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## Assessment of antiinflammatory properties of chlorophytum laxum R. Br.

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### ABSTRACT

*Medicinal plants are those plants which are used in various traditional systems of medicines throughout the world that provide people with medicines - to prevent disease, maintain health or cure ailments. These medicinal plants consider as a rich resource of ingredients which contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Chlorophytum laxum R.Br. (commonly called as "Neeruvatti") belongs to the family Liliaceae is one of the important medicinal plant used by Kani tribes. The present study aims to scientifically evaluate the anti-inflammatory potential of Chlorophytum laxum R. Br. In this study, Carrageenan and Formalin-induced hind paw oedema models were used for in vivo anti-inflammatory study and HRBC membrane stabilization assay was used as in vitro anti-inflammatory study. The extract was administered at different doses and the maximum percentage inhibition of paw oedema in the right hind limb was shown by 450 mg/kg in both in vivo methods. The extract at a higher concentration protects significantly the hypotonicity induced hemolysis of RBC by in vitro anti-inflammatory analysis. These reveal the anti-inflammatory potential of Chlorophytum laxum. Hence the present study substantiates the medicinal properties of Chlorophytum laxum which is having antiinflammatory potential that have to be used commercially for the drug preparation.*

**Keywords**— Medicinal plants, Chlorophytum laxum, Antiinflammatory

### 1. INTRODUCTION

Medicinal plants are those plants which are used in various traditional systems of medicines throughout the world that provide people with medicines - to prevent disease, maintain health or cure ailments. Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of the indigenous system of medicine. Green plants synthesis and preserve a variety of biochemical products, many of which are extractable and used as chemical feedstocks or as raw material for various scientific investigations.

Inflammation is a vital part of the body's immune response. Inflammation is a biological response to harmful stimuli, such as pathogens, damaged cells or irradiation. It is a protective attempt by the organism to remove injurious stimuli and to initiate the healing process. It is characterized by pain, redness, heat, swelling, and disturbance of function. Antioxidants are exogenous (natural or synthetic) or endogenous compounds acting to reduce or diminish the oxidative stress. They are a defense mechanism produced by the body to neutralize the effects of ROS. Antioxidants inhibit the rate of reaction of an oxidizable substrate with oxygen by reacting with a free radical early in the oxidation process.

*Chlorophytum laxum* R.Br. (commonly called as "Neeruvatti") belongs to the family Liliaceae. It is one of the important medicinal plant used by Kani tribes of Kerala, Karnataka, Maharashtra and Madhya Pradesh for inflammation, insect bites, and snake bites. Ethnobotanically the tubers of *Chlorophytum laxum* have been used as a folk medicine for the treatment of traumatic injury, poisonous snake bites, swelling and pain, diarrhoea, dysentery, insect bite, and snake bites. The present study aims to scientifically evaluate the anti-inflammatory and antioxidant potential of *Chlorophytum laxum* R. Br.

### 2. MATERIALS AND METHODS

#### 2.1 Plant material

The tuber of *Chlorophytum laxum* R. Br. was selected based on the ethnobotanical claim of Kani tribe of Thiruvananthapuram district, Kerala. The tubers of *Chlorophytum laxum* R. Br. were collected from the hills of Western Ghats.

## 2.2 Preparation of extract

Solvent extracts (Ethanol) were prepared by refluxing 100g of the tubers of *Chlorophytum laxum* with an appropriate solvent in the ratio 1:10.

## 2.3 Determination of extract yield

Extract yield was calculated from the difference between the initial weight and final weight of the round bottom flask in which extract was concentrated.

## 2.4 Determination of solubility

A pinch of the extract was mixed in few drops of distilled water, 0.1 % Tween-80, 0.5 % Tween-80 and 1% Tween-80 in each mortar and pestle respectively. The extent of solubility was observed in each medium.

## 2.5 Acute toxicity study

Acute toxic effect of ethanolic extract of *Chlorophytum laxum* (ECL) was evaluated in Swiss albino mice as per OECD guidelines on acute oral toxicity test (Suja *et al.*, 2004). The crude extracts were suspended in a vehicle (0.5 % Tween 80) with distilled water before administration. Five groups (A- E), consisting of six animals each were used for the study. Oral administration of the drug was carried out in 4 groups and one group was maintained as control. The animals were then observed for once a day for the next 14 days. Animals were weighed and visual observations for mortality, behavioral pattern, and changes in physical appearance, injury, pain and signs of illness were monitored.

