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STUDIES ON THE ANTI-INFLAMMATORY AND ANALGESIC EFFICACY OF *SCINDAPSUS OFFICINALIS* (ROXB.) SCHOTT IN LABORATORY ANIMALS

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ABSTRACT: This research was focused on the qualitative and quantitative evaluation of anti-inflammatory and analgesic effects of ethanolic extract of *Scindapsus officinalis* (EESO) fruit in laboratory animals and whether these effects were of any statistical significance. Carrageenan-induced Hind Paw Edema test in long evans rat was the experiment for anti-inflammatory activity of the ethanolic extract of *Scindapsus officinalis* fruit while hot plate test was carried out to assess its analgesic activity in swiss albino mice. At two different doses of 250 and 500 mg/kg body weight, the analgesic test was evaluated on mice and the anti-inflammatory test was evaluated on rats by the ethanolic extract of the fruit. Phytochemical analysis of ethanolic extract of *Scindapsus officinalis* has indicated the presence of steroid, carbohydrate, flavonoid, alkaloid, tanin, saponin and terpenoid-compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us for its possible analgesic and anti-inflammatory activities. The experimental activities for the ethanolic extract of *Scindapsus officinalis* fruit exhibited statistically significant ($p<0.05$) anti-inflammatory activity in Carrageenan-induced Hind Paw Edema in long evans rat and statistically significant ($P<0.05$) analgesic activity in swiss albino mice in a dose-dependent manner. In conclusion, these observations provide evidence and possible mechanisms of action for the anti-inflammatory and analgesic properties of fruit of *Scindapsus officinalis* claimed in Ayurveda medicine. Further studies should be undertaken to correlate the pharmacological activities with the chemical constituents of the fruit of *Scindapsus officinalis*.

INTRODUCTION: *Scindapsus officinalis* (Roxb.) Schott. is one of the plant used in Indian system of medicine which belongs to family Araceae. It is common in the Midnapore district of west Bengal and cultivated vegetatively for its fruit, which is cut into transverse pieces, dried and used medicinally.

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Fruit is very important part of the plant and accepted as raw drug of known properties in both Ayurvedic and Unani system of medicine. The fruit of *Scindapsus officinalis* is known as Gajpeepal in Ayurveda. Gajpeepal consists of dried, transversely cut pieces of mature female spadix of *Scindapsus officinalis* (Fam. Araceae). It is found all along the sub-Himalayan tract between an altitude of 330-1000 m in West Bengal, Orissa, Andhra Pradesh and the Andaman Islands. Fruit occurs in transversely cut circular pieces of about 2.0-3.0 cm in diameter and 2.0-3.5 cm thick, brownish grey, rough and scaly. It has the significant antioxidant property due to presence of flavonoids and phenolic compound and has ability of cytoprotection due to antioxidant property [1].

The present study was undertaken to find out the possible actions of ethanolic extract of *Scindapsus officinalis* fruit for its anti-inflammatory and analgesic activity using hot plate method in swiss albino mice and Carrageenan-induced hind paw edema method in long evans rat respectively.

MATERIAL AND METHOD: Hot Plate (Model – 35100, UGO BASILE, Italy), Balance, Refrigerator, Beakers, Petri dishes & glass wrought, Safety rat handling gloves, Mortar & pestle, Hypodermic , Syringes, Holder & test tube, Hot waterbath, Plethysmometer

Medicinal plants (extracts): Extract were examined in two concentrations of 500mg/kg and 250mg/kg body weight of animal

Control & Positive Control:

Analgesic activity:

1. Control – distilled water
2. Positive control – Diclofenac sodium

Administered dose – 50mg/kg body weight animal

Anti-inflammatory activity:

1. Control – Normal saline
2. Positive control – Diclofenac sodium

Administered dose – 50mg/kg body weight animal

Experimental animal: Swiss albino mice (male and female), weighing 20-30g bred in International Centre for Diarrheal Diseases and Research, Bangladesh(ICDDR,B) and grown in the Animal House of the Department of Pharmacy, North South University (NSU). Long Evans rats (male and female), weighing 80-200g of either sex, bred in NSU and ICDDR,B and grown in the animal house of the Department of Pharmacy, NSU.

