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Evaluation of a Unani herbal formulation containing colchicum, ginger and aloe for anti-inflammatory activity

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Abstract

With an increased incidence of drug toxicity and resistance to allopathic drugs, natural products from plants could be interesting alternatives. Some plant extracts and phytochemicals are known to have anti-inflammatory properties, and can be of great significance in treatment of inflammatory disorders. These considerations require the scientific evaluation of the most important and commonly used traditional herbal formulations. A study has been done to find anti-inflammatory activity of Unani formulation derive from medicinally important plants like *Zingiber officinal* (Ginger), *Colchicum luteum* (Colchicum), and *Aloe vera* (Aloe). In this proposed work, we had modified powder of different mentioned plants into its solid state (tablet) by using gum *Acacia*. Its 50% alcoholic extract and aqueous extract were used to determine its anti-inflammatory activity by carrageenin induced oedema test and cotton pellet induced granuloma test. Efficacy of Unani formulation was compared with a standard referent drug, Diclofenac sodium. The obtained results using carrageenin oedema test showed decrease in the left hind paw volume significantly ($p<0.001$) after 3 hours of carrageenin injection. In cotton pellet induced granuloma test, animals in all the test and standard drug treated groups, shows reduction in granuloma formation significantly ($p<0.001$). Thus, our results clearly indicate that, modified form of the test formulation possesses significant anti-inflammatory activity in both acute and sub-acute phase.

Keywords: *Zingiber officinal*, *Colchicum luteum*, *Aloe vera*, Unani medicine, Anti-inflammatory.

INTRODUCTION

The Unani System of Medicine, an Indian variant of Greco-Arabic system is being practiced in India for centuries; not only its simple medicaments but also the polypharmaceutical preparations have great significance in the treatment of inflammatory conditions. Natural products in current use possess nearly every conceivable type of biological activity. WHO estimates that 4-billion people from all over the world use herbal medicines. The discovery of a vegetable extract of medicinal benefit leads to the isolation of active principle and its subsequent chemical characterization [1]. Despite the potential of plants to provide us with useful pharmaceutical agents, the field is still poorly studied. Only an estimated 5–10% of the approximately 3lacs–5lacs plant species worldwide have been screened for one or more bioactivities [2]. Inflammation is generally considered as an essentially protective response to tissue injury caused by noxious physical, chemical or microbiological stimulus. It is a complex process involving various mediators, such as prostaglandins, leukotrienes and platelet activating factor etc. Thus, inhibition of the prostaglandins and other inflammatory mediators could be employed as criteria to evaluate potential anti-inflammatory compounds. The current management of inflammatory diseases is limited to the use of anti-inflammatory drugs whose chronic administration is associated with several adverse effects. Plant-derived products are slowly emerging as a viable alternative because they are cheap, abundantly available and relatively less toxic [3]. Also, solid form of drug (tablet) has many advantages over conventional powder states owing to its ease of inhalation, taste and more accurate dosage form. Therefore, in the present study Pharmacological characteristic of a Unani compound formulation mentioned in Unani Pharmacopoeia, "Ilaj ul Amraz" [4] was investigated which is not generally used in clinical practices. For the study it is modified for use in the form of Tablet and additionally gum *Acacia* (S. d. Fine Chemical Ltd.) was used as excipient. According to the "Ilaj ul Amraz" the Unani formulation contains (i) Ginger (*Zingiber officinale* Linn.— Dried Rhizome- 3.5 g), (ii) Colchicum (*Colchicum luteum* Baker— Dried Corm- 3.5 g) and (iii) Aloe (*Aloe vera* Linn.—Dried Exudate- 7.0 g). All the three ingredients possess anti-inflammatory and anti-arthritic properties. They also have the action as diuretic and laxative. Colchicum and Aloe have purgative property also and many more actions are attributed to these drugs, described in Unani texts as well as scientific reports [3,5,6,7,8,9].

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MATERIALS AND METHODS

Collection of plant material

The raw materials were purchased from the local market of Aligarh and the sample were authenticated in Pharmacognosy section of the Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh and found within range of standards as mentioned in the Unani and Ayurvedic Pharmacopoeia of India [6,10,11].

Animal maintenance

The present study was undertaken to evaluate the anti-inflammatory and analgesic activity in healthy albino rats of either sex weighing 150-200g. Animals were housed in groups of 6 animals in cages under hygienic conditions. All experiments were conducted during light phase between 8.00 and 13.00 hrs. All procedures were conducted according to the guidelines of the International association for the study of pain [12]. All the animals were fed, standard animal diet and water, ad-libitum.

Drugs and chemicals

Diclofenac sodium (Voveran, Novartis, India), Carrageenan Type II (Sigma chemical company, USA), Normal Saline (E. Merck Ltd, India), 50% Alcoholic and Aqueous extract of tablets.