## 2.6 Preliminary phytochemical tests

**2.6.1 Standard phytochemical screening tests:** The qualitative phytochemical screening was carried out by using standard procedures of Harborne (1998).

**2.6.2 Total phenolic content:** The total phenolic content (TPC) was determined by spectrophotometry according to the method of Sakat *et al.*, (2009). 0.2 mL of the extract ECL (1mg/mL) was transferred in tubes containing 1.0 mL 10 % Folin-Ciocalteu's reagent. After 10 min, 0.8 mL of sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min and absorbance read at 743 nm. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 25 to 400 µg/mL.

## 2.7 Antiinflammatory studies

**2.7.1 In a *vitro* anti-inflammatory study:** The HRBC membrane stabilization has been used as a method to study the antiinflammatory activity (Gandidasan.R, 1991). Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alseiver solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water) the blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85 %, pH 7.4) and a 10% v/v suspension was made with isosaline. The assay mixture contains the drug (at various concentrations as mentioned in the table), 1 ml phosphate buffer (0.15 M, pH 7.4) and 2 ml of hypo saline (0.36%) and 0.5 ml of HRBC suspension. Diclofenac sodium was used as the reference drug. Instead of hypo saline, 2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage of hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

$$\% \text{ Protection} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

### 2.7.2 In vivo antiinflammatory studies

**i. Carrageenan induced paw oedema study:** The anti-inflammatory activity of the ethanolic extract of *Chlorophytum laxum* (ECL) was studied by the method, Carrageenan - induced rat paw oedema (Amdekar *et al.*, 2011). The animals were separated into five groups with six animals in each cage and were fasted overnight. 30 min after ECL/STD administration, 0.1 ml 1% carrageenan (in saline) was injected into the right hind paw, under the plantar apo-neurosis sub-cutaneously. The volume of hind paw was measured using a plethysmometer just before and 3h after carrageenan injection. The difference in the paw volumes was recorded, which indicates the degree of inflammation. The percentage inhibition of paw oedema was calculated by the given formula;

$$\% \text{ inhibition} = [(\text{control-test}) / \text{control}] * 100$$

**ii. Formalin induced paw oedema study:** The anti-inflammatory activity of the ethanolic extract of *Chlorophytum laxum* (ECL) was studied by the method, Formalin - induced rat paw oedema in wistar rats (Chau, 1989). The animals were divided into five groups of six animals each and fasted overnight. 30 min after ECL/STD administration, 0.1 mL formalin (2%) was injected into the right hind paw of the rats by sub plantar route. The drug was dispensed during an interval of 24h for consecutive 7 days. Formalin injection was given on the 3<sup>rd</sup> day after the 1<sup>st</sup> day injection. The paw thickness was measured plethysmo graphically on the 7<sup>th</sup> day after 1h of the experimental period. The mean difference in paw thickness (mm) was obtained from the difference in paw thickness before and after the induction of inflammation for each group. The difference in the paw volumes was recorded, which indicates the degree of inflammation.

$$\% \text{ Inhibition} = [(\text{Control-Test}) / \text{Control}] * 100$$

## Statistical analysis

The results obtained were expressed as the mean ± standard deviation (SD) and presented as graphs and tables. Data were analyzed by using a statistical software called GraphPad 5.0 (GraphPad Software, Inc., San Diego, CA) with one way analysis of variance (ANOVA) followed by Duncan's test. ANOVA was performed to compare the significant differences between groups and Duncan's test was carried out for pair-fed comparisons between groups. The level of significance was set at \*\*\* $p < 0.05$ .

### 3. RESULTS

**3.1 Extract yield:** The % yield of ethanolic extract of the whole plant was found to be 5.02.

**3.2 Extract miscibility:** The extract showed maximum solubility in distilled water and this solvent was selected as the vehicle for drug preparation.

**3.3 Oral acute toxicity studies:** The ethanolic extract of *C. laxum* (ECL) was administered to mice orally for the acute toxicity study at doses of 5, 50, 300 and 2000 (mg/kg body weight) for each group. No mortality and behavioural changes were observed even at the highest dose of 2000 mg/kg.