All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 20C, and 12 hours light dark cycle).

The animals were fed with standard diet from ICDDR, B and had free access to filtered water [2].

Plant Extraction method:

Collection: The plant sample of *Scindapsus officinalis* was collected from an Ayurvedic Institution 'Back to Nature' during 18.06.2012 in the form of fruit shavings. The fruit of the plant was collected and washed with water several times.

Drying and grinding: The collected fruit was washed with water, separated from undesirable materials or plant parts, partially dried by fan aeration and then fully dried in the oven at below 40°C for 2 days. The fully dried fruit was then grinded to a powdered form and stored in the refrigerator at +4°C for a few days.

Cold extraction (Ethanol extraction): 542gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 2300 ml of 80% ethanol, sealed and kept for a period of 2 days with occasional shaking and stirring. It was then filtered first by cotton material and twice through whatman filter paper to obtain a finer filtrate. The filtrate (Ethanolic extract) obtained was evaporated by Rotary evaporator (Eyela n 1000, Tokyo Rikaki kai co.ltd, Rotary vacuum, Japan) at 4 to 5 rpm and at 65^{o}c temperature. The separated filtrate was found to be a precipitate of dark brown chocolate color and the gummy concentrate was designated as the crude ethanolic extract of the fruit of *Scindapsus officinalis*. It was then dried in the freeze drier and preserved at +4°C for two weeks.

Analgesic activity of *Scindapsus officinalis*:

Study design: Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV consisting of 6 mice in each group. Individual weighing was done to adjust individual doses. Here, distilled water was given to group I, 50 mg/kg Diclofenac sodium for group II, 250 mg/kg for group III and 500mg/kg for group IV of the crude extract of *Scindapsus officinalis*.

Mice Screening: Young Swiss-albino mice aged 4-5 weeks, average weight 20-30 gm were used for this study. They were kept in standard environmental condition for one week in the animal house of the

Department of Pharmacy, North south University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water ad libitum and maintained at natural day night cycle.

Mice screening was performed before Hot plate test. In that experiment mice with significant response action (Licking, Shaking and Jumping) and response time (at the range of 0-20 seconds) were selected.

Hot Plate Test method: The hot-plate test employed for measurement of analgesic activity which was previously described by Lanhers et al. (1992) and modified by Mahomed and Ojewole (2004). A comparison of Hot plate test was made between positive control (Diclofenac sodium), control and test sample given orally 30 minutes after hot plate induction. Positive analgesic activity was shown when sample animal gave longer number of stimuli than the control, or the sample. The temperature of the metal surface of the hot plate was maintained at 55 ± 0.2°C. Latency to a discomfort reaction (licking, shaking or jumping) was determined before and after drug administration. The cut-off time was fixed at 15s to avoid the damage to the animal paw.

The latency was recorded at 0, 30, 60, 120, 180, 240 min following oral administration of the agents. The prolongation of the sample latency time compared with that of control was used for statistical comparison. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced nociceptive pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken (reaction time). Each mouse served as its own control [3, 4] . Before treatment, its reaction time was taken once. The mean of these values on determination constituted initial reaction time before treatment of the mouse.

Each of the test mice were thereafter treated with either distilled water, diclofenac sodium (50mg/kg of body wt) and ethanol extract at the doses of *Scindapsus officinalis* 250 mg/kg and 500 mg/kg body wt. orally. Thirty min after treatment, the reaction time of each group mice were again evaluated five times individually in one hour interval on this occasion. Percent analgesic score was calculated as:

$$(PAS) = Tb\text{-}Ta/Tb \times 100$$

where, Tb= Reaction time (in second) before drug administration, Ta = Reaction time (in seconds) after drug administration

Anti-inflammatory Effect of *Scindapsus officinalis*

Preparation of inflammatory agent: Carrageenan was used as inflammatory agent in this experiment. It was obtained from Jahangirnagar University. Carrageenan powder was suspended in 5 ml saline to make 0.1% suspension and kept in water bath for proper homogenization. The tube was kept in hot water (50±2˚c) containing beaker to prevent transformation into a jelly like compound. Long Evans rats (male and female), weighing 80-200g of either sex were collected from ICDDRB for the study and were kept in standard environmental condition for weeks in the animal house of the Department of Pharmacy, North south University, Bangladesh for adaptation after their purchase.

