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PHARMACOLOGICAL POTENTIAL OF *TECOMELLA UNDULATE* IN ACUTE AND CHRONIC INFLAMMATION IN RAT

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ABSTRACT

Inflammation is a biological reaction attributed with several acute and chronic pathological conditions. The study revealed the pharmacological effect of *Tecomella undulate*: bark against carrageenan induced paw edema and cotton pellet induced granuloma in rat. Wistar albino rats of either sex (180-240 g) were employed into the study. Acute inflammation was induced by injecting carrageenan (1%) in rat paw and estimated using plethysmograph. Chronic inflammation was induced by cotton pellet induced granuloma method. Serum nitrate/nitrate estimation was also done as an index of inflammatory reactions. Acute toxicity study was also done using Swiss albino mice. Butanolic and water fractions of *Tecomella undulate* (200 & 400 mg/kg) and indomethacin (10 mg/kg) were used as test drugs. Carrageennan caused a marked increase in rat paw volume due to edema formation. *T. undulate*: butanolic fraction significantly inhibited paw volume in successive hours similar to indomethacin. Interscapular implanted cotton caused significant increase in granuloma wt. and serum nitrate/nitrite level in control group. However, the test drugs lowered the effects of cotton pellet induced chronic inflammation. Therefore, the results may conclude that the bark of *T. undulate* is having a pharmacological potential to treat acute and chronic inflammation in rat.

INTRODUCTION: Inflammation, a defensive response of living mammalian tissues to any injurious agent, is characterized with marked edema, leukocyte infiltration and granuloma formation [1]. The recruitment of macrophages and neutrophils, leads to induction of cyclooxgenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [2], are responsible for the initiation and progression of acute or chronic stages of inflammation3. Nitric oxide (NO), released due to the activation of iNOS4, is responsible for vasodilatation, increased vascular permeability, edema and granuloma formation. The modern medication as steroidal and non- steroidal anti-inflammatory drugs (NSAID's), are attributed with certain side effects and toxic manifestations. Therefore, various plants have been preferred, as recommended by alternative systems of medicine, for treating inflammatory pathological conditions [5].

Tecomella undulate Seem. (Bignoniaceae) commonly called Rohitaka, is a small deciduous shrub or tree. Bark is majorly constituted into various herbal formulations like Livo-plus, Liv- 52, Herboliv, Amylcure etc. for curing inflammatory hepatic diseases. It is also used in rheumatism, muscular pain[6]. It is reported to contain iridoid glucosides- undulatin, tecomelloside and tecoside[7]; chromone glycosides – undulatoside - A & B[8]; 6- O- veratryl catalposide [9]; ferulic esters: lapachol, tectol, tectoquinone and β-sitosterol[10]. Therefore, the present study was designed to evaluate the anti-inflammatory effects of *Tecomella undulate*: bark in carrageenan induced paw edema and cotton pellet induced granuloma respectively in rat.

MATERIALS AND METHODS:

Plant material: Rohitaka bark was collected from rural areas near Jamnagar, Gujarat and authentified by prof. A.k. Rawat, National Botanical Research institute (NBRI), Lucknow, India (NBRI-SOP-202).

Preparation of the plant extracts: the powdered rohitaka bark (500g) was extracted with 95% ethanol using a soxhlet extractor. The extract was concentrated using rota- evaporator. This ethanolic extract (yield 8.6 %, w/w) of *Tecomella undulate* bark was further fractioned to obtain butanolic and aqueous fractions.

Animals: the experiments were carried out using wistar albino rats (180-240 g) and Swiss albino mice (25-30 g) of either sex procured from animal house, ISF College of Pharmacy, Moga, Punjab. They were maintained on food pellets and water ad libitum. The temperature and humidity were maintained at 20 - 25^{o}c and 45-50% respectively. Animals were housed fewer than 12 - 12 hrs light/dark cycles. The animals were acclimatized for at least 5 days to the laboratory conditions before doing experiments. The experimental protocol was approved by the institutional animal ethics committee (IAEC) and the care of laboratory animals was taken as per the guidance of CPCSEA.

