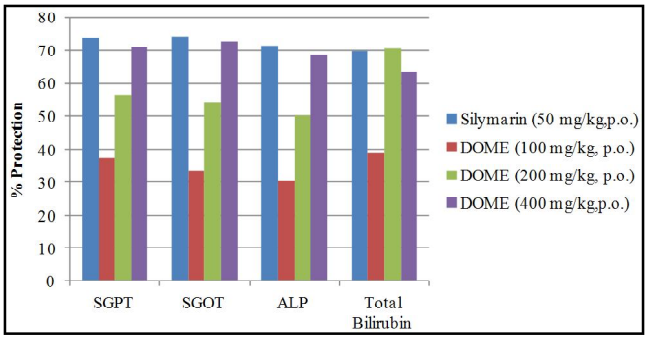
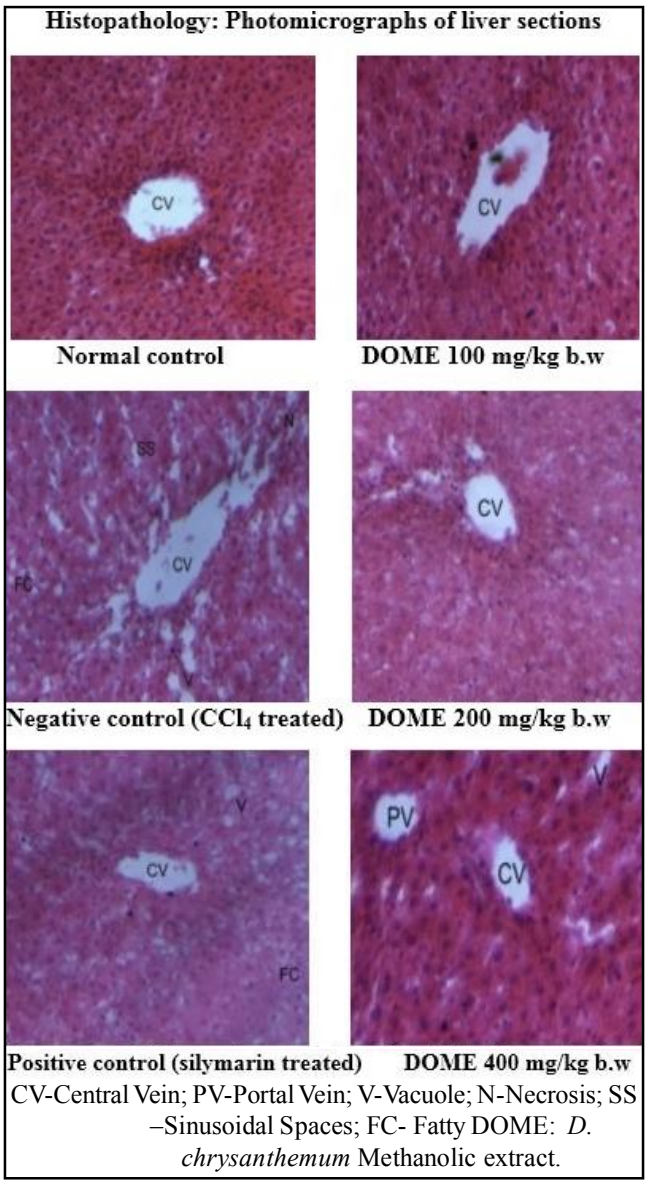


Fig. 1: Percentage reduction of various serum biochemical parameters due to treatment with *D. chrysanthemum* methanolic extract (DOME) against CCl_4 induced hepatotoxicity in albino rats.



CV-Central Vein; PV-Portal Vein; V-Vacuole; N-Necrosis; SS –Sinusoidal Spaces; FC- Fatty DOME: *D. chrysanthemum* Methanolic extract.

Fig. 2: Effect of methanolic extract of *D. chrysanthemum* against CCl_4 induced hepatotoxicity in albino rats.

one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analysed using Dunnet's *t*-test. A *p-value* < 0.05 was considered to be significant. All the values were expressed as mean ± SEM.

Results and Discussion

The methanolic extract of *D. chrysanthemum* at dose levels of 100, 200 and 400 mg/kg, p.o., were tested using silymarin (50 mg/kg, p.o.,) as a standard. As indicated from the results CCl_4 induced animals showed an increase in the activities of SGPT, SGOT, and ALP and the content of total bilirubin when compared to the normal control group. The tested doses exhibited significant hepatoprotective activity against CCl_4 induced liver intoxicated rats by reduction in increased serum levels of SGOT, SGPT, ALP and total bilirubin. A slight decrease was found after the treatment with 100 mg/kg b.w., when compared to the CCl_4 induced group. However administration of doses at 200 and 400 mg/kg b.w., produced significant decrease in enzymes levels (SGOT, SGPT, ALP) and total bilirubin table 2 and Fig. 1. No abnormal appearance or histopathological changes were observed in the liver. Treatment with silymarin almost restored the normal architecture of liver whereas the rats treated with test methanolic extract of *D. chrysanthemum* at doses of 100, 200 and 400 mg/kg b.w., showed recovery from CCl_4 induced liver damage as evident from normal hepatocytes. With higher dose (400mg/kg) showed significant attenuation of inflammatory and necrotic changes and cellular architecture of liver was preserved indicating a marked protective activity (Boll *et al*., 2012 and Kasdallah-Grissa *et al*., 2007) similar to that observed in silymarin Fig. 2.

Phytochemical studies on the selected plant revealed the presence of flavonoids, alkaloids, triterpenoids, glycosides, steroids and carbohydrates. The presence of above constituents in selected plant extract alone or in combination might be responsible for the observed hepatoprotective activity. Further, this was supported by quantitative estimation of phytoconstituents. The total phenolic, flavonoid and alkaloid contents were found to be 4.51 ± 0.4, 5.34 ± 0.93 and 48.16 ± 0.46 respectively [Table 1]. Therefore, the study shows that there is a prospective future in the use of plants as a source of natural medicine for curing various diseases.

Conclusion

All these scientific observations support the traditional use of *Dendrobium chrysanthemum* for treating liver disorders. The free radical scavenging and antioxidant properties are probably due to the presence of phytoconstituents.

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