Carrageenan-induced Rat Hind Paw Edema Test: The ethanolic extract of *Scindapsus officinalis* on carrageenan induced inflammation in rat paw was investigated by following the method of Winter et al (1962) with minor modifications. Rats were randomly divided into four groups, each consisting of six animals, of which group I was kept as control giving only water .Group II was given carrageenan as inflammatory agent. Group III and group IV were given the test sample at the dose of 250 and 500 mg/kg body weight respectively.

Half an hour after oral administration of the test materials, 0.1ml 0.1% carrageenan suspension was injected subcutaneously in left hind paw of each animal leading to the formation of edema in situ (localized inflammation). The volume of paw edema was measured at 1, 2, 3, 6 and 8 hours using water plethysmometer after administration of carrageenan. The right hind paw served as a reference non inflamed paw for comparison [5,6]. The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula-

$$\% \text{ Inhibition of paw edema} = [1- (Vt / Vc)] X 100$$

where Vc and Vt represent average paw volume of control and treated animal respectively.

Statistical analysis: All the results were expressed as Mean ± Standard deviation (SD). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. P values <0.05 were considered as statistically significant.

RESULT:

Anti-inflammatory Activity:

TABLE 1: ANTI-INFLAMMATORY EFFECT OF ETHANOLIC EXTRACT OF *SCINDAPSUS OFFICINALIS* ON CARRAGEENAN INDUCED RAT PAW INFLAMMATION

Treatment	0 Hr	1 Hr	2Hr	3 Hr	6 Hr	8 Hr
Control	0.68±.048	0.87±.059	1.17±.011	1.43±.056	1.52±.055	1.60±.054
Standard	0.65±.390	0.85±.261	0.99±.036	1.24±.046	1.02±.028***	0.79±.020***
Scindapsus (250mg/kg)	0.81±.33	1.11±.062**	1.28±.073***	1.38±.205	1.14±.064***	0.99 ±.231***
Scindapsus (500mg/kg)	0.84±.368	1.14±.035***	1.31±.018***	1.40±.044	1.16±.027***	0.99±.037***

TABLE 2: PERCENT INHIBITION OF THE STANDARD AND TWO DIFFERENT CONCENTRATIONS OF THE EXTRACT COMPARED WITH THEIR RESPECTIVE MEANS AT 0 HOUR

Treatment	% inhibition 1 hour	% inhibition 2 hour	% inhibition 3 hour	% inhibition 6 hour	% inhibition 8 hour
Standard	29.96	51.68	89.90	56.26	20.48
Scindapsus (250mg/kg)	37.03	58.27	70.37	40.74	22.96
Scindapsus (500mg/kg)	41.46	55.10	66.03	38.24	18.65

