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 anti-inflammatory 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-acute 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 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 maintained at 25 ± 1°C.
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 sacrificed 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 pellet, 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 blunt 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).**

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


γ-GTP and lipid peroxides were significantly increased in cotton pellet granuloma induced inflammation. Hydrocortisone (40 mg/kg/day, sc) significantly 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 metabolic 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 involved 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 reduction 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 A₂ and also has stabilization effect on the lysosomal membrane system. Foeniculum vulgare (22) is a carminative and contains pectin and Vit. A, B₁, B₂ 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 stabilizing action of Jigrine might be responsible for its anti-inflammatory action.
REFERENCES


