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EFFECT OF *Solanum nigrum* AND *Ricinus communis* EXTRACTS ON HISTAMINE AND CARRAGEENAN-INDUCED INFLAMMATION IN THE CHICKEN SKIN

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Abstract – We studied anti-inflammatory effect of ethanolic extract of *Solanum nigrum* leaves and *Ricinus communis* root bark using chicken skin as model. Leaves of these plants were dried under shade and powdered. 5% Ethanol extracts were prepared using Soxhlet and injected intraperitoneally (400 mg/kg) 1 hour prior to the induction of inflammation. Inflammatory lesion were induced by intradermal injection of 0.02ml 0.05%w/v histamine (0-2 min, 15min, 30 min, 1hr and 6hr) and 1% w/v carrageenan (0-2 min, 30min, 1hr, 6hr, 12 hr and 48 hr) in different group of birds. Increase in vascular permeability was studied using Evans blue as a permeability marker both qualitatively and quantitatively. Cellular events were studied in skin lesions at various time intervals and cells were counted at high power objective under microscope. Both, extracts exhibited significant decrease in permeability response at an early stage (0-2min) of histamine as well as in carrageenan induced inflammatory lesions. There was a significant ($p < 0.05$) suppression in the emigration of heterophils, monocytoid cells, basophils and total leukocytosis in *Solanum nigrum* and *Ricinus communis* pretreated chicken skin lesions as compared to the control. The present study suggested antihistamine and anti-inflammatory properties of ethnolic extract of *Solanum nigrum* and *Ricinus communis.*

Key words: *Solanum nigrum*, *Ricinus communis*, anti-inflammatory, histamine, carrageenan, ethanol extract.

INTRODUCTION

Many of the non-steroidal anti-inflammatory drugs such as phenylbutazone, methysergide and indomethacin have been reported to act on acute and chronic inflammation (19). In addition to its anti-inflammatory activity, indomethacin and other similar drugs are reported to cause untoward effects leading to gastrointestinal, hepatic and renal disorders in man and animals (54). There has been an increasing interest, recently for identifying safer treatment against inflammatory diseases.

Solanum nigrum, locally known as 'Makoi', is commonly used in traditional medicine as sedative, useful in the diseases of liver, heart and eyes and is also effective against piles, fever and dysentery (46). *Solanum nigrum* is commonly used in the Indian system of medicine and it has been reported to possess hepatoprotective, antiseptic, antispasmodic, immunomodulating and anticonvulsant activities (39, 52, 34, 45). The leaves of this plant are used for the treatment of open wounds and are known to possess hypotensive effect (57). Leaves were employed as poultice over rheumatic and gouty joints, in heart diseases, piles, gonorrhea, inflammatory swellings and chronic inflammation of the liver and spleen and also in the remedy of various skin diseases (35). Whole plant juice of *Solanum nigrum* is used for curing patients with liver disorders like enlargement and swelling (49). Leaf and tender shoot are used for dropsy and swollen testicle (24). The leaves of *Solanum nigrum* are used for the treatment of swelling of the body locally the disease is known as 'Jaherbad' (16).

Ricinus communis Linn. (Euphorbiaceae) is a soft-wooded small tree widespread throughout tropics and warm temperature regions of the world (20). This plant is locally known as 'Arandi' and in ethno clinical practices it plays a role in the treatment of inflammatory pains, bronchitis, wound healing, boils, and acute and sub-acute inflammation (9, 7, 2, 6). In the Indian system of medicine, the leaf, root and seed oil of

this plant have been used for the treatment of inflammation and liver disorders (30), hypoglycemic (13), laxative (10), diuretic (1) and antibacterial (28, 56). *Ricinus communis* contains a naturally occurring toxin derived from the beans, which is considered to be potential chemical weapon (8). The anti-inflammatory and free radical scavenging activity are well demonstrated (18) and its antifertility properties are widely reported in humans (48).

No scientific data is available on the mechanism of action of *Solanum nigrum* and *Ricinus communis* on vascular and cellular events. Using in vivo model it has been reported that number of plants exhibit anti-inflammatory activity. However, studies on mechanism of action in avian species are scanty. The present investigation was designed to evaluate the effects of *Solanum nigrum* extract on histamine and carrageenan-induced acute inflammatory response using chicken skin.

MATERIALS AND METHODS

Chemicals

Carrageenan was procured from Sigma Chemical Co.(St. Louis. USA) and histamine acid phosphate form BDH chemicals, Poole England. All other analytical grade laboratory chemicals and reagents were procured from Hi Media Laboratories Pvt. Limited (Mumbai, India). All the samples were stored and refrigerated in desiccators to avoid decomposition.