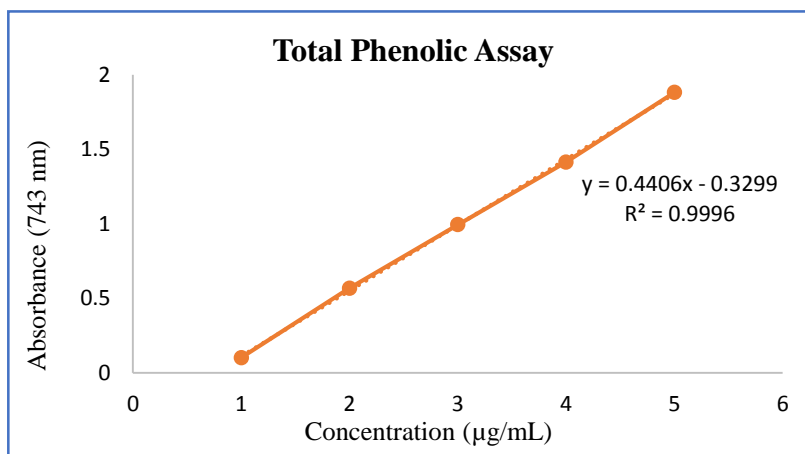
#### 3.4 Preliminary phytochemical test

**3.4.1 Standard phytochemical screening tests:** Qualitative analysis of the roots indicated the presence of alkaloids, phenols, saponins, tannins, proteins, sugars, glycoside and steroids (Table 1).

**3.4.2 Total phenolic content:** The values are expressed as Gallic acid equivalents (GAE). The total phenolic content of ethanolic extract of tubers of *C. laxum* was found to be 5.12 mg GAE/g of extract (Figure 1).

**Table 1: Phytochemical analysis of *C. laxum***

S. No.	Test	Results
1	<b>Alkaloids</b>	+
	Mayer's test	
	Wagner's test	
	Hager's test	
	Dragendorff's test	
2	<b>Flavonoids</b>	-
	Alkaline reagent test	
	Shinoda test	
	Lead Acetate test	
3	<b>Phenols</b>	+
	Lead acetate test	
4	<b>Tannins</b>	+
	Braymer's Test	
5	<b>Saponins</b>	+
	Foam test	
	Forth test	
6	<b>Carbohydrates</b>	+
	Fehling's test	
7	<b>Proteins</b>	+
	Xanthoproteic test	
	Biuret test	
8	<b>Steroids</b>	+
	Salkowski's test	
9	<b>Anthocyanins</b>	-
10	<b>Glycosides</b>	+
	Killer killiani test	
11	<b>Phlobatanins</b>	-



**Fig. 1: The total phenolic content of the ethanolic extract of *C. laxum* R. Br.**

Values are expressed as mean  $\pm$  SD, for n=6  
 The TPC of extract = 5.12 mg GAE/g of extract

3.5 Antiinflammatory studies

3.5.1 *In vitro* antiinflammatory study: The inhibition of hypotonicity induced HRBC membrane lysis was taken as a measure of the antiinflammatory activity. Ethanolic extracts of *C. laxum* at different concentrations (50, 100, 200 µg/mL) showed significant stabilization of HRBC membrane. The ethanolic extract of *C. laxum* at a concentration of 200 µg/mL offered maximum HRBC membrane protection. It showed the maximum inhibition of 55.69 % at 200 µg/mL. The EC<sub>50</sub> value of extract was found to be 162.47 µg/mL. The results were shown in table 2.

Table 2: *In vitro* anti-inflammatory activity of *C. laxum* R. Br.

Concentration (µg/mL)	(%protection) ECL	EC <sub>50</sub> value (µg/mL)	(%protection) Standard	EC <sub>50</sub> value (µg/mL)
Control	-	162.47	-	84.32
50	33.67 ± 0.66		41.65 ± 1.03	
100	40.34 ± 0.51		55.20 ± 0.78	
200	55.69 ± 0.74		72.93 ± 0.72	

Values are expressed as mean ± SD, for n=6.