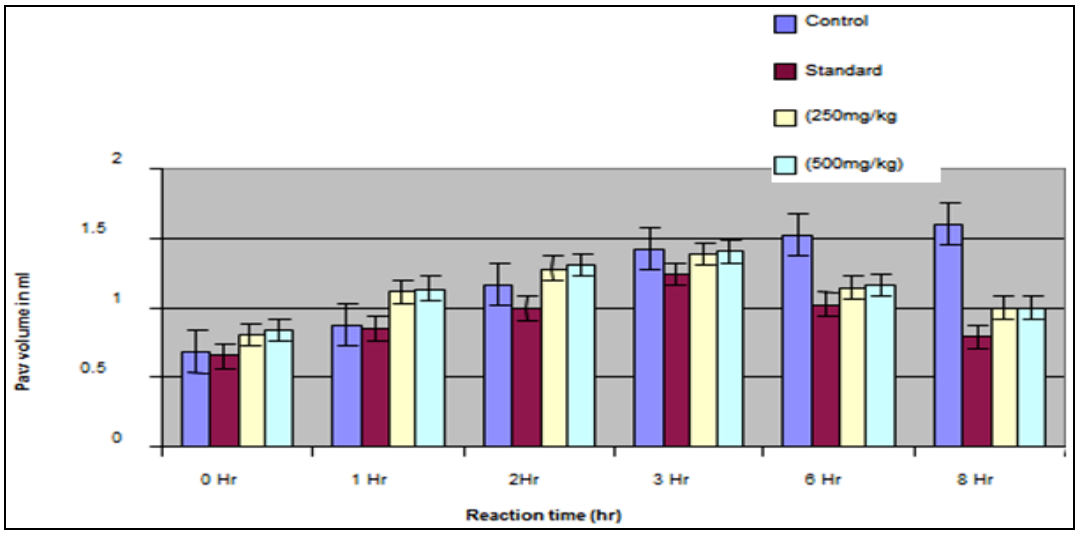

FIGURE 1: ANTI-INFLAMMATORY ACTIVITY OF SCINDAPSUS BY PAW EDEMA METHOD

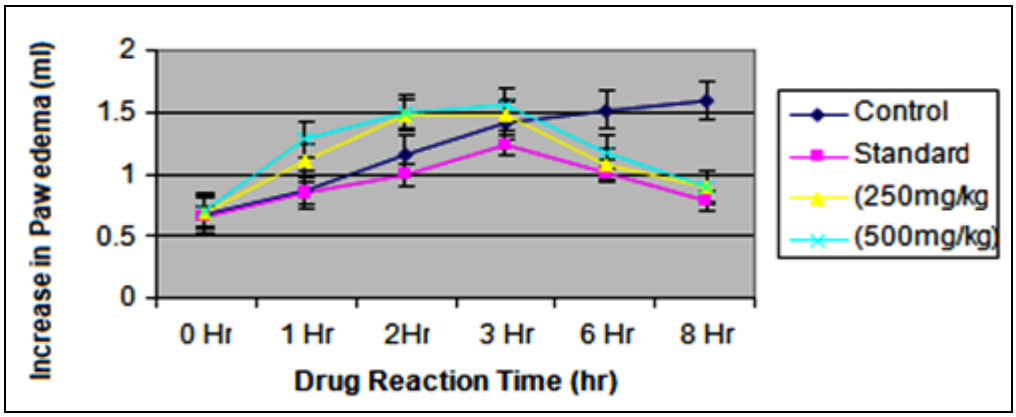

FIGURE 2: ANTI-INFLAMMATORY ACTIVITY OF *SCINDAPSUS* BY PAW EDEMA METHOD

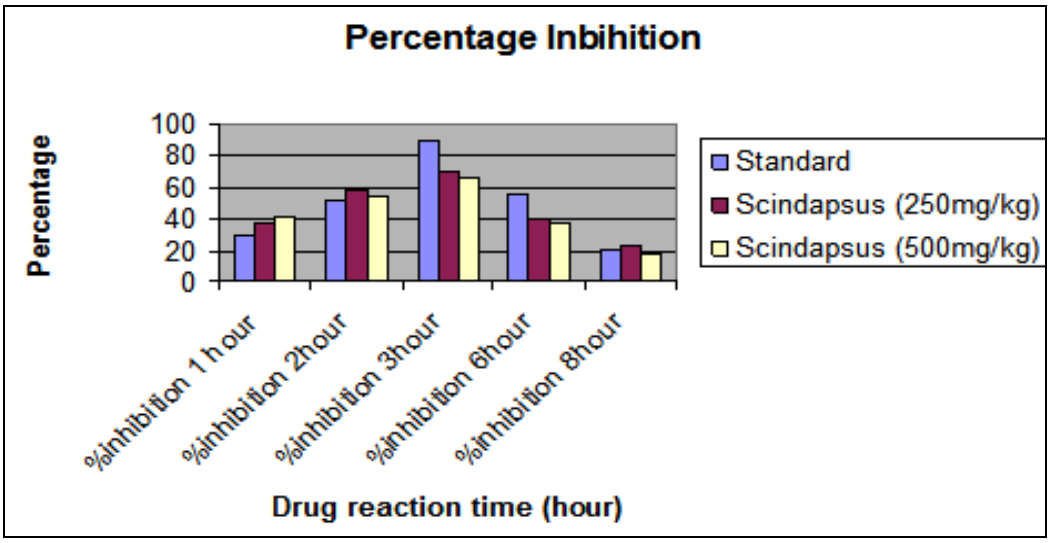

FIGURE 3: PERCENTAGE INHIBITION OF *SCINDAPSUS OFFICINALIS*

Effect of plant extract on Carrageenan-induced Hind Paw Edema: The ethanolic extract of *Scindapsus officinalis* exhibited statistically significant ($p<0.05$) anti-inflammatory activity in Carrageenan-induced Hind Paw Edema of rat. This was determined by analyzing data using one way ANOVA followed by Dunnett's test. In control animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach a maximal intensity four hours after the injection of the phlogistic agent. Ethanol extract of *Scindapsus officinalis* showed a significant dose depended reduction at both 250 and 500mg/kg body weight. However significant inhibition of edema was found to be 40.74% and 38.24% at six hour of study at a dose of 250 and 500mg/kg body weight respectively. Further significant inhibition was to be 22.96% and 18.65% at eight hour of study at a dose of 250 and 500mg/kg body weight respectively.

