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Research Article

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Study of antioxidant and anti-inflammatory activities of methanol extract of *Desmodium gyrans* (DC)

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ABSTRACT

Desmodium gyrans (DG) is a medicinal plant used for a number of properties in traditional medicine. GC-MS analysis and study on antioxidant and anti-inflammatory activities of DG were done using 70% methanol extract of the whole plant. The IC_{50} values of DG for scavenging superoxide, DPPH, Hydroxyl and ABTS radicals and inhibiting lipid peroxidation were found to be 12.6, 4.0, 13.5, and 1.95 and 74.0 µg/ml respectively. Both acute and chronic anti-inflammatory studies were performed in hind paw of Balb/c mice. The percentage inhibitions were found to be 13.02 and 17.39 in acute study and 13.9 and 31.71in chronic anti-inflammatory experiments. The results showed that 70% methanol extract of DG has excellent antioxidant activity and anti-inflammatory activity, especially during chronic inflammation. GC-MS analysis showed the presence of phytochemicals active in inhibiting inflammation thus validating the anti-inflammatory activity of Desmodium gyrans extract

Key words: *Desmodium gyrans*, antioxidant, anti-inflammatory.

INTRODUCTION

In recent years, people have recognised the use of many medicinal plants and a tendency to depend on herbal medicine is on the rise. This has resulted in screening plant products in search of novel therapeutic agents[1]. *Desmodium gyrans* DC is an annual herb belonging to family Fabaceae found in Indian forests. The plant is also known as 'The Indian telegraphic plant' since it makes automatic movements of its leaves. *D. gyrans* is used in traditional and folk medicine since its leaves have diuretic, febrifugal and tonic properties and roots are used in Indian medicine as remedy for asthma, coughs, as anti dysenteric and as emollient. It is believed to have cardio-tonic properties and remarkable wound healing effect[2]. *D. gyrans* has a long history of use in Chinese traditional medicine to treat various ailments[3]. Closely related species *D. gangeticum* is used in Indian system of medicine as a bitter tonic, febrifuge, digestive, anticatarrhal, antiemetic, in inflammatory conditions of chest and various other inflammatory conditions[4]. It has proven antioxidant property[5] and also has hypolipidemic, cardio protective properties and gives protection against cardiac reperfusion injury [6].

Metabolic reactions taking place in our cells generate reactive oxygen species which are capable of critically damaging biomolecular structures[7]. In recent times one of the areas which have attracted a great deal of attention is antioxidant research and management of diseases arising as a result of free radical stress. Although free radicals like superoxide radicals, hydrogen peroxide and highly reactive hydroxyl radicals and others are important in

processes like phagocytosis, cell signaling and energy production,[8] these can also lead to a variety of acute and chronic diseases including diabetes, cardiovascular disorders, cancer and a number of inflammatory diseases [9]. The presence of safe and bioactive natural antioxidants which can act as scavengers of free radicals raises a substantial interest in deploying medicinal plants in curing of these diseases.

Many medicinal plants having antioxidant properties have been reported to have anti-inflammatory activity also[10]. The carrageenan-induced paw edema in rat is most widely used for the screening of promising anti-inflammatory agents. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Formalin induced paw edema in rats is widely used for screening potential anti-inflammatory agents. Formalin induced chronic inflammation and edema is also biphasic with an early neurogenic component involving release of bradykinin being followed by a later tissue-mediated response involving release of histamine, 5-hydroxytryptamine and prostaglandin.

The study is designed to make a probe into the traditionally assumed medicinal effects of *D. gyrans* which may be attributed to its hitherto unknown antioxidant and anti-inflammatory properties which have to made known to the world especially since it has remained a 'virgin plant' and not been the subject of much research works.

EXPERIMENTAL SECTION

1.1. Animals
Balb/c mice (20-25 gms) used for study were procured from SABS, Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India. The animals were acclimatised for a period of 14 days in uniform hygienic well ventilated cages under standard environmental setting.

1.2. Approval of Institutional Animal Ethics Committee
All experiments in the study were carried out with prior approval of Institutional Animal Ethics Committee (IAEC) and were conducted strictly as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division of Government of India.