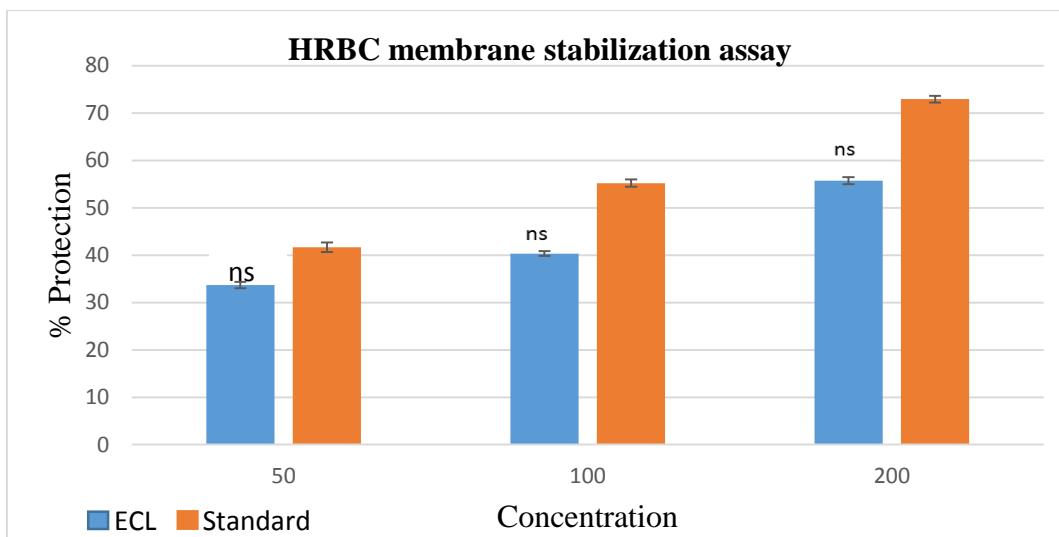


Fig. 2: *In vitro* antiinflammatory activity *C. laxum* R. Br

Values are expressed as mean ± SD, for n=6, one way ANOVA followed by Duncan's multiple comparison tests, ns- no significant difference compared with the standard.

3.5.2 *In vivo* antiinflammatory study

i. Carrageenan induced paw oedema in Wistar rats: The effect of ethanolic extract of *C. laxum* on carrageenan induced paw oedema was examined in adult Wistar rats. The percentage of inhibition of paw oedema volume is shown in Table 3 and the difference in paw oedema volume is shown graphically in Figure 3. The maximum percentage of inhibition of paw oedema (80.98%) was exhibited by a dose of ECL (450 mg/kg). The standard drug Indomethacin exerted a promising level of inhibition (87.61%) at a dose of 10 (mg/kg) body weight.

Table 3: Effect of ethanolic extract of *C. laxum* on Carrageenan induced paw oedema.

S. No.	Concentration (mg/kg)	Percentage of inhibition
1	Control	----
2	Indomethacin (10 mg/kg)	87.61 %
3	ECL (50 mg/kg)	33.34 %
4	ECL (150 mg/kg)	61.90 %
5	ECL (450 mg/kg)	80.98 %

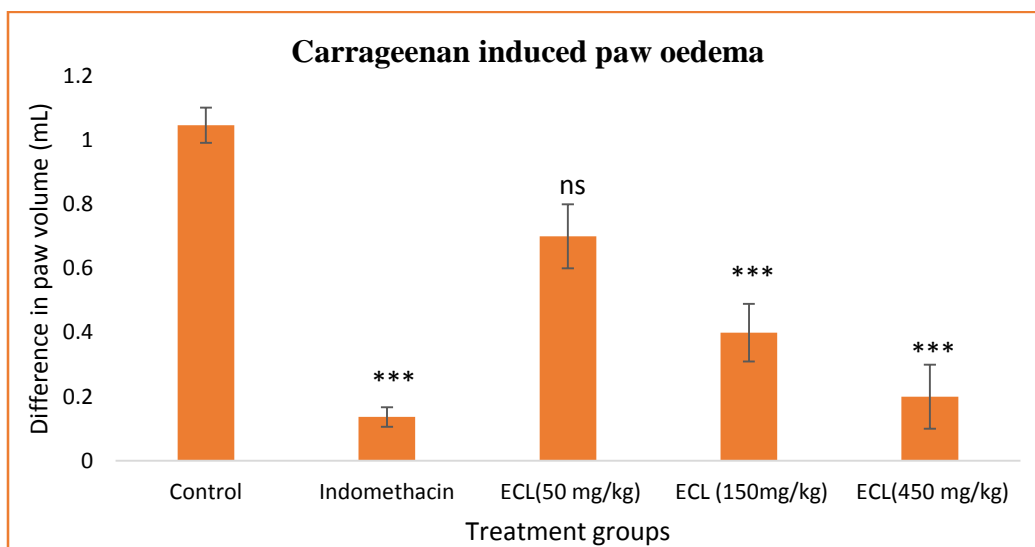
Values are expressed as mean ± SD, for n=6