The plausible interpretation of such effect exerted by the plant extract could be the presence of flavonoids in the fruit as data support the inhibition of arachidonic acid metabolism as one of the mechanisms by which flavonoids exert their anti-inflammatory effects [7].

Analgesic activity:

TABLE 3: ANALGESIC EFFECT OF THE ETHANOL EXTRACT OF *SCINDAPSUS OFFICINALIS* USING THE HOT – PLATE METHOD. STATISTICAL EVALUATION OF THE RESULTS SHOWN IN TABLE

Treatment	0 min	30 min	60 min	120 min	180 min	240 Hour
Control	10.70±.85	9.66±.94	8.00±.81	6.58±.64	5.52±.54	5.00±.44
Standard	9.14± .52	11.02±1.00	12.60±.95***	14.16±1.08***	15.96±.68***	12.48±.70***
Drug 250 mg/kg	6.30±.67	7.66±.51	8.68±.35	9.84±.31**	11.08±.42***	8.59±.74**
Drug 500 mg/kg	7.58±.64	9.54±.45	11.08±.58**	12.38±.71***	13.88±.86***	10.53±.65***

Values in the results are expressed as mean ± SEM., a Significantly different in comparison with control at $P<0.05$.

TABLE 4: PERCENT INHIBITION OF THE STANDARD AND TWO DIFFERENT CONCENTRATIONS OF THE EXTRACT COMPARED WITH THEIR RESPECTIVE MEANS AT 0 HOUR

Treatment group	% Inhibition				
	½ Hour	1 Hour	2 Hours	3 Hours	4 Hours
Standard	20.56	37.00	54.90	74.61	36.54
Scindapsus (250 mg/kg)	21.58	37.7	56.1	75.87	36.35
Scindapsus (500 mg/kg)	25.85	46.17	63.32	83.11	38.91