Preparation of Alcoholic Extracts

Plants were collected in the month of February 2009 form the Jabalpur (M.P.) region of India and identified by Botanist from the herbarium (Acession No. 6199 for *Solanum nigrum* and 9236 for *Ricinus communis*) of Central Research Institute Ayurveda (under Central Council for Research in Ayurveda and Siddha) Gwalior-9 (M.P.)

Fresh leaves of *Solanum nigrum* and root bark of *Ricinus communis* were dried under shade, powdered, and further extracted with absolute alcohol using Soxhlet apparatus till a colourless solvent was found in the reservoir of the apparatus. Absolute alcohol was then redistilled from the extract (17).

Acute Toxicity Studies

Alcoholic extracts of *Solanum nigrum* and *Ricinus communis* were dissolved in normal saline to prepare 5% (w/v) solution, which were administered intraperitoneally (2 g/kg body weight) to a group of 4 chickens. The birds were monitored for 14 days for any gross change in behaviour and mortality, if any. The mean effective dose (ED_{50}) was calculated on the basis of median lethal dose (LD_{50}), which was considered to be 1/5 of the LD_{50}. The mean effective dose of extract of *Solanum nigrum* was used for further studies.

Animals and Treatment

Healthy commercial male broiler chickens (aged 8-12 weeks), weighing approximately 2 kg were used in this study. These birds were procured from All India Coordinated Research Project on poultry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Adhartal, Jabalpur. They were maintained at ambient environmental conditions and housed individually in wire mesh cages. Birds had free access to water and standard finisher ration (Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Jabalpur). The institutional animal ethical committee of College of Veterinary Science and Animal Husbandry Jabalpur approved experimental protocol.

A total of 36 broiler chickens were utilized for the present study. Chickens were acclimatized for a period of one week before the start of experimental procedure. They were randomly divided into 12 groups of three chickens each and treated as below

Group I & VII Intra dermal histamine + normal saline intra peritoneal (control)
Group II & VIII Intra dermal histamine + *Solanum nigrum* intra peritoneal
Group III &IX Intra dermal histamine + *Ricinus comunis* intra peritoneal
GroupIV& X Intra dermal carrageenan+normal saline intra peritoneal (control)
Group V & XI Intra dermal histamine + *Solanum nigrum* intra peritoneal
Group VI & XII Intra dermal histamine + *Ricinus comunis* intra peritoneal

Birds from group I to VI were used for studying increased vascular permeability while the remaining birds (Group VII to XII) were used for histopathology of skin sections to study cellular events.

In the experimental groups alcoholic extract of *Solanum nigrum* and *Ricinus comunis* was given 1 hour prior to the induction of inflammation (Pretreatment).

Preparation of skin

One day before induction of inflammation, feathers from lateral thoracic region of the chicken were plucked, skin was cleaned by a soft cloth moistened with sterile distilled water and was allowed to dry as described by (3, 25). On the following day cleaning of the skin was repeated.

Induction of inflammation

A. Histamine acid phosphate**:** 0.05 % (w/v) solution in normal saline, 0.02 ml per site was injected intradermally at various time intervals (0-2 min, 15 min, 30 min, 1 hour, 3 hour and 6 hour) for induction of inflammation (3).

B. Carrageenan: 1.0 % (w/v) solution in normal saline, 0.02 ml per site was injected intradermally at various time intervals (0-2 min, 1 hour, 6 hour, 12 hour, 24 hour and 48 hour) for induction of inflammation (3).

Estimation of increased vascular permeability

An increase in vascular permeability in the cutaneous lesions was estimated by Evans blue dye technique. Evans blue solution was injected intravenously (I/v) simultaneously along with the induction of lesion of 0-2min, at a dose of 15 mg /kg body weight as 1.5 % w/v solution in normal saline (25, 5, 26). The injected bird was referred as blued chicken. Leakage of the dye at the site produced a distinct blue patch indicating an increase in vascular permeability. The development of blueing in the cutaneous lesions was observed for a period of 30 min and birds were

sacrificed by exsanguination. The permeability was evaluated by visual and quantitative methods.

(a) Visual assessment

The visual assessment of increased vascular permeability was based on the size and intensity of blue area both on epidermal and under surface of the lesion. The values were scored on an arbitrary scale from 0 to ++++ (55).

(b) Quantitative assessment

The Evans blue exuded in the cutaneous lesions was determined quantitatively using the technique of Pillai *et al.*(41). Briefly, each lesion was excised, weighed and chopped into fine fragments and placed in 3 ml formamide for 48 hours at 37^0C for extraction of the dye. The mixture was then filtered through a fine muslin cloth, and the concentration of the dye in the filtrate was measured by spectrophotometer (Systronics Digital spectrophotometer 166 Sr. No. 576) at 620 µm wavelength. The total content of exuded Evans blue in the lesions was calculated by the formula:

E = C x V – (W x F)

Where,

E = the total content of exuded dye in µg in the lesion

C = the concentration of the extracted dye in µg/ml measured by spectrophotometer

V = volume of formamide used in extraction

W = the weight of the tissue in grams

F = a conversion factor

The value of 'F' was calculated by measuring the optical density of formamide extracts of aliquots of 36 uninjected skin sites from different chicken injected intravenously with the standard dose of Evans blue.

