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INVESTIGATIONS OF ANTI-INFLAMMATORY ACTIVITY OF JIGRINE

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Abstract: *Jigrine*, a polypharmaceutical herbal formulation containing 14 medicinal plants is used in the Unani system of medicine for the treatment of liver ailments. The antiinflammatory activity of *Jigrine* (0.5 ml and 1.0 ml/kg, po), was evaluated against acute inflammation caused by carrageenin (injecting 0.1 ml of 1% carrageenin in 0.9% NaCl solution into plantar surface of the hind paw of the rat) and the effect of *Jigrine* (1 ml/kg/day, po for 7 days) was also studied on the sub-acute inflammation induced by cotton pellet granuloma. The paw volume, biochemical parameters like tissue AST, ALT, γ-GTP and lipid peroxides and dry wt. of granuloma were measured to assess the anti-inflammatory activity. It showed a significant anti-inflammatory activity as evidenced by lowering the elevated levels of paw volume and biochemical parameters. But it could not reduce the sub-actute inflammation caused by cotton pellet granuloma. The study suggests that *Jigrine* has significant effect only on acute phase of inflammation caused by carrageenin. Antioxidant and membrane stabilizing action of *Jigrine* might be responsible for its anti-inflammatory effect.

Key words : inflammation, antioxidant, gamma glutamyl transpeptidase, *Jigrine*, silymarin

INTRODUCTION

Earlier studies in our laboratory showed that *Jigrine* a polypharmaceutical herbal formulation containing the aqueous extracts of 14 medicinal plants namely *Cichorium intybus, Tamarix dioica, Solanum nigrum, Rheum emodi, Rubia cordifolia, Vitex negundo, Cassia occidentalis, Fowniculum vulgare, Cuscuta reflexa, Careya arborea, Phyllanthus niruri, Plantago major, Rosa damascena* and *Solanum xanthocarpum* is known to posses antihepatotoxic properties. *Jigrine* decreased the gamma-glutamyltranspeptidase (γ-GTP) level in the liver cells of rats treated with alcohol-carbontetrachloride. The measurement of γ-GTP has recently emerged as a promising marker in inflammatory conditions. Therefore, experiments were designed to find out whether *Jigrine* modulates inflammatory reactions produced by carrageenin as well as cotton pellet granuloma.

METHODS

Male albino rats (Wistar strain) of 120–200 g body wt. were used. They were grouped into 14 groups of 5 animals each and maintained under standard laboratory conditions. They had a free access to commercial pellet diet and water. The room temperature was maintianed at 25 ± 1°C.

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Carrageenin-induced oedema

Rats of group I to V were injected 0.1 ml of 1% carrageenin in 0.9% w/v sodium chloride solution into the hind paw according to the method of Winter et al. (1) to induce oedema. The foot volume was measured in rats by the modified plethysmographic method described by Singh and Ghosh (2). Animals of the groups II to V were given drug treatment as described below exactly one hour before the carrageenin injection. Group I animals were treated with normal saline and served as carrageenin control group. Group II was given *Jigrine* (0.5 ml/kg, po). Group III was given a higher dose of *Jigrine* (1 ml/kg, po). Group IV was given silymarin (25 mg/kg, po). Group V was given indomethacin (5 mg/kg, po).

Biochemical studies

Rats in Group VI were given normal saline (1 ml/kg, po) and after exactly three hours they were sacrificed and liver removed for enzyme estimations. Rats in groups VII to XI were given 0.1 ml of carrageenin (1% w/v) in normal saline intraplantarly and the animals were sacrified exactly three hours after carrageenin injection. The liver was removed and the tissue homogenates 10% for γ-GTP (3) and lipid peroxides; 0.5% for AST and ALT were prepared in 0.15 M potassium chloride (4). Animals from these groups were also given drug treatment, one hour before carrageenin injection as described below. Out of these, Group VII rats were given normal saline and this group served as carrageenin control group; Group VIII was given *Jigrine* (0.5 ml/kg, po); Group IX was given a higher dose of *Jigrine* (1 ml/kg, po), Group X was given silymarin (25 mg/kg, po) and Group XI was given indomethacin (5 mg/kg, po).