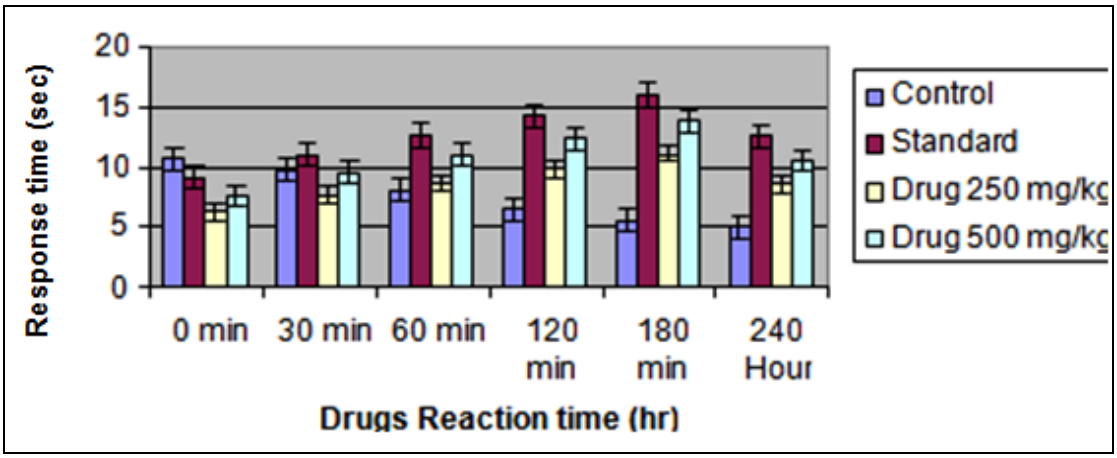

FIGURE 4: ANALGESIC ACTIVITY OF *SCINDAPSUS* BY HOTPLATE METHOD

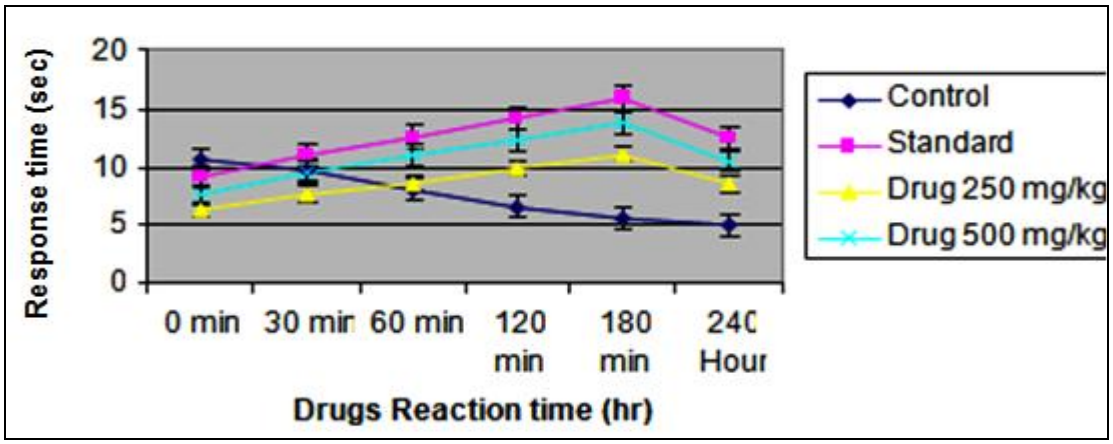

FIGURE 5: ANALGESIC ACTIVITY OF SCINDAPSUS BY HOTPLATE METHOD

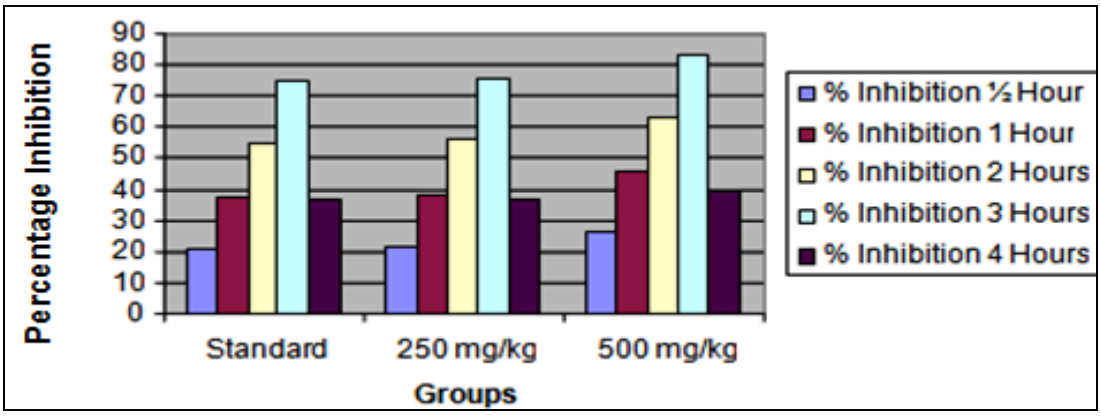

FIGURE 6: % INHIBITION OF SCINDAPSUS

Effect of plant extract on Hot-Plate test: The ethanolic extract of *Scindapsus officinalis* exhibited statistically significant ($p > 0.05$) analgesic effect in hot plate test of white albino mice. This was determined by analyzing data using one way ANOVA followed by Dunnett's post hoc test. However, the data shows that the dose dependent effect reached 83.11% at 180 minutes and 75.87% at the 180 minutes at the doses of 500 and 250 mg/kg-body weight respectively.

Oral administration of graded doses (250 & 500mg/kg) of the ethanol extract of *Scindapsus officinalis* to rats and mice did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period.

No mortality was recorded in any group after 24h of administering the extract to the animals.

DISCUSSION: As a result of adverse side effects, like gastric lesions,caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all the cases. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs [8, 9].

Carrageenan-induced edema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin all of which also cause pain and fever (Asongalem *et al.* 2004). Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms [10]. In the present study, it has been shown that the ethanol extract of the *Scindapsus officinalis* possess a significant anti-edematogenic effect on paw edema induced by carrageenan.

Effect of ethanol extract of *Scindapsus officinalis* in hot plate method is shown in the figures. It is one of the most common test for evaluating the analgesic efficacy of drugs/compounds. The paws of mice and rats are very sensitive to heat at temperature which is not damaging to the skin. The responses are shaking, jumping, withdrawal of the paws and licking of the paws. The time until these response is prolonged after administration of centrally acting analgesics (Ghosh MN,1987). *Scindapsus officinalis* extract at the dose of 250 and 500 mg/kg showed the significant ($P<0.05$) increase in latency time as compared to control. Positive control Diclofenac Na showed significant ($P<0.05$) analgesic activity at the dose of 10 mg/kg.