1.3. Preparation of extract
The plant was collected from Peechi forest area and identification was done by Dr. Sasidharan, Taxonomist, Kerala Forest Research Centre, Peechi, India. A voucher specimen was deposited in the herbarium of Amala Cancer Research Centre (ACRH No.036). Extract was made in 70% methanol from powdered and dried entire plant using soxhlet apparatus. The extract was dried by vaporisation and the yield of solvent free extract was 12% (w/w). The extract was re-suspended in double distilled water and used for further studies on animals.

1.4. Phytochemical analysis
The extract was screened for the presence of alkaloids (Dragendorff's test), phytosterols (Libermann and Barchards test), phenolic compounds and tannins ($FeCl_3$ test), terpenoids (Salkowski test) and flavonoids (NaOH test).

2.5. Acute oral toxicity study
D. gyrans extract at the dose range of 500 mg to 2000 mg per kg body weight were orally administered to different groups of rats, comprising of 6 animals in each group. Mortality was observed for 7 days and acute toxicity was determined. Blood was collected from each group and renal and liver function tests were performed in serum.

2.6. GC-MS analysis
The GC-MS analysis of selected samples was done with Varian GC-MS –Saturn 2200. The inert gas helium (99.9995%) was used as carrier gas, at flow rate of 1.0 ml/min, Split ratio 20:1; sample size, 1µL injected using the splitless injection procedure; fused capillary silica columnVF5 ms (30 m × 0.25 mm × 0.25 µm). Temperatures: injector: 250˚C, detector: 300˚C, column: 100˚C, 10˚C min–1, 270˚C (20 min). The overall GC running time was at 63 min.

The MS was performed at 70 eV. The MS scan parameters included a mass range ofm/z 40-600, a scan interval of 0.5 s, a scan speed of 2000 amu s–1, and a detector voltage of 1.0 kV. Identification of constituent compounds was conducted using the database of NIST Libraries. Mass spectrum of individual unknown compounds was compared

with the known compounds stockpiled in software database Libraries. The name, molecular weight and structure of the components of the test materials were determined.

2.7. Estimation of *in vitro* antioxidant activity

The antioxidant properties of *D. gyrans* were analysed by determining the scavenging effect of the generation of free radicals such as superoxide, DPPH, hydroxyl, ABTS radicals and inhibition of lipid peroxidation in various *in vitro* assay system.

2.8. Anti-inflammatory activity

For assessing the anti-inflammatory activity, carrageenan induced paw edema (acute inflammation) and formalin induced paw edema (chronic inflammation) model were employed.

2.8.1. Carrageenan induced paw edema.

Female Balb/c mice were divided into five groups comprising six animals in each group. Group I with sub-plantar injection of 0.02 ml freshly prepared 1% suspension of carrageenan on right hind paw served as positive control. Group II was administered with diclofenac (10 mg/kg b.wt) intraperitoneally, as standard reference drug one hour prior to injection of carrageenan. Group III and group IV were treated with *D. gyrans* (100 and 250 mg/kg b.wt) orally for five consecutive days. On the fifth day, acute inflammation was induced by sub-plantar injection of freshly prepared carrageenan on right hind paw along with the other groups[11]. The inflammation was measured using vernier calipers one hour before and for 5 hours after carrageenan injection on an hourly basis (Table 3). Blood was collected by tail vein bleeding before the start of carrageenan injection and at 3rd and 5th hr after it. Serum was used for the analysis of high sensitive C- reactive protein (hs CRP) using turbidimetric method and determination of nitric oxide [12].

2.8.2. Formalin induced paw edema.

Female Balb/c mice were divided into five groups comprising six animals in each group. Group I with sub-plantar injection of 0.02 ml freshly prepared 2% formalin on right hind paw served as positive control. Group II was administered with diclofenac (10 mg/kg b.wt) intraperitoneally, as standard reference drug one hour prior to injection of 2% formalin. Group III and group IV were treated with *D. gyrans* (100 and 250 mg/kg b.wt) orally for five consecutive days. On the fifth day, acute inflammation was induced by sub-plantar injection of 0.02 ml of freshly prepared 2% formalin on right hind paw along with the other groups[13]. The inflammation was measured using vernier calipers before and after injection of formalin and for six consecutive days. Increase in thickness as a measure of inflammatory edema was calculated using the formula,

$Pt - P_0$ where P_o is the initial thickness prior to induction. Percent inhibition of inflammation is calculated by the formula $P_c - P_t/P_c \times 100$. Where P_t is the increase in thickness of the treated. P_c is the control. Graph is also plotted using mean and SD was calculated .