Chemicals: Carrageenan was purchased from Sigma Aldrich, USA. All the chemicals or reagents obtained from reputed firms in India of analytical grade were freshly prepared.

Preliminary Phytochemical Screening: Qualitative screening of the extract was made to investigate the major chemical classes of components present. It was screened for the presence of alkaloids, anthraquinones, tannins, flavonoids, glycosides, terpenes, phenols and steroids.

Acute Toxicity Studies: Acute oral toxicity studies were performed according to OECD guidelines. Swiss albino mice (n= 5) of either sex selected by random sampling technique were employed in study. The animals were fasted for 4 h with free access to water only. Rohitaka: butanol and water fractions (suspended with 8% propylene glycol and distilled water respectively) were administered orally at the dose of 50 mg/kg initially and mortality was observed for 3 days. If mortality was not observed, the procedure was then repeated with higher doses such as 200, 1000 and 2000 mg/kg.

ACUTE ANTI-INFLAMMATORY STUDIES:

Carrageenan Induced Paw Oedema in Rats: The rats were divided into five groups: control, Rohitaka: butanolic extract (200 and 400 mg/kg), Rohitaka: water extract (200 mg/kg), and Indomethacin (10 mg/kg) as standard, each comprising six animals. Acute inflammation was induced by subplantar administration of 0.1 ml of carrageenan (1%). Test drugs were administered orally, 1 hr before administration of carrageenan. The paw volume was measured prior to injection of carrageenan (0 hr) and then at predetermined interval of 1 hr upto 3 hrs. Paw volume was measured using Plethysmograph [11].

Change in paw volume was measured using the formula: % Inhibition of Inflammation= [1- (Vt/Vc) x100], where Vt: the change in the paw volume in test drug treated group; Vc: the change in paw volume in control group.

CHRONIC ANTI- INFLAMMATORY STUDIES:

Cotton-Pellet Induced Granuloma in Rats: The Wistar albino rats were divided into following groups (n= 6).

Group	Treatment
Control	Normal saline + cotton-pellet
R.BE.200	Rohitaka: But.Ext. 200mg/kg + cotton-pellet
R.BE.400	Rohitaka: But.Ext. 400mg/kg + cotton-pellet
R.WE.200	Rohitaka: W.Ext. 200mg/kg + cotton-pellet
Indom.10	Indomethacin: 10mg/kg + cotton-pellet

The rats were anesthetized with intraperitoneal administration of Kitamine hydrochloride (70 mg/kg, i. p.). After shaving the fur, 50 mg of sterile cotton pellets were surgically implanted in both sides of interscapular region. The test drugs, vehicle and standard drug were allowed to administer for 7 consecutive days. On the 8th day, blood was collected

through retro orbital plexus for serum nitrite/nitrate estimation. Rats were sacrificed; cotton pellets were removed surgically and dried at 60 ^{o}C for 8 hrs. Dry weight of the pellet was taken as measure of granuloma formation[12].

Measurement of Serum Nitrite/Nitrate Concentration: Total nitrite/nitrate in serum, an indicator of nitric oxide (NO) synthesis, was measured by Griess reaction. Briefly, add 500 µl of Griess reagent [0. 1 % (w/v) naphthylethylendiamide dihydrochloride in H_2O and 1% (w/v) sulphanilamide in 5 % (v/v) concentrated H_3PO_4, 1:1] to 100µl of serum sample. The optical density at 540 nm was measured using UV spectrophotometer. Nitrite concentration was calculated by comparison with OD540 of standard molar solutions of sodium nitrite [13].

Statistical Analysis: Results expressed as Mean±SD, were analyzed using one-way analysis of variance (ANOVA) followed by Dunnets multiple test as post-hoc analysis. $P < 0.05$ was considered as statistically significant.