Preparation of extracts

The Test formulation (tablets) was coarsely powdered in an iron mortar for extraction. The powdered drug was extracted separately in 50% Alcohol (Hydro-alcoholic) and in Distilled Water (DW) with Soxhlet's apparatus for 6 hours. The extracts were filtered and dried by evaporation on water bath. The yield percentage was calculated with reference to test drug and was found to be 26.75% for hydro-alcoholic extract (Halc) and 32.35% for aqueous extract (Aq.) respectively. Fresh suspension of extracts was prepared in distilled water with 2% gum *Acacia* powder (S. d. Fine Chemical Ltd.), which was administered orally in the animals with the help of feeding canula after shaking the suspension well. The dose for the animal was calculated by extrapolating the human dose of test drug by conversion factor of 7 for rat [13]. Hence the two different doses selected for the study of hydro-alcoholic extract of the test drug were 225 mg/kg and 335 mg/kg and for aqueous extract of the test drug were 270 mg/kg and 410 mg/kg.

Determination of anti-inflammatory activity
Carrageenin induced oedema test

Oedema represents the acute phase of inflammation in carrageenin-induced paw oedema test. The effect of test drug on carrageenin-induced oedema in rat paw was studied by the method of Winter et al. [14]. It is the simplest and most widely used model for studying the anti-inflammatory activity. Albino rats of either sex, weighing 150-200 gm, were divided in to 6 groups of 6 animals each. The volume of left hind paw was measured plethysmographically before giving the drugs. Animals in Group I served as control and were administered with 20 ml/kg distilled water. The reference drug, Diclofenac Sodium was given to the animals in Group IInd, in a dose of 5 mg/kg, orally. IIIrd and IVth group was treated with 225 mg/kg and 335 mg/kg of Halc. extract and animals in group Vth and VIth were administered with 270 mg/kg and 410 mg/kg of Aq. extract of Test formulation in the form of suspension respectively. One hour after the drug/vehicle treatment, 0.1 ml of 1% aqueous solution of Carrageenin in distilled water was injected under the plantar aponeurosis of the left hind paw. The volume of the paw was again measured at 1, 2, 3, 4 and 5 hours after Carrageenin injection. The percentage inhibition of oedema was calculated with reference to negative control by the formula described by Newbould (1963) [15].

$$I = 100\left[1 - \frac{a - x}{b - y}\right]$$

Where,

I = Percentage of inhibition
a = Mean left hind Paw volume of Test / Standard animals after Carrageenin injection.
b = Mean left hind Paw volume of control animals after Carrageenin injection.
x = Mean left hind Paw volume of Test / Standard animals before Carrageenin injection.
y = Mean left hind Paw volume of control animals before Carrageenin injection.

Cotton pellet induced granuloma test

Cotton pellet test was carried out by the method of Winter et al. [16]. The six groups of albino rats of either sex weighing 150-200 g, six in each group was included in this study. After shaving off the fur, the animals were anaesthetized by ether. Sterile pre weighed cotton pellets (20 ± 1mg) were implanted in the ventral region of rats, one near each axilla through a single needle incision. Animals in group I served as control and were administered with 20 ml/kg distilled water. The standard drug was given to the animals in group II, in a dose of 5 mg/kg, orally. Third and fourth group was treated with 225 mg/kg and 335 mg/kg of Halc. extract and animals in group fifth and sixth were administered with 270 mg/kg and 410 mg/kg of Aq. extract of test formulation in the form of suspension respectively. Test formulation was administered orally to the respective group of animals for seven consecutive days, from the day of cotton-pellet implantation. On the day one test formulation was administered 30 minutes before cotton pellet implantation. On the eighth day, the animals were anaesthetized again; the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37 °C for 24 h and dried at 60 °C to constant weight. The increase in the dry weight of the pellets was taken as a measure of granuloma formation.

Statistical analysis

The results are expressed as Mean ± SE from n=6 observations. The findings were also analyzed for determining significance of difference by one-way ANOVA test followed by pair-wise comparison of various group by LSD. The analysis was carried out by using the online available software [17].

RESULTS

The results of carrageenin induced oedema test are presented in Table-1 and the comparison of percentage of inhibition among all the treated groups is shown in Figure-1. In the carrageenin oedema test, 5mg/kg of the standard drug, Diclofenac sodium, higher and lower doses of Halc. and Aq. extract of the test formulation were found to decrease the left hind paw volume significantly ($p<0.001$) 3 hours after carrageenin injection. The percentage inhibition of oedema was found to be 69.09% with Standard drug treated group. Whereas, in the test drug treated groups the inhibition was found as 56.36% and 72.73% with 225mg/kg and 335 mg/kg of Halc. extract, and 63.63% and 74.55%

with 270mg/kg and 410 mg/kg of Aq. extract, respectively.