2.9. Statistical analysis

All values were assessed as the mean ± standard error of the mean (SEM). The data were subjected to one way analysis of variance (ANOVA) and comparison was made by *Dunnetpost hoc* test for multiple comparisons among groups. A ‘p’ value less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

3.1. Acute oral toxicity study

Mortality was not reported in the acute toxicity study after observation for 7 days. Blood collected from each group was subjected to renal and liver function tests gave normal results.

3.2. Preliminary phytochemical screening

As reported in Table 1, phytochemical analysis on extracts prepared with different solvents showed the presence of phytochemical constituents like sterols, triterpenoids, flavonoids, phenols, saponin, alkaloids and tannins. Methanolic extract of *D. gyrans* showed the presence of alkaloids, sterols, terpenoids, tannins, flavonoids and phenolic compounds. Ethyl acetate extract, chloroform extract and acetone extract also showed the presence important constituents.

Table 1. Qualitative phytochemical analysis of *Desmodium gyrans* extracts in different solvents

Phytochemical Tests	Petroleum ether	Toluene	Chloroform	Ethyl acetate	Acetone	Methanol	Water
Alkaloids	+	-	-	-	-	+	+
Phytosterols	+	+	+	-	-	+	-
Saponins	-	-	-	-	-	-	-
Tannins	-	-	-	+	-	+	+
Steroids	-	-	+	+	+	-	-
Terpenoids	+	-	+	+	-	+	+
Flavonoids	-	+	+	+	+	+	-
Volatile oils	-	+	+	-	-	-	-
Phenolic compounds	-	-	-	-	+	+	+

The result suggested that methanol is more efficient solvent for extraction of constituents from *D. gyrans* whole plant, when compared to other solvents. Petroleum ether extract provided very less extractable phytochemicals.

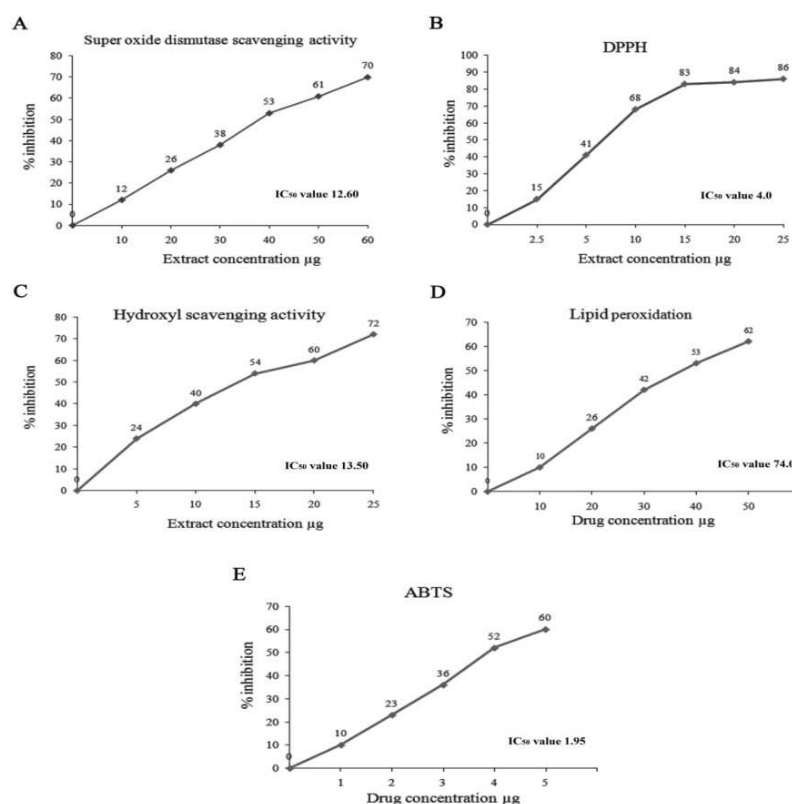

Figure 1. ***In vitro*** **free radical scavenging activity of** ***Desmodium gyrans***

