Synergistic Analgesic Effects of Ginger, Colchicum, and Nux Vomica Extracts in a Unani Polyherbal Combination

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ABSTRACT:
Ginger, colchicum, and detoxified nux vomica extracts are combined in an effective dose ratio to evaluate analgesic activity. This combination has not been previously utilized by Unani practitioners. Three unprocessed herbs (Ginger, Colchicum, and Nux vomica) were separated using hydro-alcoholic (50-50%) and aqueous solvents. Every extract, including aqueous and hydro-alcoholic extracts, had its established LD_{50}. To create aqueous and hydro-alcoholic dosage forms, the corresponding extracts were now combined in an efficient dosage ratio. Analgesic effectiveness of both formulations (aqueous and hydro-alcoholic) is assessed using Eddy's hot plate test, analgesiometer test, and formalin test. The reference drug diclofenac sodium was used to examine the effectiveness. The groups administered either the standard dose or the test drug had the highest increase in reaction time at 120 minutes in Eddy's hot plate test. The degree of significance was $p<0.001$ with a larger dose of aqueous extract (1100 mg/kg), therefore maximum tolerance of pain was observed. The combination containing the larger dose of hydro-alcoholic extract (580 mg/kg) had the longest reaction time (4.97 seconds) on the analgesiometer at 75 minutes. The flinches at 14.81 seconds in the formalin test were considerably lessened by the larger dose of the aqueous extract (1100 mg/kg) than by the lower dose ($P<0.05$). In summary, our research validates the analgesic properties of polyherbal extracts combination. As a result, when used together, these medications have the potential to effectively treat acute, subacute, and chronic nociception. Thus, suitable dosage formulations for the treatment of algesia may be created in the future.

GRAPHICAL ABSTRACT

Comparative Study of Analgesic Activity against Different Models

1. Introduction

Having been used for millennia in India, the Unani medical system is extremely old. Its single and multiple formulations are very important for their analgesic properties. Natural products are biologically active in almost every way. Nearly five billion people worldwide rely on herbal medication, according to the WHO. Analgesia or pain perception can be associated with various problems including chronic pain, neuropathic pain, acute pain, cancer pain, psychogenic pain, phantom limb pain, referred pain, central pain syndrome, headaches and migraines, and inflammatory pain. Here we are treating inflammation pain mainly arthritis (Wajaul Mafasil) is associated with joint pain.[1] the immune system attacks the joints causing inflammation, pain, and swelling. The body secretes more mediators at the site of inflammation in response to external stimulation including prostaglandins and leukotrienes.[2] since these mediators also make inflammation worse, it is important to suppress them. Currently, analgesic drugs are designed to relieve such problems.[3]

Ginger (Zingiber Officinale, Zingiberaceae)

Dried ginger, known as sonth, is made from the dried rhizome of Zingiber officinale Roxb., a plant widely cultivated in India. The rhizomes are typically harvested between January and February, after which the buds and roots are removed.[4] the rhizomes are then soaked in water overnight, peeled, sometimes treated with lime, and dried. Ginger is a perennial herb that can grow up to 90 cm tall, with a large, solid, and tough rhizome that is stout, tuberous, and horizontal, consisting of many round joints. Its roots are numerous, large, cylindrical, fleshy, thick, and brittle.[5] The leaves are alternately arranged, narrow, subsessile, and 1-2 cm wide, with long sheaths that stand away from the stem and spread across the ground. The plant produces greenish flowers with a small dark purple or purplish-black lip, borne in radical spikes 3.8-7.5 cm long and about 2.5 cm in diameter, on peduncles 15-30 cm long.[6] the stem is erect, leafy, and stands between 0.6 and 1.2 meters tall, entirely covered with leafy stems. The rhizome has a brownish, nodule-like appearance, a sharp taste, and a distinctive aroma. It is widely recognized in Unani and Ayurvedic texts as a therapeutic herb. It is said that the rhizome contains more than 400 distinct chemicals. The rhizomes of ginger contain carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic chemicals.[7] Zingiberene, α-farnesene, α-curcumin, β-bisabolene, and β-sesquiphellandrene come under the terpene components whereas gingerol, shogaol, and parasols come under the phenolic components.[8] Compared to other constituents, gingerols (23–25%) and shogaols (18–25%) are more prevalent. Besides them, amino acids, proteins, phytosterols, raw fibers, vitamins (e.g., nicotinic acid and vitamin A) and minerals