Histopathology

For sequential histopathological study, the skin lesions were excised, pinned on cardboard to preserve the original shape, keeping epidermal surface downward. The entire preparation was then fixed in Carnoy's fluid for 48 – 72 hours. Pieces were excised at right angles to the epidermal surface from middle of lesions, with a sharp blade. The tissues were dehydrated in acetone, cleared in benzene and embedded in paraffin. The sections were cut at 5 µm thickness (32).

Skin sections were stained with haematoxylin and eosin, 0.05% solution of toluidine blue for basophils and mast cells as described earlier (32, 14).

Tissue leukocytosis

Quantifying tissue leukocytosis assessed the cellular reaction. A total of 6 lesions of the same age were obtained from 3 different birds and processed for histopathology. One section from each lesion of a particular interval was examined for counting different emigrated leukocytes per 40X microscopic field. In each section the cells were counted in five representative fields as described earlier (25, 26). The cited results thus represent the mean value ± standard error of 30 microscopic fields of each time interval. All leukocytes present in the intervascular space in one microscopic field were counted. Intravascular and intramural leukocytes were excluded.

Statistical Analysis

The values were presented as mean and standard errors. The analysis of each parameter representing inflammatory process was analysed by using the method for factorial randomized block design. The differences between treatments and between intervals within each treatment were tested statistically for their significance (51).

RESULTS

Acute Toxicity Studies

Intraperitoneal administration of alcoholic extracts of leaves of *Solanum nigrum* and root bark of *Ricinus communis*, did not produce any signs of toxicity up to 14 days. It was, thus assumed that LD_{50} for both extracts was more than 2g/kg body weight. On a safer side approximate dose was selected as $1/5^{th}$ of LD_{50} i.e. 400 mg/kg body weight for both the extracts which was administered intraperitoneally as pretreatment to experimental birds.

Estimation of increased vascular permeability

In histamine-induced inflammation the intensity of blueing increased at rapid rate within first 15-20 min, and thereafter it was gradual within group. Table I and figure 1 shows visual assessment of increased vascular permeability, Intensity of blueing was very less in *Solanum nigrum* and *Ricinus communis* pretreated chickens compared to control at 0-2 minute interval than at other time intervals between groups.

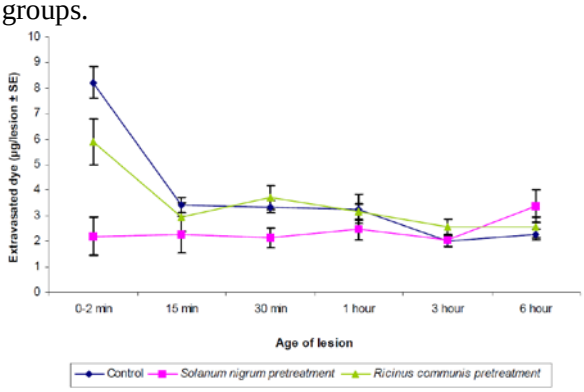

Figure 1. Time-course pattern of increased vascular permeability at the site of histamine-induced inflammation in the chicken skin

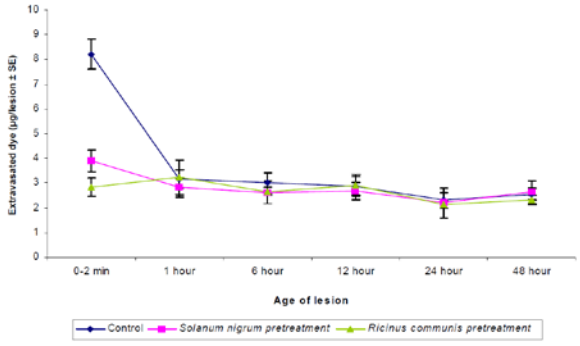

Figure 2. Time-course pattern of increased vascular permeability at the site of carrageenan-induced inflammation in the chicken skin

Table I. Qualitative assessment of increased vascular permeability in histamine-induced inflammation of the chicken skin

Time intervals	Control (without pretreatment)	*Solanum nigrum* pretreatment	*Ricinus communis* pretreatment
0-2 minute	++++	+±	+++
15 minute	++	+±	+
30 minute	++	±	+
1 hour	+	±	±
3 hour	-	-	-
6 hour	-	-	-

Table II. Qualitative assessment of increased vascular permeability in carrageenan-induced inflammation of the chicken skin

Time intervals	Control (without pretreatment)	*Solanum nigrum* pretreatment	*Ricinus communis* pretreatment
0-2 minute	++++	++	+
1 hour	++	+	++
6 hour	+	±	+±
12 hour	±	-	-
24 hour	-	-	-
48 hour	-	-	-

Quantitative assessment of increased vascular permeability showed that at 0-2 minute interval there was maximum exudation of dye as compared to other time intervals within group. However, there was a significant decrease of vascular permeability noticed at later phase in all the groups irrespective of the treatment given. *Solanum nigrum* and *Ricinus communis* pretreated group at 0-2 min interval shows significant decrease in the amount of dye exudation compared to control (figure 2).