RESULTS:

Preliminary Phytochemical Screening: The phytochemical studies indicated that the butanolic fraction of *T. undulate*: bark contains glycosides (iridoid), tannins, phenolics, flavonoids, and terpenes. Tests for alkaloids, steroids and reducing sugar were shown negative. Moreover, the water fraction only showed the presence of tannins, phenolics.

Acute Oral Toxicity Test: Butanolic and water fractions of *Tecomella undulate* did not produce any mortality even at highest dose (2000 mg/kg, p. o.) employed. Moreover, two doses (200 and 400 mg/kg, p. o.) were selected for further pharmacological studies.

ACUTE ANTI- INFLAMMATORY STUDIES:

Effect of Drug Treatment on Carrageenan Induced Rat Paw Edema: In acute inflammation animal model, the subplantar injection of carrageenan caused a time dependent increase in paw volume at 1 h, 2 h and was maximal at 3 h in control group. However, the entire test drugs except RWF - 200, showed significant reduction in paw volume during 1st and 2nd h. Moreover, pretreatment with butanolic fractions of Rohitaka (200 & 400 mg/kg) and indomethacin markedly attenuated ($p < 0.005$) the carrageenan induced paw edema during 3rd h by 26.6%, 17.6% and 10.18% respectively (Figure 1).

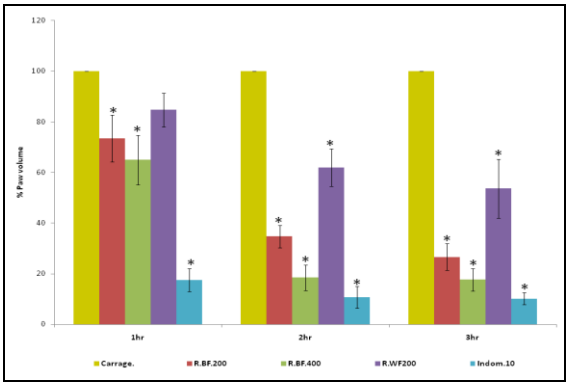

Fig. 1: Effect of Drug treatment on Carragennan induced rat paw edema

Data: Mean ± SD; $p<0.05$ statistically significant; * vs carragennan control; [Carrage.: Control; RBF-200: Rohitaka: But.Fract.200 mg/kg; RBF-400: Rohitaka: But.Fract.400 mg/kg; R.WF-200: Rohitaka: Water Fract.200 mg/kg; Indom.10: Indomethacin 10 mg/kg].

CHRONIC ANTI- INFLAMMATORY STUDIES:

Effect of Drug Treatment on Cotton-Pellet Induced Granuloma in Rat: In this model, interscapular implantation of sterile cotton (50 mg) caused a significant increase in granuloma wt. in control group. However, the Rohitaka: butanolic fractions (200 & 400 mg/kg) inhibited ($p < 0.05$) granuloma by 45.98%, 57.31% respectively. RWF-200 showed 16.13% reduction in granuloma. The standard: indomethacin (10 mg/kg) also depleted the granuloma wt. upto 72.96% (Table: 1).

Table: 1: Effect of Drug Treatment on Cotton-pellet induced Granuloma in rat

Group	Dose (mg/kg, *p .o.*)	Granuloma wt (mg)	% inhibition
Control	-	291.4 ± 30.21	-
R.BF.200	200	157.4 ± 7.53*	45.98
R.BF.400	400	124.4 ± 9.58*	57.31
R.WF.200	200	244.4 ± 10.78*	16.13
Indom.10	10	78.8 ± 12.71*	72.96

Data: Mean ± SD; p<0.05 statistically significant; *vs Control. [Control: Cotton-pellet control, R.BF.200: Rohitaka: But.Fract.200 mg/kg; R.BF.400: Rohitaka: But.Fract.400 mg/kg; R.WF.200: Rohitaka: Water Fract.200 mg/kg; Indom.10: Indomethacin 10 mg/kg]

EFFECT OF DRUG TREATMENT ON COTTON-PELLET INDUCED SERUM NITRITE/NITRATE LEVEL: After chronic induction of cotton-pellet induced granuloma, a marked increase ($p < 0.05$) in serum nitrite/nitrate level was noted as compared to saline control. However, Rohitaka: Butanolic fractions 200 and 400 mg/kg significantly ($p < 0.05$) decreased nitrite/nitrate level, similar to indomethacin (Table 2).