Note: (a) Superoxide radical scavenging activity (b) DPPH scavenging activity (c) Hydroxyl radical scavenging activity (d) Inhibition of lipid peroxidation (e) ABTS radical scavenging activity

3.3 Antioxidant and anti-inflammatory activity

In vitro analysis of *D. gyrans* extract revealed the antioxidant potential of *D. gyrans* (Figure 1). It showed the superoxide scavenging activity of different concentrations of extract and IC_{50} value for this extract was found to be

12.6 µg/ml. The IC_{50} value of the extract in scavenging the generated DPPH and hydroxyl radical were found to be 4.0 µg/ml and 13.5 µg/ml respectively. The dose dependent activity of the extract in scavenging ABTS radicals was given by IC_{50} value of 1.95 µg/ml. The efficiency in inhibiting lipid peroxidation was shown by an IC_{50} value of 74 µg/ml. All these values were compared with that of a standard antioxidant namely Vitamin C.

The results revealed antioxidant efficacy of 70% methanolic extract of *D. gyrans* in scavenging free radicals generated in antioxidant assays and in inhibition of lipid peroxidation. Superoxide is generated as an unwanted byproduct of mitochondrial respiration as well as by activity of several cellular enzymes [14]. The oxygen radical may have several effects either directly or through generation of other free radicals all of which lead to oxidative damages, endothelial dysfunction and altered gene transcription. Reactive Oxygen Species (ROS) are reported to be involved in diabetes, hypertension, atherosclerosis, heart failure and cancer [15].

Table 2. Acute anti-inflammatory Study

Groups	Initial paw thickness (mm)	Paw thickness on 3rd hr (mm)	Increase in paw thickness (mm)	% inhibition
Control	1.786 ± 0.0873	3.39 ± 0.1149	1.604	
Diclofenac 10 mg/kg b.wt	1.946 ± 0.1303	2.728 ± 0.1519	0.782	51.24
D. gyrans 100 mg/kg b.wt	1.815 ± 0.0880	3.21 ± 0.1956	1.395	13.02
D. gyrans 250 mg/kg b.wt	1.795 ± 0.1249	3.12 ± 0.0867	1.325	17.39

Table 3. Chronic anti-inflammatory Study

Groups	Initial paw thickness (mm)	Paw thickness on 6th day (mm)	Increase in paw thickness (mm)	% inhibition
Control	1.864 ± 0.072	3.302 ± 0.165	1.438	
Diclofenac 10 mg/kg b.wt	1.73 ± 0.050	2.324 ± 0.0859	0.594	58.6
D. gyrans 100 mg/kg b.wt	1.823 ± 0.084	3.061 ± 0.078	1.238	13.9
D. gyrans 250 mg/kg b.wt	1.843 ± 0.094	2.825 ± 0.0148	0.982	31.71

The percentage inhibitions were found to be 13.02 and 17.39 in acute anti-inflammatory study and 13.9 and 31.71 in chronic anti-inflammatory experiments as summarised in Table 2 and 3 (Figures 2 and 3). Inflammation is basically a protective mechanism of the body and is considered as the second line of defense in the functioning of immune system. But inflammation can become harmful when it turns hypersensitive. Inflammations can be acute or chronic depending on the severity and duration of the development. The significant antioxidant activity of the extract observed in the current study can be considered contributing to its anti-inflammatory property also.