are also present. Studies conducted on animals and in vitro have shown that ginger is likely to have 5-HT3 antagonistic effects.[9] In addition, ginger has anti-arthritic and anti-inflammatory properties.[10] The ginger component, 6-gingerol has been demonstrated to produce an anti-inflammatory action through the modulation of NF-κB.[11] According to reports, the ginger component i.e. 6-shogaol, lowers the inflammatory response and shields the femoral cartilage from harm in a Freund's adjuvant mono-arthritic model of the rat knee joint.[12] In LPS-stimulated macrophages, ginger extract decreased the production of pro-inflammatory cytokines such as IL-12, TNF-α, IL-1β, RANTES, and MCP-1. Ginger was also proven to generate a strong anti-inflammatory impact in both acute and subacute types of inflammation.[13]

Colchicum (Colchicum Luteum, Liliaceae)

Its corm is used in this study. The corm is the root of a small herbaceous plant, with narrow, elongated annual leaves that emerge from the same nodes. The plant can be propagated using either seeds or corms. Starting in May, seeds are sown in beds or boxes and lightly covered with soil.[14] the plant resembles an onion in appearance. Those with white flowers are considered of the highest quality. When the flower appears, the leaves fall off. The leaves are sparse, lorate, linear-oblong or oblanceolate, obtuse, emerging with the flowers, and short during flowering, reaching 15-30 cm during fruiting.[15] Between August and September, the plant produces two to six saffron-like flowers with a lilac or purple hue. When fully open, the flowers have a diameter of 2.5-3.8 cm, a golden yellow perianth, a tube of 7.5 cm (3-4 inches), stamens shorter than the perianth, and filaments much shorter than the long yellow anthers. The filiform style is significantly longer than the perianth. It is reported that colchicine, lumicolchicine, chlorogenic acid, and 3,4,5,7-tetrahydroxy flavone are the major chemical constituents based on multiple current spectroscopic techniques. The majority of reported alkaloids were nevertheless colchicines.[16] In Unani literature, it is a well known anti-inflammatory and antidote for arthritis among other uses.[15,16] Colchicine (marker chemical) acts against the inflammatory response and is helpful in the treatment of gout.[17] It lowers NF-κB expression and prevents the NLRP3 inflammasome from activating in response to inflammatory microcrystals.[18] It lowers serum uric acid and prevents uric acid (urate) crystal formation. It also considerably reduces serum TNF-α levels.[19]

Nux Vomica (Strychnos Nux-Vomica, Loganiaceae)

Its seeds are used after undergoing a detoxification process. This tree can reach a height of 12.5 meters. The leaves are orange-ovate, five-nerved, glabrous, and measure 7×5 cm, with an obtuse base and a 0.5 cm petiole. The inflorescence is a terminal cyme with a peduncle length of 1-5 cm. The flowers are greenish-white, and the bark ranges from greyish to yellow.[20] the cyme measures up to 25 cm in diameter, with pedicels barely visible, and the corolla tube is 0.6-0.8 cm long. The anthers are oblong and glabrous, the style is long and glabrous, and the stigma is small and capitate. The fruits are orange-yellow, globose berries up to 4 cm in diameter, containing many seeds (4-5). The seeds, 1.5 cm in diameter, are discoid, compressed, very hard, and covered on both sides with fine, appressed grey silky hairs radiating from the center. The seeds are grey on the outside and whitish on the inside, with a very bitter taste. α-amyrin, vomicine, stearic acid, β-sitosterol, vanillin, ethyl gallate, methylgallate, novacine, daucosterol, chloromethochlorid, loganic acid, geniposide, loganin,strychnine, and brucine are the chief constituents identified in the literature as being present in the seed. Additionally, nux vomica contains anti-inflammatory and anti-arthritic qualities.[21,22] These neurotoxins act centrally. Strychnine increases muscle reflex excitability by competitively opposing the post-synaptic binding of the inhibitory transmitter i.e. glycine.[23]