Table: 2: Effect of Drug Treatment on Cotton-Pellet induced serum nitrite/nitrate level

Group	Dose (mg/kg, *p.o.*)	Serum Nitrite/Nitrate (µM/ml)
Saline control	Normal saline	0.778 ± 0.192
Control	-	3.15 ± 0.432[a]
R.BF.200	200	1.652 ± 0.084[b]
R.BF.400	400	1.378 ± 0.189[b]
R.WF.200	200	2.42 ± 0.27[b]
Indom.10	10	0.974 ± 0.132[b]

Data: Mean ± SD; p<0.05 statistically significant; a: Saline control vs Control, b: vs Control. [Control: Cotton-pellet control, R.BF.200: Rohitaka: But.Fract.200 mg/kg; R.BF.400: Rohitaka: But.Fract.400 mg/kg; R.WF.200: Rohitaka: Water Fract.200 mg/kg; Indo.10: Indomethacin 10 mg/kg]

DISCUSSION: The present study demonstrates that the bark of *Tecomella undulate* has marked pharmacological potential to attenuate Carrageennan induced acute and cotton pellet induced chronic inflammations in rat.

Carrageennan model is attributed to cause paw edema in the form of acute inflammatory response[11] which involves increased capillary permeability, leukocyte infiltration initially; migration of neutrophils and macrophages; and activation of cyclooxygenase and lipoxygenase enzymes[14]. This leads to secretion of pro-inflammatory mediators: histamine, bradykinin and prostaglandins[15]. In this study, the subplantar injection of carrageenan produced marked edema characterized with plasma protein extravasation and exudation[16]. However, the *T. undulate*: butanolic fraction was significantly prevented the states of inflammation progressively; indicating the inhibition of histamine and cyclooxygenase (COX)

induced prostaglandins release (Fig. 1). However, in another study, methanolic extract of bark did not shown any significant effect on Carrageennan induced paw edema[17].

In chronic inflammatory study, the transudative, exudative and proliferative phases are appeared[18], showing granuloma formation as evidenced in present study. Further, it is attributed with monocyte, neutrophil infiltration, fibroblast proliferation; and exudation[19]. In this study, butanolic fraction of *T. undulate* lowered weight of cotton pellets as compared to control groups (Table: 1). This may be due to the potential of *T. undulate* in preventing fibrogenesis, collagen and mucopolysaccharide synthesis, which are natural proliferative events of granulation tissue formation[20]. The inflammatory reaction is characterized with induction of PGs, cytokines and iNO synthase, producing (NO) [3]. NO is responsible for vasodilatation, increased vascular permeability and edema formation[21]. *T. undulate*: butanolic fraction markedly inhibited the level of NO in present study (Table 2). The mechanism responsible for NO inhibition may involve prevention of iNOS activity and it could be implicated in suppression of PGs generation. Moreover, the water fraction of *T. undulate* showed mild effect against acute and chronic inflammatory conditions in successive hours, and this effect may be due to less availability of active constituents, as compared to butanolic fraction.

CONCLUSION: Conclusively, the results suggest that *Tecomella undulate* has marked effect in attenuating experimental acute and chronic inflammatory reactions in rat. This effect may be due to the presence of iridoid glucosides, tannins, flavones and terpenes, present in the plant as reported earlier. In addition, the study provides a scientific rational to its therapeutic use. Future studies may be designed to isolate and characterize the active plant component and its putative mechanisms of prevention in inflammatory conditions.

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