

Effect of *Solanum nigrum* Linn on Acute and Sub-Acute Models of Inflammation

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ABSTRACT

Objective: The aim of the study was to see the effect of ethanolic extract of *Solanum nigrum* Linn on Acute and Sub-acute inflammation. **Method:** Wistar rats weighing 150-200gms were used, divided into four groups Group 1 was control group, two groups Group 2 and 3 treated with the plant extracts at 100 & 200 mg/kg b.w and Group 4 treated with indomethacin, 10 mg/kg b.w. Acute inflammation was induced by injecting 0.1 mL of 1% homogenized carrageenan suspension in normal saline to the right hind paw of the rats prior to which drug treatment was given and paw volume was measured using a digital plethysmometer. Sub-acute anti-inflammatory studies were carried out as described by winter and Porter and hematological parameters were estimated. The granuloma tissue formed during the proliferative process was isolated and wet and dry exudates formation was estimated in various treatment groups. The effect of extract and indomethacin on vital organs like liver, kidney, stomach was also studied by performing histological studies. **Results:** Study shows that the hydroalcoholic extract of whole plant of *Solanum nigrum* L. is effective only at higher dose effectively suppressing carrageenan induced inflammation suggesting the inhibition of release of prostaglandin, bradykinin, leukotrienes and polymorphonuclear cells. The histological studies further confirm the anti-inflammatory property of the extract while simultaneously maintaining normal cellular architecture of vital organs than as compared to indomethacin treated group. **Conclusion:** The ethanolic extract of whole plant of *Solanum nigrum* Linn shows promising anti-inflammatory activity on both acute and sub-acute stages of inflammation.

Key words: Acute Inflammation, Sub-acute Inflammation, Anti-inflammatory activity, *Solanum nigrum*, Hematological parameters, Histological studies.

Key Message: Inflammation is a typical reaction which gets triggered due to external influences. *Solanum nigrum* is a weed which is commonly found throughout the country and which has been claimed traditionally for its use as anti-diarroheal, analgesic and anti-hyperlipedemic. This study mainly involves *in-vivo* effect of the hydroalcoholic extract of the whole plant on acute inflammation induced using carrageenan and sub-acute inflammation induced using cotton pellet. The plant has elicited significant anti-inflammatory activity on acute as well as sub-acute stages of inflammation decreasing the paw volume and controlling the secretion of pro-inflammatory mediators.

Evaluating the plant further for its effects on biochemical biomarkers and its chemical profiling is required and is in process

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INTRODUCTION

A localized protective reaction of cells/ tissues of the body gets triggered by factors like tissue injury, bacterial infection and chemical injury, is called as Inflammation. A typical inflammatory reaction is characterized by heat, redness, swelling, and loss of function along with accumulation of leukocytes, proteins and fluids at the site of inflammation.^{1,2,3} Several gastrointestinal, cardiovascular, neurological and psychiatric disorders show involvement of pro-inflammatory mediators acting as culprits in the inflammatory process.⁴ Categorically inflammation process can be divided into three distinct phases, the first phase characterized by increased vascular permeability and exudation of fluids namely the acute phase followed by the second phase involving infiltration of leukocytes and granuloma formation called as the sub-acute phase ultimately followed by the third and last phase of chronic inflammation typically characterized by regulated production of pro and anti-inflammatory mediators like the TNF- α , (IL)-1 β and IL-6, chemokines, and inducible enzymes.⁵

Since time immemorial medicinal plants have been used as remedies on human diseases. Pain and inflammation are the most common complications encountered and several such plants and their isolates have been proved to be having anti-inflammatory properties.