In carrageenan-induced inflammation the blueing at the site started appearing 6-7 minute after intravenous injection of Evans blue. The intensity of blueing increased rapidly up to 20 min, and thereafter it was gradual. At 30 minute, when the birds were sacrificed, both extent and intensity of blue patch was highest at 0-2 minute interval lesion than other time intervals (Table II, figure 3). Intensity of blueing was very less in *Solanum nigrum* and *Ricinus communis* pretreated chickens at 0-2 minute interval as compared to control than at other time intervals between the groups.

Figure 4 shows quantitative estimation of amount of Evans blue exuded in the lesion. At 0-2 minute time interval maximum amount of dye had exuded as compared to other time interval within group both in control and pretreated group. However, there was a statistical significant difference in the amount of dye exuded in control and both the pretreated group of chickens at 0-2

Histamine-induced inflammation **Carrageenan-induced inflammation**

Figure 3. **A**. Blueing reaction at various time intervals after intradermal injection of histamine in control chicken. **B**. Blueing reaction at various time intervals after intradermal injection of histamine in *Solanum nigrum* pretreated chicken. Note decreased intensity of blueing at 0-2 minute. **C**. Blueing reaction at various time intervals after intradermal injection of histamine in *Ricinus communis* pretreated chicken. Note decreased intensity of blueing at 0-2 minute. **D**. Blueing reaction at various time intervals after intradermal injection of Carrageenan in control chicken. **E**. Blueing reaction at various time intervals after intradermal injection of Carrageenan in *Solanum nigrum* pretreated chicken. Note decreased intensity of blueing at 0-2 minute. **F**. Blueing reaction at various time intervals after intradermal injection of Carrageenan in *Ricinus communis* pretreated chicken. Note decreased intensity of blueing at 0-2 minute.