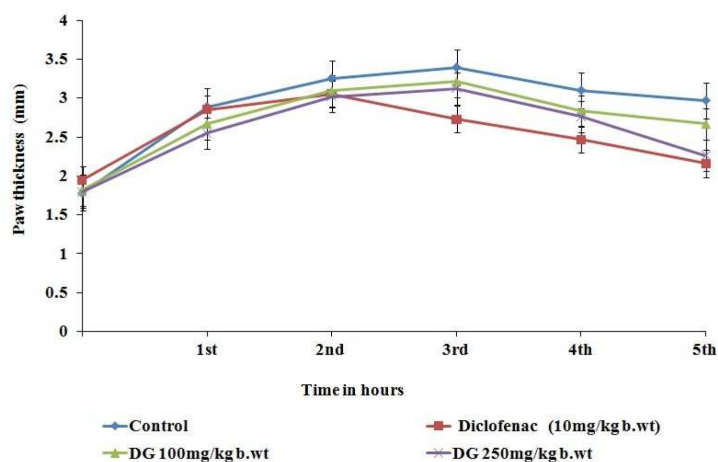

Figure 2. Effect of *Desmodium gyrans* on carrageenan induced paw edema (Acute inflammation)

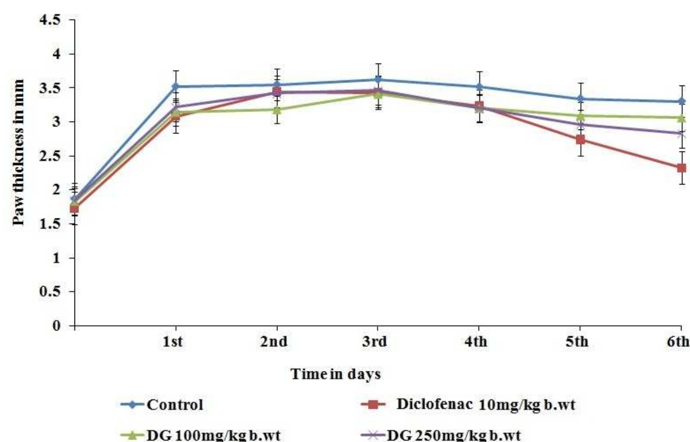

Figure 3. Effect of *Desmodium gyrans* on formalin induced paw edema (Chronic inflammation)

Carrageenan-induced edema is mediated through the release of prostaglandin and slow reacting substances of inflammatory response which peak at 3 hrs [16, 17]. Development of edema upon administration of carrageenan in the rat paw is a biphasic event[18]. In the initial phase (0-2.5 hrs after carrageenan injection, release of mediators namely histamine, serotonin and kinins occur [19]. In the second phase increased production of inducible cyclooxygenase (COX) leads to increased synthesis of prostaglandins followed by marked increase in cellular infiltration and liberation of acute inflammatory mediators such as myeloperoxidase and cytokines (IL-1β, IL-6, TNF-α) at the inflammatory site. The subsequent release of reactive oxygen species (ROS) and reactive nitrogen species, mediate the exaggerated inflammatory response [20,21]. The prostaglandin, particularly contribute to increased blood flow through a vasodilatation in this phase. The second phase is found to be sensitive to most of the clinically effective anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) [22, 11].

Table 4. Effect of *Desmodium gyrans* extract on serum CRP and nitric oxide level during acute inflammation

	hs - CRP (mg/dl)			NO (µmol)		
Time	Control	*DG* 100 mg	*DG* 250 mg	Control	*DG* 100 mg	*DG* 250 mg
0hr	0.532 ± 0.012	0.523 ± 0.011	0.525 ± 0.011	28.87 ± 1.98	29.02 ± 2.08	28.92 ± 1.58
3rdhr	1.383 ± 0.010	1.124 ± 0.010	0.978 ± 0.011	46.78 ± 1.73	38.38 ± 1.75	36.23 ± 2.09
5thhr	1.297 ± 0.011	1.032 ± 0.010	0.833 ± 0.012	39.82 ± 2.08	36.42 ± 1.86	31.62 ± 1.23