Solanum nigrum locally known as Kakamunchi in kannada is a herbaceous weed 30-45 cms in height and found throughout the country.⁶ Literature reveals the fruit extract of the plant has been evaluated for anti-oxidant and anti-hyperlipedemic activity which was found to be significant.⁷ The ethanolic extract of the dried fruit has been evaluated for anti-diarroheal, anti-hyperlipedemic, antioxidant, analgesic and cytotoxic activity.^{8,9} The literature review claims the plant to be effective on various chronic inflammatory conditions however the whole plant has been previously evaluated only for its activity on acute inflammation¹⁰ similarly isolated compounds from the extract of the plant have been studied *in-vitro* for its effect on LTC₄.¹¹ The present study has been designed to evaluate the effect of the hydroalcoholic extract of the whole plant of *Solanum nigrum* *in-vivo* on both acute as well as sub-acute phases of inflammation and effect of the extract on different vital organs was evaluated using histopathological studies.

MATERIALS AND METHODS

Drugs and Chemicals: Indomethacin was obtained from Fabrica Italiana Sintetici, Vicenza, Italy, carrageenan was obtained from Sigma Chemical Company (St. Louis, U.S.A). All other chemicals were of analytical grade.

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Plant material and extract: The whole plant of *Solanum nigrum* was collected from the surroundings of Hubballi and Dharwad, Karnataka. The plant was authenticated by Dr. A.B. Sonappanavar, Dept. of Botany, P.C. Jabin Science College, Hubballi. The collected plant material was collected; shade dried and grinded in the mixer.

The powder was stored in air tight polyethylene bag. About 50g of the powdered drug was weighed using an electronic balance and blended with about 200 mL 70% ethanol, refluxed for 1.5hrs at 65-70°C. This cycle was repeated three times. The extracts freed of the solvent gave a brown solid mass. Total percentage yield of the extract was found to be 9.045%.

Experimental Animals: Wistar rats weighing between 150-200 g were obtained from Venkateshwara Enterprises, Bangalore. All experimental procedures were approved by the Institutional Ethics Committee (IAEC). Project Code: KLEU's010/IAEC.HBL/31st Aug 2013.

Acute Toxicity Studies: The acute toxicity studies were carried out as per OECD guidelines 423. For the toxicity studies swiss albino mice were procured from the animal house of K.L.E.University's College of Pharmacy, Vidyanagar Hubballi.

Female mice 3 nos. weighing 20-30 g were selected and after acclimatisation were kept for fasting for 18 h being provided only with water. An emulsion of the extract was prepared using 0.5% CMC and dose of 2000 mg/kg b.w. was administered and the animals were observed under open-field conditions for behavioural, locomotion, muscle spasms, tremors, convulsions and mortality for 24 h and further for a period of 14 days for occurrence of any toxic symptoms¹².

Experimental Design and Drug Treatment

Two models were employed to evaluate the anti-inflammatory effect of the whole plant of *Solanum nigrum* Linn. For acute inflammation carrageenan induced paw edema technique was employed while for sub-acute inflammation foreign body induced (Grass pith and cotton pellet) granuloma technique was employed.

Carrageenan Induced Paw Edema in Rats.

Wistar rats weighing between 150-200 g were obtained from Venkateshwara Enterprises, Bangalore. The rats were divided into 4 groups, each group containing 6 rats each, total of 24 rats. Acute inflammation was produced by injecting 0.1 mL of 1% homogenised carrageenan suspension in normal saline to the left hind paw of the rats. One hour prior to this test drugs were administered.

Group I received 0.9% NaCl and served as control, Group II received indomethacin (10 mg/kg b.w.) Group III and IV were administered the test drug *Solanum nigrum* extract, 100 mg/kg and 200 mg/kg body weight respectively. Administration of indomethacin and the plant extract was done by p.o. route^{13,14}.

A mark was made at the ankle up till which the paw was dipped and paw volume was measured at interval of 1 h, 2 h, 3 h and 5 h using a plethysmometer. The mean paw volume at different intervals was measured, compared to control and percentage inhibition was calculated using:

$$\text{Percentage edema inhibition} = [(V_c - V_t) / V_c] \times 100$$

Where: Vt: Percentage difference in increased paw volume after the administration of test drugs to the rats

Vc: Difference of increased volume in the control groups

Cotton-Pellet induced Granuloma in Rats:

The method is based on granuloma formation in rats over a foreign body by subcutaneous implantation of compressed cotton pellets along with grass pith.