Table 1. Anti-inflammatory effect of the formulation tablet in carrageenin induced oedema test.

Group(s)	Increase in paw volume in ml after carrageenin injection (Mean ± SE)				
	Ist hr.	IInd hr.	IIIrd hr.	IVth hr.	Vth hr.
Control	0.40±0.16	0.43±0.17	0.55±0.16	0.44±0.09	0.41±0.07
Diclofenac sodium (5mg/kg)	0.17±0.05 x•	0.14±0.05 x•	0.17±0.04 x■	0.18±0.03 x■	0.20±0.02 x■
Alc. Ex. (225mg/Kg)	0.20±0.02 x•	0.21±0.03 x*	0.24±0.03 x■	0.25±0.06 X*	0.24±0.04 x•
Alc. Ex. (335mg/Kg)	0.21±0.05 x*	0.18±0.05 x•	0.15±0.06 x■	0.19±0.05 x•	0.21±0.05 x■
Aq. Ex. (270mg/Kg)	0.20±0.02 x•	0.18±0.03 x•	0.20±0.04 x■	0.23±0.07 x•	0.23±0.05 x•
Aq. Ex. (410mg/Kg)	0.18±0.02 x•	0.15±0.03 x•	0.14±0.06 x■	0.19±0.07 x•	0.20±0.06 x■

n=6, * = p < 0.05 ●= p < 0.01 ■ = p < 0.001, x= against control,

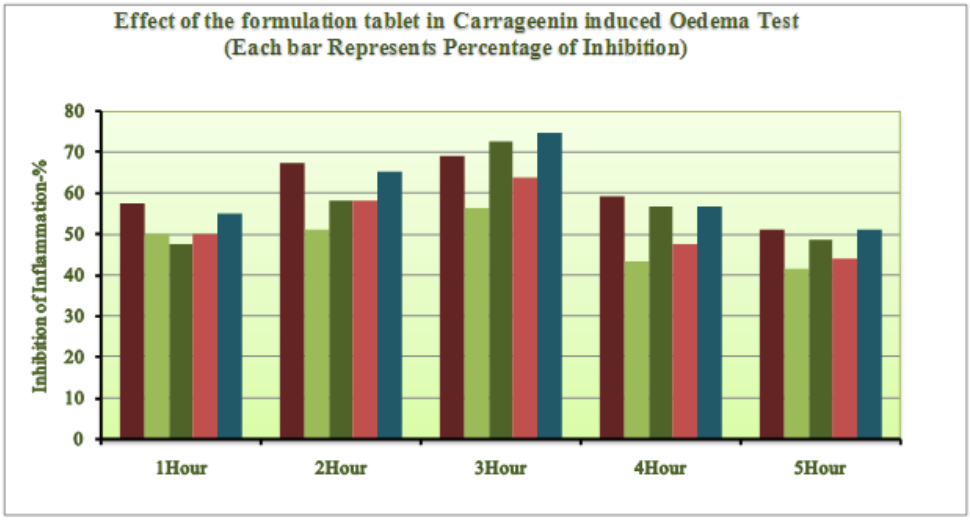

Fig 1. Effect of formulation tablet in carrageenin induced oedema test.

Diclofenac sod. 5mg/kg
Alc. Ex. 225 mg/kg
Alc. Ex. 335 mg/kg
Aq. Ex. 270 mg/kg
Aq. Ex. 410 mg/kg

In the cotton pellet induced granuloma test, 5mg/kg of the standard drug Diclofenac sodium, higher and lower doses of Halc. and Aq. extract of the test formulation were found to reduce granuloma formation significantly ($p<0.001$). The higher dose of Halc. extract of the test formulation was seem to produce significant greater effect ($p<0.001$) than that of lower dose of Halc. and Aq. extract. The higher dose of Aq. extract also produced significant greater effect ($p<0.01$) than that of lower dose of Aq. extract. The percentage inhibition of granuloma formation in albino rats was found to be 67.41% with Standard drug (Diclofenac sodium). While, with the 225 mg/kg and 335 mg/kg of hydro-alcoholic extract of tablet, the percentage inhibition of granuloma formation was found to be 43.93% and 59.75%, respectively, and with the aqueous extract of tablet having doses 270 mg/kg and 410 mg/kg the percentage inhibition of granuloma formation was calculated and found to be 39.74% and 53.04%, respectively. Results are presented in Table-2 and the comparison of percentage of inhibition among all the treated groups is shown in Figure-2.

Table 2. Anti-inflammatory effect of formulation tablet in cotton pellet test.