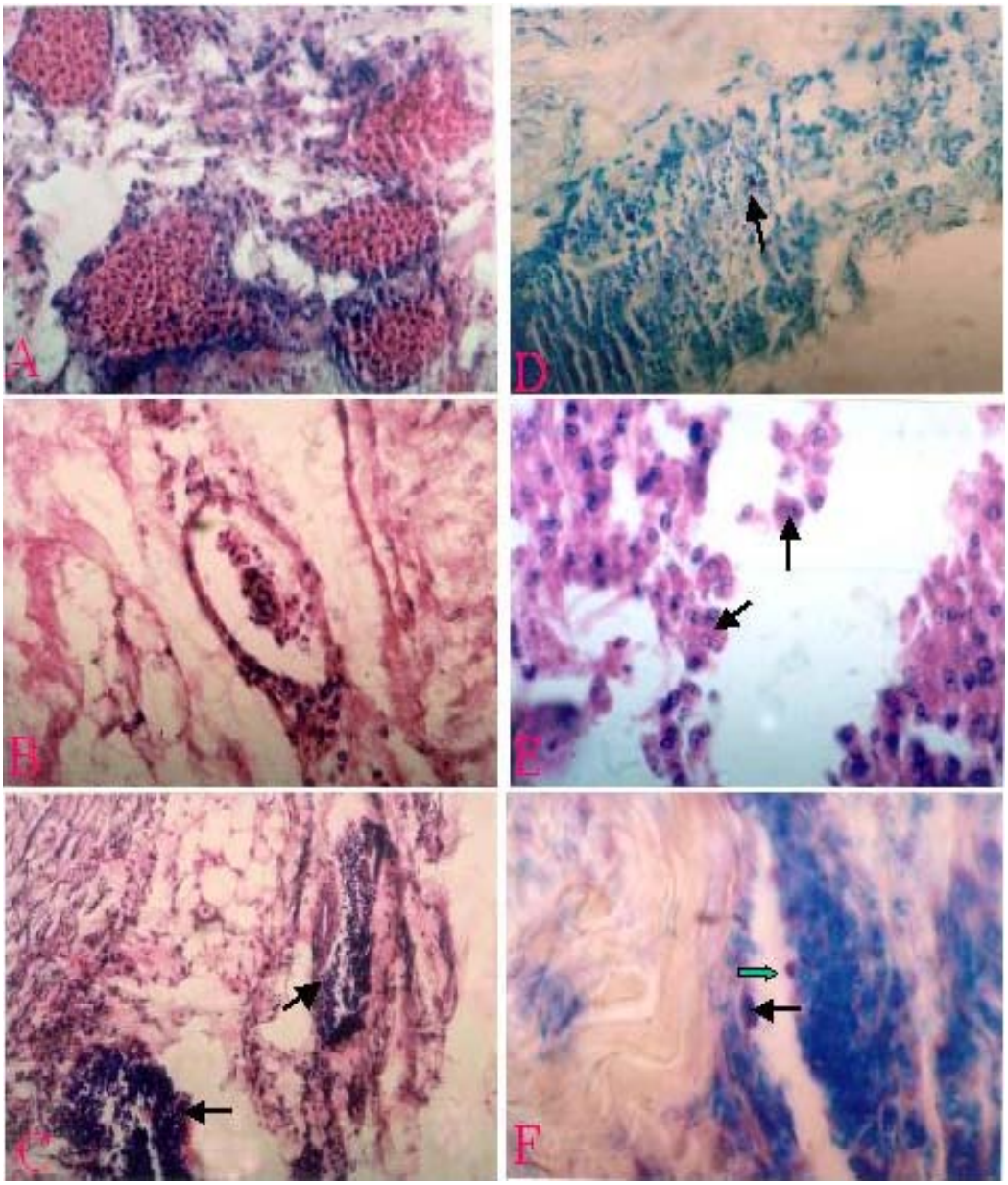

Figure 4. Section of chicken skin at various time intervals **A**. 15 minutes after intradermal injection of histamine showing extensive hyperaemia in control chicken skin H&E 40X. **B**. 15 minutes after intradermal injection of histamine showing hyperaemia, transmigration of leukocyte through vessel wall and edema in *Solanum nigrum* pretreated chicken H&E 40X. **C**. 6 hour after intradermal injection of histamine showing perivascular lymphoid aggregates in control chicken (arrow) H&E 20X. **D**. 6 hour after intradermal injection of carrageenan showing basophil (arrow) in control chicken skin toludine blue 40X. **E**. 48 hour after intradermal injection of carrageenan showing syncytia formation and few distinct giant cells (arrow) H&E 100X. **F**. 12 hour after intradermal injection of carrageenan showing mast cell (black arrow) and basophil (green arrow) toluidine blue 100X.

minute interval as compared to other time intervals between the groups.

HISTOPATHOLOGY

Histamine-induced inflammed skin sections taken at various time intervals stained with H&E and toulidine blue showed oedema of the interstitium and hyperaemia of blood vessels at 0-2 minute, which increased significantly at 15 minute and was persistent for 30 minute. However there was a reduction in oedema noticed at 1 hour and no vascular changes were noticeable in the lesion for 6 hour. Margination of leukocytes was noticed at 15 minutes time interval along with the emigration of a few heterophils. The number of heterophils increased considerably from 30 minute to 1 hour and maximum response was observed at 3 hour, though there was a substantial decrease in the number of heterophils in the lesion taken at 6 hour. Basophils were occasionally noticed from 0-2 minute to 30 minutes, at 1hour they were located perivascularly and there was a significant increase in their number at 3 hours, which depicted similar picture at 6 hour time. Monocytoid cells were rarely observed at 0-2 minute and 15 minute but their number significantly increased at 30 minute and a peak was observed at 1 hour and then remained persistent till 6 hour (Table III). Perivascular lymphocyte emigration was noticeable at 3 hour, which was more organized in the form of aggregates at 6 hour (Figure 5). A few mast cells with metachromatic granular cytoplasm were seen in toluidine blue stained sections at 15 minutes and most of the basophils and mast cells found to be in the stage of degranulation at 3 hour.

There was a marked suppression in the emigration of leukocytes in *Solanum nigrum* and *Ricinus communis* pretreated chicken skin lesions compared to the control chicken. Details of various cell counts at different intervals are shown in table III.

In carrageenan- induced inflammation, skin lesion showed intensely hyperaemic blood vessels and interstitial tissue was separated due to accumulation of oedematous fluid at 0-2 minute. Hyperaemia and oedema persisted up to 1 hour. However, at 6 hour vascular changes were absent. Margination of few leukocytes was noticeable at 0-2 minute while, erythrocytes were seen extravascularly. Carrageenan-induced a nonspecific type of metachromasia of different cells on staining with toluidine blue stain. Heterophils increased considerably from 1 hour to 6 hour and the number was maximum at 12 and 24 hours, which were diffused in the interstitium of dermis. Some of the heterophils appeared disintegrated at 6 hours and their number increased till 24 hour. A subsequent reduction in the number of heterophils was noticed at 48 hours. There was an increase in the number of monocytoid cells from 1 hour and peak was observed at 24 hour than there was a significant reduction in their number. A very few basophils were observed at 1 hour and peak was found at 6 hour. Basophils were found swollen and degranulated, their count decreased significantly from 6 hr stage than there was significant reduction in number at 12 hour that remained constant till 24 hour and further there was suppression at 48 hour. Monocytoid cell tended to form syncytia at 24 hour and a few distinct multinucleated giant cells were observed at 48 hour lesion. Lymphocytes were observed in the perivascular region at 6 hours, which seems to form aggregates at 12 hour and were found in more organized pereivascularly at 24 hour and persisted in 48 hour lesions.