Wistar rats weighing 150-200 g were procured, housed in polypropylene cages at room temperature and acclimatised. They had free access to food and water throughout the experiment. Total of 24 animals were chosen for the studies, rats were divided into 4 groups Group I received 0.9% NaCl and served as control, and Group II received indomethacin (10mg/kg b.w.). Group III and IV were administered the test drug *S.nigrum* plant extract, 100 mg/kg and 200 mg/kg body weight respectively for a period of 14 days each.

Sub-acute inflammation was produced under light ether anesthesia by shaving the ventricle aspect of the abdomen and making an incision of 3-4 cms. In this incision a subcutaneous pocket was made and cotton pellets weighing 10±1 mg each sterilised at 160°C for 30 min less than 15 lbs was inserted. Along with this grass-pith measuring 1/2 mm was also inserted. Drug treatment was started 1 h prior to cotton pellet implantation and the treatment was continued for the next 14 days.^{11, 13-15}.

On day 15, blood samples were collected through the retro-orbital route under light anaesthesia and subjected to differential leukocyte count. The animals were then sacrificed and cotton pellets removed by dissection, the granuloma tissue was identified as a firm vascular tissue. It was isolated and subjected to histological studies. Cotton pellets were dried at 60°C for 24 h and net granuloma formation was calculated by subtracting initial weight.

Granuloma percentage formation was calculated as follows:

$$\text{Granuloma inhibition (\%)} = (W_c - W_t) / W_c \times 100$$

Where: Wc and Wt represent the average weight of granuloma in the control and treated groups respectively.

Histological Studies

A 5µ section of the granuloma tissue to be subjected for histological studies was obtained with a standard microtome isolated and fixed using 10% formalin, immersed in paraffin. The tissue was then stained with hematoxylin and eosin and then subjected to histological studies.

RESULTS

Acute Toxicity Study

Animals treated with the hydroalcoholic extract of *Solanum nigrum* L. showed no behavioural changes and on administration of maximum dose of 2000mg/kg body weight there was no mortality observed even after 14days thus it was concluded that 2000mg/kg is a safe dose. 100 and 200mg/kg b.w. was used for further studies.

Effect of Carrageenan Induced Paw Edema in Rats

The percentage inhibition of inflammation at the end of 300min by 100mg/kg b.w. dose of extract was 40.36% and 68.46% by 200mg/kg b.w. of extract. Indomethacin shows 90.46% percentage inhibition Table 1.

Dry and Wet exudate formation

The extract of *Solanum nigrum* L. inhibits wet and dry exudate formation by 21.82% and 25.88% at 100mg/kg b.w. respectively at 200 mg/kg b.w. wet and dry exudate formation is inhibited at 50.76 % and 53.45 respectively Table 2.

Indomethacin shows 65.71% inhibition of wet exudate and 67.43% inhibition of dry exudate formation at dose of 10mg/kg body weight.

Evaluation of Hematological Parameters:

The mean values for Total Leucocyte Count for control group was 13733±260 cells/cm³, for group treated with 100mg/kg b.w. plant extract was 6750±123 cells/cm³ and for group treated with 200mg/kg b.w. plant extract was 7367±384 cells/cm³ respectively. Animals treated with indomethacin showed a count of 11717±444cells/cm³.