Cotton-pellete granuloma

The cotton pellet granuloma was produced in rats by the method of Winter and Porter (5) with slight modification. The pellete, weighing exactly 10 mg ± 0.5 mg each were made from 5 mm section of cotton rolls. The cotton pellet were sterilized in an autoclave for 30-45 minutes under 15 lb pressure. Under light ether anaesthesia a small incision was made in the middle of dorsal surface and a pocket was created by inserting a pair of plunt scissors into the incision, taking care that no bleeding occurs. Four cotton pellets were implanted into each animal, two on either side of the midline incision and stiched properly. Rats of Group XII were given normal saline (1 ml/kg/day, po for 7 days) and those in Groups XIII and XIV were given *Jigrine* (1 ml/kg/day, po) and Hydrocortisone (40 mg/kg/day, sc) respectively for 7 days. On 8th day animals were sacrificed, the pellets were taken out, washed and dried at 60°C–70°C for 6 hours. The granuloma weights were obtained from control and treatment groups. Liver tissues were also collected for biochemical estimations.

Biochemical parameters

Tissue homogenate obtained from various groups were used for the estimations of: I) Aspartate transaminase (6), II) Alanine aminotransferase (6), III) Gamma-glutamyl transpeptidase (7), IV) Thiobarbituric acid reactive substances (8, 9) and V) Protein/mg of tissue (10).

Statistical analysis

The mean ± SE values were calculated for each group for determining significance of inter group differences each parameter was analysed separately and Analysis of Variance (ANOVA) was carried out (11)

RESULTS

Effect of Jigrine treatment on carrageenin induced paw edema: Jigrine produced a dose

dependent inhibition of rat paw edema (Table I). The percent inhibition were found to be maximum (35% and 53%) at 3 hours of carrageenin inflammation.

Effect of Jigrine treatment on carrageenin induced biochemical changes: A highly significant increase in levels of tissue AST, ALT, γ-GTP and lipid peroxides were found due to carrageenin treatment. Oral administration of *Jigrine* (0.5 ml and 1 ml/kg, po) exhibited a reduction in carrageenin induced increase in the levels of AST, ALT, γ-GTP and lipid peroxide. Treatment with silymarin and indomethacin also reversed the carrageenin induced biochemical changes (Table II).

TABLE I : Effect of *Jigrine*, silymarin and indomethacin on carrageenin-induced rat paw edema.

Treatment	*Dose*	*Oedema (in ml)* $\bar{x}$ ± *SEM*	*Percent inhibition*
1. Control	–	0.30 ± 0.01	–
2. *Jigrine*	0.5 ml/kg, po	0.19 ± 0.05	35
3. *Jigrine*	1 ml/kg, po	0.14 ± 0.04*	53
4. Silymarin	25 ml/kg, po	0.10 ± 0.04*	65
5. Indomethacin	5 ml/kg, po	0.05 ± 0.01*	81

(n = 5); *P<0.05

Effect of Jigrine treatment on cotton pellet granuloma test: The dry wt. of grnuloma was significantly increased after 7 days of implantation. *Jigrine* (1 ml/kg, po) was not able to reduce the granuloma where as hydrocortisone (40 mg/kg/day, sc) significantly reduced the granuloma. The tissue AST, ALT,

TABLE II : Effect of *Jigrine*, silymarin and indomethacin administration on transaminases, gamma-glutamyl transpeptidase and lipid peoxidation in rat liver induced by carrageenin.

Group		*AST (units/ml)* $\bar{x}$ ± *SEM*	*ALT (units/ml)* $\bar{x}$ ± *SEM*	*γ-GTP (I.U.)* $\bar{x}$ ± *SEM*	*Lipid peroxides (n moles MDA/mg protein)* $\bar{x}$ ± *SEM*
VI	Control	57.6 ± 5.7	31.2 ± 5.08	40.94 ± 3.22	1.44 ± 0.23
VII	Carrageenin alone	225.6 ± 5.87	166.4 ± 9.74	107.54 ± 8.05	4.82 ± 0.19
VIII	Carrageenin + *Jigrine* 0.5 ml/kg, po	205.2 ± 10.04*	100.8 ± 13.28*	101.54 ± 6.74	4.22 ± 0.46
IX	Carrageenin + *Jigrine* 1 ml/kg, po	196 ± 18.55*	77.6 ± 32.2*	83.55 ± 5.76*	2.39 ± 0.3*
X	Carrageenin + Silymarin 25 mg/kg, po	215.4 ± 7.9*	66.4 ± 9.33*	90.17 ± 2.74*	2.57 ± 0.35*
XI	Carrageenin + Indomethacin 5 mg/kg, po	160.4 ± 9.98*	63.2 ± 7.28*	50.87 ± 5.68*	3.02 ± 0.29*

(n = 5); *P<0.05

TABLE III : Effect of *Jigrine* and hydrocortisone administration on transaminases, gamma-glutamyl transpeptidase and lipid peoxidation in rat liver induced by cotton-pellet granuloma.