Ginger (Zingiber officinale), Colchicum (Colchicum luteum), and detoxified nux vomica (Strychnous nux vomica) are the ingredients in this composition. The Unani combination of three aforementioned constituents work well together to boost the analgesic properties of the formulations. Qualitative screening discovered the presence of flavonoids in the extract, which

are well known for their involvement in wound healing. Additionally, flavonoids have been demonstrated to inhibit cyclooxygenase and to have analgesic properties. Alkaloids, glycosides and sterols that have analgesic properties by blocking prostaglandin pathways, are present in the composition.[24]

Thus, it may be concluded that the presence of the aforementioned groups of chemicals is responsible for at least some of the analgesic effects. Numerous medical professionals worldwide attest to the analgesic qualities of all three herbs. The Unani Physicians state that whereas ginger has gastro-protective qualities that counteract the negative effects of colchicum on the stomach and render this combination safe for long-term usage.[25]

2. Methodology

Procurement of Material

The crude herbs were procured from the Aligarh market, and were acknowledged by literature available in books and authenticated. Ginger, Colchicum, and Nux vomica with specimen voucher no. SC-0226/17, SC-0227/17 SC-0228/17 respectively submitted in the museum of the Department of Ilmul Advia (Pharmacy), A.K.T.C, A.M.U., Aligarh, for future reference. The report was found that all parameters are within range as in the literature.

Detoxification of the Strychnos Nux-Vomica

Water is used to soak the nux vomica seeds for seven nights, with new water added each night. Subsequently, the seeds underwent additional detoxification by being boiled for seven days, three hours a day, with cow milk in a dolayantra, an assembly that has a boiling pan and a cloth bundle hanging in it. The embryo and seed coat are taken off. The cotyledon needs to be finely ground after roasting in cow ghee.[26]

Combination Development Method (Dose Calculation)

To turn three raw herbs—ginger, colchicum, and detoxified nux-vomica—into powder, they were dried and pulverized using the appropriate techniques and machinery. Every powdered drug is extracted using both hydro-alcoholic (50%-50%) and aqueous solvents. As a result, we were able to extract ginger, colchicum, and detoxified nux-vomica both hydro-alcoholically and aqueously. LD_{50} in animals is determined by using Annex 2a of the OECD 423 guideline (oral toxicity) for each of these six extracts in turn (Table 1). To create an aqueous extract dosage form (aqueous formulation), aqueous extracts were combined in an effective dose ratio and hydro-alcoholic extracts were combined in an effective dose ratio to create a hydro-alcoholic extract dosage form (hydro-alcoholic formulation) reported in Table 2. Both extracts were utilized in the preclinical study in suspension form, prepared with 1% Carboxymethylcellulose (CMC). Ultimately, Eddy's hot plate test, analgesiometer test, and formalin test were used to assess the analgesic efficacy of both aqueous and hydro-alcoholic formulations. The effectiveness of the Unani formulation was compared to that of the standard reference medication i.e. diclofenac sodium.

Table 1. LD_{50} Value of Different Extracts.

Extract name	**% Yield**	**LD_{50} of drug ($mgKg^{-1}$ b.w.)**	**1/10th (High Dose) ($mgKg^{-1}$ b.w.)**	**1/20th (Low Dose) ($mgKg^{-1}$ b.w.)**
HAE of Ginger	13.45	5000	500	250
HAE of Colchicum	10.35	500	50	25
HAE of Detoxified Nux-vomica	6.13	300	30	15
AE of Ginger	10.70	5000	500	250
AE of Colchicum	7.53	5000	500	250
AE of Detoxified Nux-vomica	3.50	1000	100	50

HAE: Hydro-alcoholic Extract, AE: Aqueous Extract

Table 2. Effective Dose Formulations.

Effective dose	**Drug formulation**	**Ratio**	**Suspension ($mgKg^{-1}$ b.w.)**
LDHA	Ginger: Colchicum: Detoxified Nux-vomica	250:25:15	290
HDHA	Ginger: Colchicum: Detoxified Nux-vomica	500:50:30	580
LDA	Ginger: Colchicum: Detoxified Nux-vomica	250:250:50	550
HDA	Ginger: Colchicum: Detoxified Nux-vomica	500:500:100	1100

LDHA: Low dose hydro-alcoholic extract, HDHA: High dose hydro-alcoholic extract, LDA: Low dose aqueous extract, HAD: High dose aqueous extract