Table 1: Effect of hydroalcoholic extracts of *S.nigrum* on carageenan induced inflammation

Treatment	Dose	Mean oedema volume (mL)				% Inhibition			
		1h	2h	4h	5h	1h	2h	4h	5h
Control	0.1mL	1.2±0.09	2.0±0.11	1.9±0.02	1.7±0.03	-	-	-	-
<i>S.nigrum</i>	100mg/kg b.w.	0.86±0.09*	0.94±0.04***	0.8±0.04***	0.75±0.01***	23.75	35.35	39.28	40.36
<i>S.nigrum</i>	200mg/kg b.w.	0.70±0.05***	0.86±0.05***	0.5±0.02***	0.41±0.02***	27.11	40.26	63.68	68.46
Indomethacin	10mg/kg b.w.	0.46±0.03***	0.36±0.01***	0.29±0.01***	0.16±0.01***	48.66	75.47	83.13	90.46

Values are expressed as mean±SE (n=6)

*Significantly different from control p<0.5

***Significantly different from control P<0.001

Table 2: Effect of hydroalcoholic extract of *S.nigrum* L. and Indomethacin on Dry and Wet and Dry exudate formation

Sl. No.	Treatment Group	Inhibition of Exudate Formation	
		Wet Exudate	Dry Exudate
1.	CONTROL GROUP	--	--
2.	<i>S.nigrum</i> 100mg/kg b.w.	21.82%	25.88%
3.	<i>S.nigrum</i> 200mg/kg b.w.	50.76%	53.45%
4.	Indomethacin 10mg/kg b.w.	65.71%	67.43%

Values are expressed as mean±SE (n=6)

***Significantly different from control P<0.001

Table 3: Effect of hydroalcoholic extracts of *S.nigrum* on Differential Leucocyte Count, PMN count and Lymphocyte Count.

	Differential Leucocyte Count (cells/cmm)	% of Polymorpholeucocytes	% of Lymphocytes
Control	13733±26	37.67±2.71	58.67±3.07
<i>S.nigrum</i> (100mg/kg)b.w.	6750±12	24.83±0.5	72.67±0.42
<i>S.nigrum</i> (200mg/kg)b.w.	7367±38	24.67±0.61	72.00±1.03
Indomethacin	11717*±43	23.83*±0.74	70.50*±0.34

Values are expressed as mean±SE (n=6)

***Significantly different from control P<0.001

The percentage of PMN(Polymorphonucleocytes) and lymphocyte count for control group was 37.67% and 58.67%. It was 24.83% and 72.67% respectively. Groups treated with plant extract at lower dose and at higher dose the percentage of PMN and lymphocyte count was 24.67% and 72.0% respectively. Indomethacin treated group showed a count of 23.83% and 70.50% respectively Table 3.

Histological Studies

Light microscopic examination of granuloma tissue was done. Similarly vital organs like Stomach and kidneys were isolated and subjected to Histological studies. The study was done at 100x.

DISCUSSION

Inflammation primarily refers to events occurring on invasion of pathogen or on exposure to noxious substance.¹⁶ The present study was designed to evaluate the hydroalcoholic extract of whole plant of *Solanum nigrum* L. on inflammation and effect of the extract on vital organs.

Carrageenan a phlogistic agent induces histamine and 5-HT mediated edema and inflammation in the preliminary one hour followed by release of kinins, leukotrienes and PMN cells in the next 2h until completion of 6h.^{14,15} Carrageenan triggers a biphasic event, phase one involving release

of histamine, serotonin, with increased synthesis of prostaglandins phase two is characterised with marked release of prostaglandins, bradykinin, leukotrienes and tissue macrophages.^{17,18}

On using the cotton-pellet granuloma method it was observed that the extract interferes with the proliferative phase characterized by growth of connective tissue and collection of exudates containing neutrophils, collagen and fibroblasts. Decrease in the formation of granuloma indicates suppression of the proliferative phase.¹⁹

The extract shows dose dependent action in inhibiting vascular permeability. Dry exudate formation is correlated to positive effect of the drug on proliferative phase and the dried pellet is also indicative of the severity of inflammation.¹⁹⁻²¹ The study reveals that the extract has exhibited dose dependent activity and is at par to previous studies¹⁰ due to presence of steroids, alkaloids and triterpenoids in the extract.^{11,13-15}

Migration of WBC during the process of inflammation is a biological marker in inflammatory studies. On assessing the hematological parameters it was seen that at both the doses of extract there is decrease in leucocyte and PMN count depicting that the action is not dose dependent.