Group(s)	Increase in weight of cotton pellet in mg (Mean ± SE)
Control	52.17±2.52
Diclofenac sodium (5mg/kg)	17.00±2.09 x■a■c■d●
Alc. Ex. (225mg/Kg)	29.25±1.39 x■
Alc. Ex. (335mg/Kg)	21.00±2.18 x■a■c■
Aq. Ex. (270mg/Kg)	31.58±1.65 x■
Aq. Ex. (410mg/Kg)	24.5±2.58 x■c●

n=6, * = p < 0.05 ● = p < 0.01■ = p < 0.001
x= against Control , a= against Alc. Ex. 225mg/Kg, b= against Alc. Ex. 335mg/Kg,
c= against Aq. Ex. 270mg/Kg, d = against Aq. Ex. 410mg/Kg.

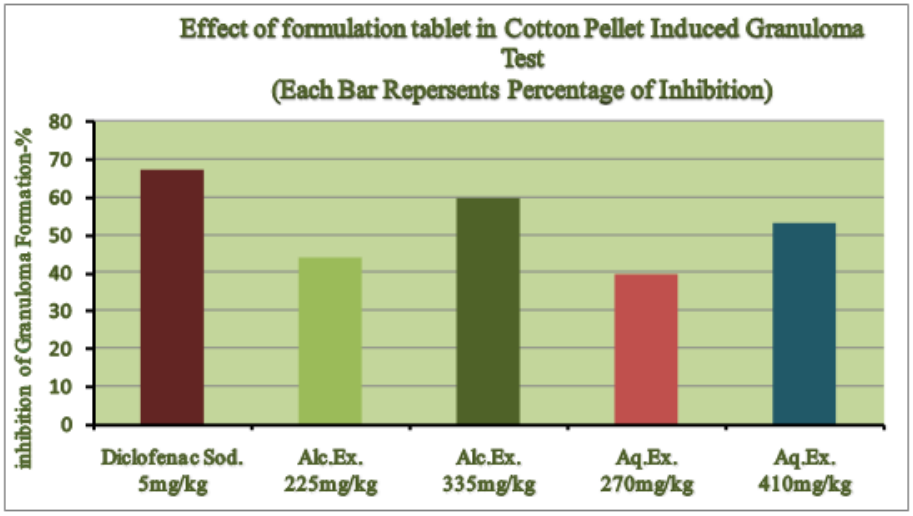

Fig 2. Effect of formulation tablet in cotton pellet induced granuloma test.

DISCUSSION

In carrageenin induced oedema test the study shows that both the doses of both extracts of the test formulation possess anti-inflammatory activity. However, there was no significant difference between the effect of the two extracts and between the two doses of each extract. Thus the above findings indicate that both hydro-alcoholic and aqueous extracts of the tablet possess good protective activity against acute inflammation, approximately same as standard drug Diclofenac sodium.

The carrageenin-induced oedema test has been a popular inflammatory model to investigate anti-inflammatory effect of compounds [18]. This test was carried out in the multi-phasic form, i.e., the inhibition was observed also at 1, 2, 4 and 5 hours after carrageenin injection. This is considered to suggest the mechanism of anti-inflammatory action as different inflammatory mediators predominate in various phases of acute inflammation. Histamine plays the major role at 1 hour, and prostaglandins at 4 and 5 hours, after the inflammatory stimulus [19] [20]. The present study reveals that the test drugs exerted inhibition at all intervals of testing. However, the inhibitory effect was lesser at 1 hours. and maximum at 3 hours. The higher dose of hydro-alcoholic and aqueous extracts of tablet at 1 hours and 2hours are approximately equally effective (not significantly different), while aqueous extracts became more effective 3 hours onwards. These findings indicate that both hydro-alcoholic and aqueous extracts of tablet oppose the action of most mediators of acute inflammation. However, aqueous extract of the tablet is indicated to have greater effect against prostaglandins.

The cotton pellet granuloma model is an indicator for the proliferative phase of inflammation [21]. In this test inflammation and granuloma developed during the period of several days. Inflammation involves proliferation of macrophages, neutrophils and fibroblast, which are basic sources of granuloma formation [22]. Hence the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by the hydro-alcoholic and aqueous extract of the test drug. These findings strongly suggest that the tablet possesses significant effect against sub acute inflammation. The Findings also indicate the dose dependent

response to test formulation, higher dose of alcoholic and aqueous extract reduce weight of cotton pellet significantly ($p<0.01$) than that of lower dose.

CONCLUSIONS

The lower and higher dose of both hydro-alcoholic and aqueous extract of the test formulation possesses significant activity against both acute and sub-acute inflammation. The study offers an improvement in Unani health care by showing the more convenient Tablet form to be effective, which may replace the existing usage of powder form which is less convenient to use.

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