The level of hs-CRP and nitric oxide are found reduced by the treatment with *D. gyrans* extract in acute anti-inflammatory study (Table 4). C- reactive protein is considered also as a causative agent for inflammatory response, besides being an inflammatory marker. So the observed reduction in CRP can be considered indicative of the anti-inflammatory properties of the extract. Inflammatory response is aggravated by activated macrophages which release NO, a powerful chemical having cytotoxic effects [23]. Despite the fact that NO is considered as a molecule which protects endothelium from injuries with its effects on vasodilation, NO is having a harmful role of getting converted into peroxynitrates which in turn cause various chemical reactions in biological structures. These include nitration of tyrosine residues in proteins, lipid peroxidation, interference during mitochondrial transport and oxidation of biological thiol molecules[24]. *D. gyrans* extract is able to closely regulate the release of NO, leading to reduction of inflammatory reactions [25]. The anti-inflammatory activity of the plant extract can be credited to the potential to slow down the mediators of inflammatory reactions, especially the production of substances involved in the second phase of acute inflammatory response in addition to free radical scavenging.

Chronic inflammation has significant role in promoting chronic diseases like cardiovascular disease, diabetes and cancer and suppressing chronic inflammation may contribute in preventing them. Formalin induced paw edema in rats is widely used for screening potential anti-inflammatory agents. Formalin induced chronic inflammation also is biphasic with an early neurogenic component involving release of bradykinin being followed by a later tissue-

mediated response involving release of histamine, 5-hydroxytryptamine and prostaglandin. *D. gyrans* extract may be imparting an inhibitory role in these processes rendering protection against chronic inflammation.

The deployment of *D. gyrans* in asthma, cough, as emollient and in wound healing is justified since the study verifies antioxidant and anti-inflammatory properties of the extract. The reason for successful traditional use of *D. gyrans* in heart ailments is possibly due to its anti-inflammatory properties which assist in inhibiting atherogenesis since atherosclerosis is considered also as an inflammatory disorder [26,27].

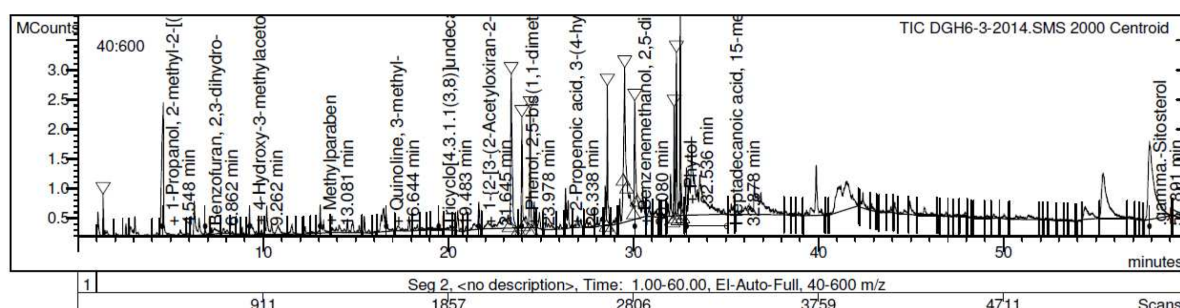

Figure 4. Chromatogram of GC-MS analysis

GC-MS analysis showed the presence of phytochemicals active in inhibiting inflammation thus validating the anti-inflammatory activity of *D. gyrans* extract (Figure 4). Some of the fragmented compounds revealed in GC-MS like, Hexadecanoic acid, 2,3,dihydro benzofuran,[28] phytol,[29,30] 2,6-dihexadecanoate, ascorbic acid, dexamethazone, 9,12,15-Octadecatrienoic acid are having hypolipidemic, antioxidant, anti-inflammatory effects. Hexadecanoic acid inhibit inflammation by way of inhibiting phospholipase A(2) responsible for ester bond hydrolysis of membrane phospholipids and consequent release of fatty acids which is believed to initiate inflammation [31]. 9,12,15-Octadecatrienoic acid may reduce cardiovascular risk through a variety of biologic mechanisms, including regulation of platelet function, inflammation and endothelial cell function [32]. The presence of these identified compounds and other phytochemicals in the plant may be responsible for the medicinal properties of *D. gyrans*.

CONCLUSION

The medicinal properties of *Desmodium gyrans* can be credited to its distinct antioxidant properties and anti inflammatory activities. These findings validate the use of this plant in traditional medicine.

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