A predominant migration and deposition of macrophages, mast cells, neutrophils, and mixed inflammatory mediators was seen in tissue of animals belonging to control group, less deposition of macrophages

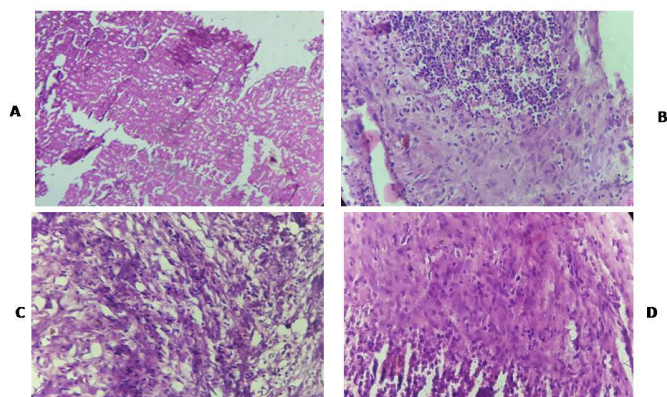


Figure 1: T.S. of Granuloma Tissue of A: Rats belonging to untreated group B: Rats treated with 200 mg/kg b.w. of *S. nigrum* C: Rats treated with 100 mg/kg b.w. of *S. nigrum* D: Rats treated with Indomethacin.

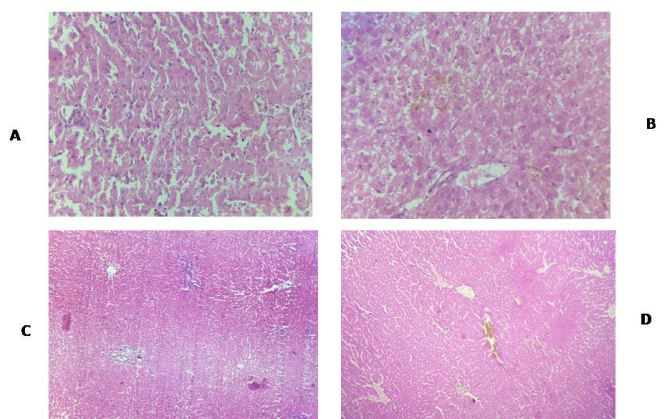


Figure 2: T.S. of Liver of A: Rats belonging to untreated group B: Rats treated with 200 mg/kg b.w. of *S. nigrum* C: Rats treated with 100 mg/kg b.w. of *S. nigrum* D: Rats treated with Indomethacin

and mast cells and a predominant deposition of collagen fibres signifies healing process and the anti-inflammatory activity of extract.

Granuloma Tissue: Examination of granuloma tissue of animals belonging to control as well as treated groups was done. A predominant migration and deposition of macrophages, mast cells, neutrophils, mixed inflammatory mediators was seen in tissue of animals belonging to control group while animals treated with hydroalcoholic extracts of *Solanum nigrum* depict less deposition of macrophages and predominant deposition of collagen fibres which aid in healing process and signify anti-inflammatory activity Figure 1.

Histological Analysis of Liver: The examination of section of liver belonging to animals treated with hydroalcoholic extract of whole plant of *Solanum nigrum* L. at two different doses viz. 100mg/kg and 200mg/kg body weight showed normal appearance of hepatic lobules and hepatic cells. However the sections of Liver obtained from animals treated with indomethacin depict acute congestion Figure 2.