Group		*Dry wt. of granuloma (mg)* $\bar{x}$ ± *SEM*	*AST (units/ml)* $\bar{x}$ ± *SEM*	*ALT (units/ml)* $\bar{x}$ ± *SEM*	*γ-GTP (I.U.)* $\bar{x}$ ± *SEM*	*Lipid peroxides (n moles MDA/mg protein)* $\bar{x}$ ± *SEM*
XII	Control	19.4 ± 0.41	383.2±1.95	133.6±12.81	100±3.55	3.09±0.20
XII	*Jigrine* 1 mg/kg, po	18.6 ± 0.35	329.6±18.74	100±14.08	84.44±2.75	1.65±0.10*
XIV	Hydrocortisone 40 mg/kg, po	14.4 ± 0.31*	234.4±13.71*	72±6.19*	58.73±7.55*	2.11±0.08

(n = 5); *P < 0.05

γ-GTP and lipid peroxides were significantly increased in cotton pellet granuloma induced inflammation. Hydrocortisone (40 mg/kg/day, sc) sigficantly reduced the elevated levels of tissue AST, ALT, γ-GTP and lipid peroxides. But with *Jigrine* (1 ml/kg/day, po) no significant reduction was found (Table III).

DISCUSSION

Carrageenin administration into the plantar region is known to produce inflammation due to release of various autocoids like histamine, 5-hydroxytryptamine kinins, and prostaglandins (12). Carrageenin induced inflammation in rats is accompanied by a significant increase in lipid peroxides by the liver due to some as yet uncharacterized metablic reaction in the liver (13). Carrageenin induced inflammation is known to increase the liver AST and ALT levels (14). Gammaglutamyl transpeptidase (γ-GTP) a membrane bound enzyme is present on the external surface of the cell membranes in variety of anatomical locations. The enzymes γ-GTP is invloved in the biosynthesis of leukotrienes (15). At pico or nano molar concentrations these leukotrienes act as inflammatory mediators (16). In our study we also found that γ-GTP levels were raised in the liver of carrageenin treated group, which was not reported in the literature earlier. The rise in γ-GTP in the liver indicate that carrageenin could cause damage might be caused either due to carrageenin or due to release of autocoids. The decrease in the γ-GTP levels indicates the membrane stabilising effect of *Jigrine*. The reduction of peroxide formation in the *Jigrine* treated group indicate the anti-oxidant property of this unani medicine.

The increase in lipid peroxide formation, γ-GTP, AST, and ALT levels in liver during cotton pellet granuloma in the rat indicate that inflammation at different site might have an adverse effect on the liver, probably due to the release of autocoids. The reduction in the dry wt. of granuloma by hydrocortisone shows, the anti-inflammatory effect of hydrocortisone in sub-acute inflammatory condition. *Jigrine* could not produce significant reducation of dry wt. of granuloma, indicating that *Jigrine* may not be exhibiting a steroid like anti-inflammatory effect.

The crude extracts of the medicinal plants *Solanum nigrum* and *Cichorium intybus* have been reported to inhibit free radical mediated DNA damage (17). some of the ingredients of *Jigrine* are known to possess anti-inflammatory activity e.g. *Tamarix dioica* contains flavonoids and these flavonoids have anti-inflammatory property. *Rubia cordifolia* (18, 19) has anti-inflammatory activity apart from immunomodulant, anti-PAF and anti-peroxidative activities. *Vitex negundo* (20) contains triterpenoids and exhibits anti-inflammatory activity. *Cassia occidentalis* (21) contains chrysophenols and exerts anti-inflammatory activity by inhibition of phospholipase A_2 and also has stabilization effect on the lysosomal membrane system. *Foeniculum vulgare* (22) is a carminative and contains pectin and Vit. A, B_1, B_2 and C has antispasmodic and also antihistaminic activities. *Cuscuta reflexa* (23) possess spasmolytic activity, *Careya arborea*, *Plantago major* and *Rosa damascena* are known to possess anti-inflammatory activity. The decrease in carrageenin induced paw oedema caused by *Jigrine* might be due to presence of the above mentioned anti-inflammatory drugs. This study suggests that *Jigrine* has significant effect on acute phase of inflammation caused by carrageenin. Anti-oxidant and membrane stablizing action of *Jigrine* might be responsible for its anti-inflammatory action.

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