ii. Formalin induced paw oedema in Wistar rats: The effect of ethanolic extract of *C. laxum* on formalin induced paw oedema in rats was studied. The percentage of inhibition of paw oedema volume on 1<sup>st</sup> and 7<sup>th</sup> day are shown in Table 4 and difference in paw oedema volume on 1<sup>st</sup> and 7<sup>th</sup> day are shown graphically in Figure 4. The degree of inflammation was found to be significantly reduced in the groups treated with 150 and 450 mg/kg ECL, where they showed maximum inhibitions of 60.34% and 72.41% respectively after 7 days of formalin treatment. The standard drug Indomethacin exhibited a percentage inhibition of 77.58% at a dose of 10 mg/kg. The results indicate the potential antiinflammatory activity of *C. laxum*.

Table 4: Effect of ethanolic extract of *C. laxum* on Formalin induced paw oedema

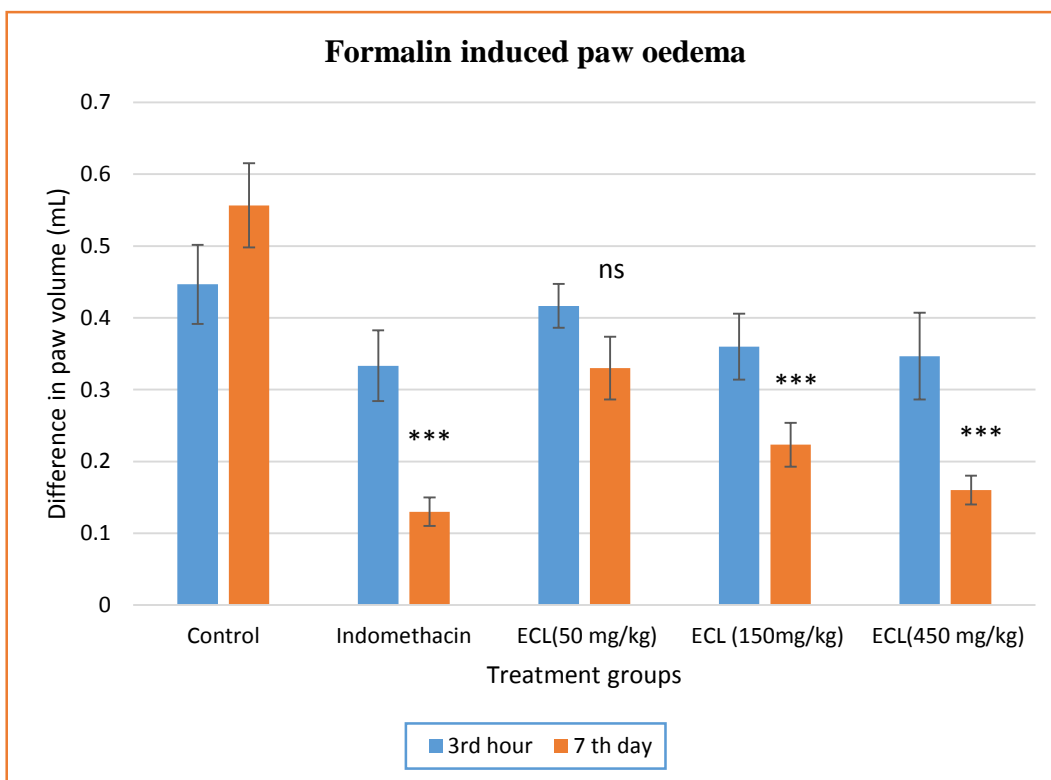
S. No.	Group	% Inhibition 1 <sup>st</sup> day	% Inhibition 7 <sup>th</sup> day
1	Normal	----	----
2	Standard (Indomethacin)	31.11	77.58
3	ECL (50 mg/kg)	8.88	48.27
4	ECL (150 mg/kg)	17.77	60.34
5	ECL (450 mg/kg)	24.44	72.41

The difference in paw volume of animals treated with ECL compared with the control group. Results are expressed as mean ± SD, for n=6.