There was significant suppression in emigration of heterophils, basophils and monocytoid cells in *Solanum nigrum* and *Ricinus communis* pretreated chicken skin lesions as compared to the control chicken (Table IV). Similarly, there was a significant reduction in the total leukocytosis in *Solanum nigrum* pretreated chicken skin lesions compared to the control chicken.

DISCUSSION

The pathology and pathogenesis of the acute inflammatory response in the chicken, induced by various non-immunological and immunological stimuli, have yielded valuable information on vascular and cellular events, unfolding the nature of several basic processes. Several experimental investigations have been made on the avian inflammatory process (55). Effect of certain anti-inflammatory drugs on various aspects of avian inflammation (19, 42, 21, 22, 23), and inflammatory-reparative response in the chicken (26, 27, 11, 43, 44, 29) too were made. However, information on the role of herbal drugs on the inflammatory process in the chicken is lacking.

The present work was undertaken to assess the effect of leaf extract of *Solanum nigrum* and *Ricinus communis* root extract on vascular and

Table III. Leukocyte response at the site of histamine-induced inflammation in the chicken skin

Time intervals	**Leukocytes per high power (x 400) microscopic field (Mean ± SE)**											
	Heterophils			**Monocytes**			**Basophils**			**Total Leukocytes**		
	C	*S*	*R*	*C*	*S*	*R*	*C*	*S*	*R*	*C*	*S*	*R*
0-2 minute	1.49 ± 0.035^{a}	1.100 ± 0.075^{ab}	1.160 ± 0.031^{b}	1.010 ± 0.341^{a}	0.700± 0.487^{a}	0.933 ± 0.307^{a}	0^{a}	0^{a}	0^{a}	2.503 ± 0.064^{a}	1.800 ± 0.068^{ab}	2.093 ± 0.036^{b}
15 minute	4.36 ± 0.526^{a}	1.360 ± 0.022^{b}	1.472 ± 0.023^{b}	3.767 ± 0.494^{a}	1.367 ± 0.352^{a}	1.267 ± 0.053^{a}	1.000 ± 0.240^{a}	0.867 ± 0.052^{ab}	0.700 ± 0.068^{b}	9.127 ± 0.246^{a}	3.594 ± 0.348^{c}	3.439 ± 0.857^{bc}
30 minute	15.91 ± 0.216^{a}	10.83 ± 0.014^{b}	8.07 ± 0.022^{b}	9.173 ± 0.927^{a}	6.403 ± 1.096^{b}	7.237 ± 0.703^{b}	1.233 ± 0.240^{a}	0.933 ± 0.140^{b}	0.800 ± 0.213^{b}	26.323 ± 1.060^{a}	18.169 ± 0.935^{c}	16.111 ± 0.784^{bc}
1 hour	31.96 ± 0.492^{a}	11.633 ± 0.363^{c}	10.937 ± 0.347^{b}	27.517 ± 1.168^{a}	10.867 ± 0.918^{c}	8.733 ± 0.825^{b}	1.967 ± 0.175^{a}	1.467 ± 0.482^{b}	0.967 ± 0.141^{c}	61.451 ± 4.465^{a}	23.967 ± 0.224^{c}	20.637 ± 0.237^{bc}
3 hour	49.23 ± 4.046^{a}	25.917 ± 0.275^{c}	29.583 ± 0.429^{b}	25.933 ± 1.901^{a}	20.933 ± 1.658^{c}	22.667 ± 1.487^{b}	2.400 ± 0.369^{a}	1.767 ± 0.375^{b}	0.733 ± 0.084^{c}	77.566 ± 3.631^{a}	48.617 ± 0.569^{c}	52.983 ± 0.491^{b}
6 hour	42.56 ± 1.380^{a}	21.600 ± 1.616^{c}	25.967 ± 1.024^{b}	21.933 ± 0.541^{a}	18.717 ± 0.843^{c}	20.233 ± 1.285^{b}	2.167 ± 0.260^{a}	1.670 ± 0.397^{b}	0.867 ± 0.257^{c}	66.667 ± 1.669^{a}	41.987 ± 1.211^{c}	47.067 ± 0.954^{b}