The analgesic activity was expressed as mean increase in latency after drug administration ±SEM. *Scindapsus officinalis* exhibited potent analgesic activity at the dose levels of 250 and 500mg/kg. These extracts show analgesic activity at low dose of 250mg/kg even in first hour in test. These result indicate that ethanolic extract of *Scindapsus officinalis* can produce significant analgesic effect.

It has been reported that a number of flavonoids posses anti-inflammatory and analgesic activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatpry effects. Since, prostaglandins are also involved in the pain perception, inhibition of their synthesis might be possible reason for the analgesic activity of the ethanolic extract [11, 12] .

The presence of flavonoid identified might be responsible for the analgesic and anti-inflammatory activities in the ethanolic extract of *Scindapsus officinalis*.

CONCLUSION: The present study indicated that the ethanol extract of Scindapsus officinalis may have potential use in medicine. In our study, the ethanolic extract of the plant showed significant dose dependent inhibition of paw edema and significant analgesic effect. On a more precise note, the results of the experiments suggested that *Scindapsus officinalis* may be used as an alternative or supplementary herbal remedy for the treatment of analgesic and anti-inflammatory diseases. The present study warrants further investigation involving components of Scindapsus officinalis for possible development of new class of analgesic and anti-inflammatory drugs [13].

These observations provide evidence for the anti - inflammatory and probable analgesic properties of fruit of Scindapsus officinalis claimed in Ayurveda medicine. Further studies should be undertaken to correlate the pharmacological activities with the chemical constituents of the fruit of Scindapsus officinalis and uncover specific mechanisms of action so that we may find a viable natural alternative to the traditional NSAIDs [14, 15].

Thus, it is concluded that the ethanolic extract of fruit of Scindapsus officinalis produce significant anti-inflammatory and analgesic activities in dose dependant manner.

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