**Fig. 3: Effect of ethanolic extract of *C. laxum* on Carrageenan induced paw oedema in rats**

Values are expressed as mean ± SD, for n=6, one way ANOVA followed by Duncan's multiple comparison tests, \*\*\* $P \leq 0.05$  and ns- no significant difference compared with control group.



**Fig. 4: Effect of ethanolic extract of *C. laxum* on Formalin induced paw oedema in rats**

Values are expressed as mean ± SD, for n=6, one way ANOVA followed by Duncan's multiple comparison tests, \*\*\* $P \leq 0.05$  and ns- no significant difference compared with control group.

#### 4. DISCUSSION

*Chlorophytum laxum* R.Br. is basically used for the treatment of piles and as astringent and the tuberous roots are being used as a well-known tonic and an aphrodisiac and for the treatment of diarrhea, dysentery, also used as demulcent and galactagogue. The tubers of *Chlorophytum laxum* is used for the treatment of insect bite and snake bites (Padal *et al* 2012). To meet the objective of the present study, antioxidant and anti-inflammatory properties of the *Chlorophytum laxum* has been investigated.

##### 4.1 Oral acute toxicity study

The objective of toxicity studies is to elucidate the toxic profile of a herbal drug. The acute toxicity studies are done in experimental animal models such as mice or rats. The acute toxicity study was carried out by orally administering 4 doses of ECL extract (5, 50, 300 and 2000 mg/kg) which showed no symptoms of toxicity or change in the behavioural or physiological pattern even at the highest dose (2000 mg/kg). So the plant extract could be considered safe for oral administration at this range. A similar study conducted by Jaijoy and his colleagues revealed that the water extract from the fruits of *Piper chaba* was found to be non-toxic even at the highest dose tested of 2000 mg/kg in Sprague-Dawley rats (Jaijoy *et al.*, 2011).

#### 4.2 Preliminary phytochemical screening

**Quantitative phytochemical analysis:** Phytochemicals are naturally occurring compounds, which work with nutrients and fibers to act against diseases or more specifically provide protection against diseases (Devasagayam *et al.*, 2004). The preliminary phytochemical screening of the plant was performed using standard methods. The medicinal value of plants has been claimed to lie in their phytochemical components including alkaloids, tannins, flavonoids, and other phenolic compounds, which produce a definite physiological action on the human body.

A preliminary phytochemical evaluation result of *C. laxum* has revealed the presence of carbohydrates, phenols, alkaloids, proteins, steroids, saponin, tannin, saponins and glycosides. Antimicrobial, analgesic, anti-inflammatory, anticancer activities have been reported for glycosides (Dembitsky 2006) and tannins. Saponins have shown anticancer activity, antimicrobial activity, analgesic and anti-inflammatory activity (Moharram and El-Shenawy, 2007). Hence the phytochemicals present in the plant may be responsible for its medicinal properties.

#### 4.3 Total phenolic content

Phenolic compounds are the largest group of phytochemicals and responsible for the antioxidant activity of plants or plant products (Ji *et al.*, 2011). Phenolic compounds are ubiquitous secondary metabolites in plants which are known to have antioxidant activity (Tepe *et al.*, 2006). The results obtained in this study showed a considerable level of phenolic content. From the experiment, the content of total phenols in the ethanolic extract of *C. laxum* expressed as Gallic acid equivalents per gram of dry extract is 5.12 mg GAE/g of extract.

#### 4.4 Antiinflammatory studies

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al.*, 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al.*, 2010). The complex inflammatory reactions involve the release of a wide variety of inflammatory mediators i.e. prostaglandins, thromboxanes, and leukotrienes. When tissue cells become injured they release kinins, prostroglandins, and histamine. These work collectively to cause increased vasodilation (widening of blood capillaries) and permeability of the capillaries.

*In vitro* as well as *in vivo* methods are used in determining antiinflammatory activity by plant extracts. For the present study, Carrageenan and Formalin induced hind paw oedema models were used for *in vivo* anti-inflammatory study. HRBC membrane stabilization assay was used as *in vitro* anti-inflammatory study. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) was used as the standard for antiinflammatory studies.

#### 4.5 Carrageenan induced hind paw oedema

Carrageenan is a mucopolysaccharide derived from Irish Sea moss, *Chondrus*. The inflammation induced by carrageenan is acute, non-systemic effects, non-immune as well as a high degree of reproducibility (Hafeez *et al.*, 2013). The development of oedema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin, and prostaglandin like substances (Vinegar *et al.*, 1969).