Note: *Same superscripts indicate non-significant difference between treatments within same time interval at P<0.05.
C- Control; S- *Solanum nigrum* (pretreated); R- *Ricinus communis*(pretreated)

Table IV. Leukocyte response at the site of carragenan-induced inflammation in the chicken skin

Time intervals	**Leukocytes per high power (x 400) microscopic field (Mean ± SE)**											
	Heterophils			**Monocytes**			**Basophils**			**Total Leukocytes**		
	C	*S*	*R*	*C*	*S*	*R*	*C*	*S*	*R*	*C*	*S*	*R*
0-2 minute	1.100 ± 0.578	0.750 ± 0.206	1.650 ± 0.198	0.500 ± 0.210^{a}	0.167 ± 0.166^{a}	0.333 ± 0.341^{a}	0^{a}	0^{a}	0^{a}	1.600 ± 0.610	0.917 ± 0.245	1. 983 ± 0.350
1 hour	29.667 ± 3.648	18.900 ± 3.546	20.400 ± 3.540	5.267 ± 0.443^{a}	4.600 ± 0.478^{a}	4.900 ± 0.337^{a}	0.767 ± 0.224^{b}	1.467 ± 0.098^{a}	0.900 ± 0.215^{b}	35.100 ± 3.848	24.967 ± 3.757	26.200 ± 2.519
6 hour	48.650 ± 3.325	41.783 ± 3.416	43.067 ± 6.670	20.267 ± 1.705^{a}	14.000 ± 1.548^{b}	17.367 ± 1.878^{ab}	2.333 ± 0.171^{a}	1.900 ± 0.177^{ab}	1.000 ± 0.158^{b}	72.650 ± 3.917	59.350 ± 3.619	61.433 ± 3.079
12 hour	55.160 ± 2.619	48.593 ± 6.830	49.460 ± 5.850	24.467 ± 1.519^{a}	18.167 ± 0.642^{b}	20.967 ± 1.518^{ab}	1.767 ± 0.160^{a}	1.733 ± 0.272^{a}	0.867 ± 0.166^{b}	81.450 ± 5.227	68.493 ± 7.208	71.293 ± 5.450
24 hour	56.200 ± 6.420	45.067 ± 1.920	46.933 ± 3.080	34.733 ± 1.148^{a}	23.467 ± 1.732^{c}	29.367 ± 1.123^{b}	1.733 ± 0.239^{a}	0.967 ± 0.221^{b}	1.167 ± 0.262^{b}	92.667 ± 3.401	69.500 ± 2.952	77.600 ± 3.301
48 hour	34.067 ± 3.420	27.067 ± 2.670	33.633 ± 2.360	25.150 ± 1.548^{a}	17.450 ± 1.875^{b}	18.850 ± 1.760^{b}	1.067 ± 0.217^{a}	0.900 ± 0.169^{a}	0.667 ± 0.133^{a}	60.250 ± 5.463	45.417 ± 3.687	53.217 ± 2.477

Note: * Same superscripts indicate non-significant difference between treatments within same time interval at P<0.05.
C- Control; S- *Solanum nigrum* (pretreated); R- *Ricinus communis*(pretreated)

cellular events of inflammation induced by intradermal histamine and carrageenan. It was envisaged that the information obtained might be of help to explain certain aspects of avian inflammation and contribute towards a better understanding of the pathology and pathogenesis of poultry diseases in general along with the role of herbal drug used as a preventive measure for subsequent treatment regime.

Inflammation is generally defined as the response of living tissue to an injurious stimulus (15). The usual features of inflammation include the activation of epithelial cells and resident macrophages, and the recruitment and activation of inflammatory cells (37). Chemically induced edema represents an acute local inflammation eliciting a complex series of physiological events involving many processes in which components of a plant extract may interact, inhibiting kinins and prostaglandins on vascular permeability that appear to be involved with inflammatory process (38). In the present experiment histamine and carrageenan were used in aqueous solution for the induction of acute inflammatory response in chicken's skin as a test system.

Induction of acute inflammation by histamine and carrageenan in the chickens evoked an immediate increase in vascular permeability. The permeability response was short lived; peak was noticed only at 0-2 minute, then showed significant decline. Using the terminology of Kumar *et. al.*(31), from amongst the three patterns of increased vascular permeability, namely: immediate-transient, immediate-sustained and delayed-prolonged, the present response apparently falls into the first category. It was found that increased vascular permeability due to histamine and 5-HT decreased after 30 min, and the normal permeability was restored after 1 hour. The subsequent decrease of permeability, in the present work was abrupt, and normal permeability was restored after 30 min. Simlarly, in carrageenan induced inflammation the peak was observed at 0-2 minute, which declined significantly up to 48 hour. Present results are in agreement with the earlier findings of (4, 19).