Histological Analysis of Kidneys: *Solanum nigrum* treated animals show normal renal cytoarchitecture with normal renal corpuscles, renal tubules, glomeruli and Bowman's capsule. TS of Kidneys of animals treated with indomethacin show congestion, vacuolation of cell lining and glomerular contraction Figure 3.

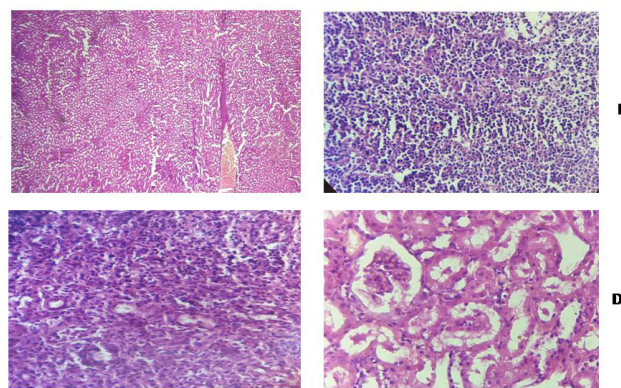


Figure 3: T.S. of Kidney of A: Rats belonging to untreated group B: Rats treated with 200 mg/kg b.w. of *S. nigrum* C: Rats treated with 100 mg/kg b.w. of *S. nigrum* D: Rats treated with Indomethacin

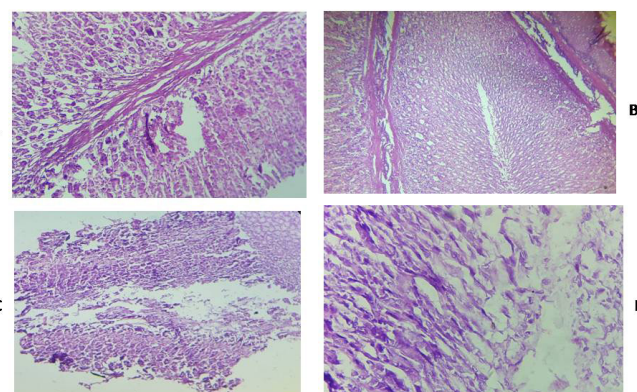


Figure 4: T.S. of Stomach of A: Rats belonging to untreated group B: Rats treated with 200 mg/kg b.w. of *S. nigrum* C: Rats treated with 100 mg/kg b.w. of *S. nigrum* D: Rats treated with Indomethacin.

Histological Analysis of Stomach : animals of control group exhibit acute mucosal necrosis, congestion, ulceration and infiltration of neutrophils *Solanum nigrum* treated group of animals at lower dose exhibited unremarkable cellular structure, abundant epithelia and normal mucin producing glands. *Solanum nigrum* at (200 mg/kg) shows ulceration, destruction of the epithelial cells and absence of intracellular glandular mucin. Animals treated with indomethacin show gastric mucosa with ulceration, mixed inflammatory cells, necrosis of mucosa Figure 4.

CONCLUSION

The present experimental study has shown that the hydroalcoholic extract of leaves of *Solanum nigrum* L. has elicited significant anti-inflammatory activity against both acute and sub-acute models of inflammation. The haematological parameters indicate the extracts to be possessing potent anti-inflammatory activity and histological studies confirm this. The histological studies also indicate that hydro alcoholic extract of whole plant of *Solanum nigrum* has organ protection properties and indicate that the extract is safe for prolonged use at 100 mg/kg b.w. dose. At the dose of 200 mg/kg b.w. the anti-inflammatory activity is significant but at this dose the extract is seen to cause necrosis and ulceration in the stomach.

The anti-inflammatory effect could be contributed to the presence of steroidal alkaloids and steroidal saponins in the plant.²⁵

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CONFLICT OF INTEREST

There is no Conflict of Interest

ABBREVIATION USED

RBC: Red Blood Corpuscles, WBC: White Blood Corpuscles, *S. nigrum*: *Solanum nigrum*, PGE₂: Prostaglandins.

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