The ethanolic extract of *C. laxum* showed inhibition of carrageenan induced paw oedema in a dose dependent manner and the maximum inhibition was observed at 450 mg/kg (80.98%), which showed a significant similarity to the standard drug Indomethacin (87.61%). The effect of the extract may be due to the inhibition of inflammatory mediators such as prostaglandins, histamines, cytokines etc., either in an independent way or in a synergistic approach by the secondary metabolites in the plant extract.

#### 4.6 Formalin induced hind paw oedema

Formalin induced paw edema in rats is one of the most suitable procedure to screen the acute inflammation and it is a biphasic event. Formalin induction causes the changes in connective tissue metabolism, is one of the major biochemical events during the process of inflammation. These changes are effected in the alteration of the relative composition of various constituents of connective tissue such as mucopolysaccharides, glycoprotein, hexosamine, and hydroxy proline, sialic acid (Houck and Jacob, 1960).

In case of ECL administration, the maximum reduction in paw volume was observed at the dose 450 mg/kg after the 7<sup>th</sup> day (72.41%), which was significantly comparable that of the standard drug Indomethacin (77.58%). The results suggest a possible inhibition of inflammatory mediators in the inflammation cascade by the phytochemicals present in *C. laxum*.

#### 4.7 *In the vitro* antiinflammatory study

The HRBC membrane stabilization has been used as a method to study the *in vitro* anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane (Shenoy *et al.*, 2010) and its stabilization implies that the extract will stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. HRBC membrane is similar to lysosomal membrane components the prevention of hypo tonicity induced HRBC membrane lysis is taken as a measure of the anti-inflammatory activity of drugs (Rajendran and Lakshmi, 2008).

The results showed that ECL at a concentration of 200 µg/mL protects significantly hypotonicity induced haemolysis of RBC. At the highest concentration (200 µg/mL), ECL showed maximum inhibition of 55.69 %, as compared with the control Diclofenac sodium which showed 72.93 % inhibition of RBC haemolysis. The EC<sub>50</sub> value of extract was found to be 162.47 µg/mL as compared with a standard having an EC<sub>50</sub> value of 84.32 µg/mL.

## 5. CONCLUSION

*Chlorophytum laxum* R. Br. (Neeruvatti) of family Liliaceae is one of the important medicinal plant used by Kani tribes of Kerala. The tubers of *Chlorophytum laxum* R. Br. were collected from the hills of Western Ghats and maintained at JNTBGRI to conduct the pharmacological studies. Extraction procedures were carried out to prepare the drugs of different doses for the study. Acute oral toxicity studies in mice and antiinflammatory studies in rats were done as pharmacological analysis. In the preliminary phytochemical investigation, standard phytochemical screening and total phenolic content of *Chlorophytum laxum* were carried out. The preliminary phytochemical screening of tubers of *Chlorophytum laxum* has shown the presence of secondary metabolites like carbohydrates, phenols, alkaloids, proteins, steroids, saponin, tannins, saponins and glycosides that may be responsible for its medicinal properties. The study also showed the presence of phenolic content in the tuber extract from the results obtained. Toxicity studies of *Chlorophytum laxum* tuber extract were investigated in Swiss albino mice for 14 days by the administration of 4 doses (5, 50, 300 and 2000 mg/kg body weight) and no symptoms of toxicity were seen in the animals even up to the highest dose.

The antiinflammatory potential of tuber extract was investigated *in vivo* by carrageenan induced paw oedema and formalin induced paw oedema and *in vitro* by HRBC membrane stabilization assay. The ECL extract was administered at doses (50, 150 and 450 mg/kg) of body weight orally in adult wistar rats and the maximum percentage inhibition of paw oedema in the right hind limb was shown by ECL 450 mg/kg in both the methods. ECL at higher concentration protect significantly the hypotonicity induced haemolysis of RBC by *in vitro* antiinflammatory analysis. These reveal the antiinflammatory potential of *C. laxum*.

The present study substantiates the medicinal properties of *C. laxum* which is having antiinflammatory and antioxidant potential that have to be used commercially for the drug preparation. Further studies in future are to be needed to explore the exact mechanism behind the action of *Chlorophytum laxum* R. Br. in analyzing their bioactivities.

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