The dye exudation in *Solanum nigrum* and *Ricinus communis* pretreated group was significantly lower than the control group in both histamine-induced inflammation and carrageenan-induced inflammation of the chicken skin. Since information relating to the role of *Solanum nigrum* and *Ricinus communis* as permeability suppressant is lacking, a comparison of results was not possible. The maximum suppression of vascular permeability at early phase by these plant extracts suggested there role in antagonizing the action of histamine and 5-HT. Observations of Reddy (47) and Nadeem and Hussain (34) further support our findings as they have reported anti-inflammatory activity of *Solanum nigrum* in acute inflammatory reactions by carrageenan-induced hind paw edema model. Antiinflammatory activity of *Ricinus communis* was also reported by Ilavarasan *et. al.*(18) in carrageenan-induced hind paw edema model in rat and Valderramas *et. al.* (53) in mouse ear edema model and formalin-induced paw edema in mice. Thus, suppressed vascular permeability in the chicken skin may also be co-related with these conditions. It can be concluded that *Solanum nigrum* and *Ricinus communis* plant extracts exerted antihistaminic and anti-inflammatory activity through suppression of vascular permeability in chickens.

The cellular events following chemical, thermal, bacterial, punched wounding and immunological injuries in the chicken skin have been investigated by number of workers (25, 26, 55, 29). However, information on the ameliorative effect of herbal products in cellular events of avian inflammation is lacking. Thus, the present work was designed to investigate the anti-inflammatory effect of *Solanum nigrum* and *Ricinus communis* extract over the cellular events of histamine and carrageenan-induced inflammation in the chicken skin.

In histamine-induced inflammation, chicken skin sections of control group showed hyperaemia and oedema with margination and early emigration of few leukocytes. The leukocyte emigration continued to increase, reaching its maximal count at 3 hour stage where heterophils were noticed as predominant cell type. The monocytoid cells migrated concurrently with the heterophils, followed by basophils. Infiltration of eosinophil was not noticed at any time interval. Present findings are in conformity with the observations of (25, 26, 11) who have reported similar cell kinetics during early inflammatory reaction in the chicken.

The early emigrated leukocytes at carrageenan-induced inflammed site comprised of heterophils and monocytes which was soon followed by migration of basophils. A similar mixed cellular exudate has been reported to occur in the early stages of avian inflammation induced

by various non-immunological stimuli (25), and immunological stimuli (40). The findings also endorse the view of Vegad and Katiyar (55) that early emigration of heterophils and monocytoid cells and participation of basophils appears to be characteristic feature of the early inflammatory reaction in the chicken.

A remarkable feature of the present work, in sharp contrast to its mammalian counterpart, appears to be the formation of prominent perivascular lymphoid foci both in histamine induced and carrageenan induced inflammatory lesion. It could also be indicated that the lymphoid foci formed at site of inflammation may possibly be functioning similar to the mammalian lymph nodes as suggested by Nair (36). The formation of perivascular lymphoid aggregates in present carrageenan-induced reaction also supports the findings of earlier workers reviewed by Vegad and Katiyar (55).

The control group of present investigation revealed that migrated monocytoid cells in the later phase tended to coalesce and formed syncytia after 24 hour, and by 48 hour formed distinct multinucleated giant cells. Several workers have described giant cell formation during the late stage of avian inflammation using different stimuli (14, 29, 50). This is in marked contrast to the situation occurring in mammals, where giant cell formation does not seem to occur in acute inflammation, and are generally seen in certain chronic specific inflammations (31). In general, it may be suggested that concurrent early emigration of monocytes with heterophils, and having a longer half-life of monocytes makes chicken more prone to giant cell formation.

In the present study sequence of leukocytic migration in *Solanum nigrum* and *Ricinus communis* treated groups was similar to untreated groups. However, the overall intensity of leukocyte migration was found to be significantly lower than control group both in histamine induced and carrageenan induced inflammation in the chicken skin. Observations on the pathogenesis of acute inflammation in tissues revealed three stages in the escape of leukocytes from blood vessels (i). Adhesion to vascular endothelium (ii). Transmigration across the endothelium and (iii). Migration in interstitial tissue towards a chemotactic stimulus (33). Thus, above findings can be correlated with impaired adhesion of leukocyte or their failure to pass through the vessel wall to the site of injury due to some interference produced by the herbal extracts which is responsible for their anti inflammatory activity. Since information relating to the role of *Solanum nigrum* and *Ricinus communis* modulating cellular emigration is lacking, a comparison of results was not possible.

In the present study both the plant extracts in early stages make blood vessels impermeable and thereafter prevent cellular emigration. Since, vascular permeability and cellular response are independent of each other and there was marked suppression of both these events suggest anti-inflammatory activity of both the plant extracts. Thus, the present study establishes the therapeutic rationale of using *Solanum nigrum* leaves and *Ricinus communis* root in various anti-inflammatory multiherbal formulations.

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