Animal Housing

The purpose of the current investigation was to assess an analgesic activity in Wistar albino rats. We chose animals weighing between 100 and 150 g both male and female equally. Animals were procured from All India Institute of Medical Sciences (AIIMS), New Delhi. There were six animals in a cage with Standard food and fresh water procured from Catalyst Life sciences, New Delhi as per CPCSEA guidelines. Housing conditions were kept according to CPCSEA guidelines: The optimal temperature between 20-26°C, Relative humidity between 40-70%, a regular light-dark cycle maintained, typically 12 hours of light followed by 12 hours of darkness and adequate ventilation. Surgery was carried out between 9 and 14 hours during the day. The procedures used during the experiments (Registration No. 1979/GO/Re/S/17/CPCSEA) complied with CPCSEA guidelines for the use and care of lab animals.

Chemicals and Reagents

Diclofenac sodium salt-Voveran (Novartis, India), and normal saline (E. Merck Ltd., India).

Determination of Analgesic Activity

Eddy's Hot Plate Test

The hot-plate test was conducted using Eddy and Leimbach's approach. 100–150 g albino rats of both sexes were split up into 5 groups of 6 animals each. Diclofenac sodium was given orally to animals in Group I as standard at a dose of 5 mg/kg. Hydro-alcoholic extract formulation was administered at doses of $290mgKg^{-1}$ and $580mgKg^{-1}$ to the second and third groups respectively. Animals in groups four and five were given 550 $mgKg^{-1}$ and 1100 $mgKg^{-1}$ of the aqueous extract formulation respectively in the form of a suspension (1% CMC) Table 4. Each rat was first timed to react by placing it on Eddy's hot plate between 55 and 55.50°C. A cutoff period of 20 s was observed to avoid damage to the paw in rats. The duration between placing the animals on the hot plate and their jumping or licking of their paws was used to calculate reaction time. Following a one-hour drug administration period, the response time of each animal was measured every fifteen minutes for ninety minutes.[27] Using an ANOVA test and LSD, the reaction times at each post-treatment interval within a group were statistically compared to the beginning reaction times.

Table 3. Different Groups and Treatments Received.

Groups	Name	Treatment received
I.	Control	Carboxymethylcellulose (CMC) 2mlKg^{-1}, Orally
II	Standard	Diclofenac sodium 5 mgKg^{-1}, Orally
III	LDHA	Low dose hydro-alcoholic extract 290 mgKg^{-1}, Orally
IV	HDHA	High dose hydro-alcoholic extract, 580 mgKg^{-1}, Orally
V.	LDA	Low dose aqueous extract 550mgKg^{-1}, Orally
VI	HDA	High dose aqueous extract 1100 mgKg^{-1}, Orally

Analgesiometer Test

An analgesiometer test was conducted using D'amour and Smith's methodology. 100–150 g albino rats of both sexes were split up into 4 groups of 6 animals each. The first and second groups received treatment with 290 mg^{-1} and 580 mg^{-1} of hydro-alcoholic extract formulation. Animals in groups three and four were given 550 mgKg^{-1} and 1100 mgKg^{-1} of an aqueous extract formulation in the form of suspension (1% CMC) respectively Table 5. Placing the tail of each rat on the analgesiometer's nichrome wire and placing it in a rat holder allowed researchers to measure the first reaction time for each rat. After adjusting the variac until the reaction time was determined to be between three and six seconds, the matching variac reading was recorded. To test the same animal again later, the variac was placed at the same point. For 120 minutes, the responses of each animal were noted at 15-minute intervals.[28] An ANOVA test was used, followed by LSD, to statistically compare each post-treatment group's reaction time with its starting reaction time.

Formalin Test

Using the formalin test, antinociception was evaluated. Six groups of six albino rats, each weighing between 100 and 150g, were created. Group I animals were given distilled water at a rate of 20 mlKg^{-1} as controls. The animals in Group II received an oral dosage of 5 mg/kg of the conventional medication i.e. diclofenac sodium. Hydro-alcoholic extract formulations for the third and fourth groups were 290mgKg^{-1} and 580mgKg^{-1} respectively. Animals in the fifth and sixth groups were given 550 mgKg^{-1} and 1100 mgKg^{-1} of the test aqueous extract formulation respectively in the form of suspension (1% CMC) Table 6. Thirty minutes before the formalin injection, the test medication was given orally. A 30-gauge needle was used to inject 0.1 ml of diluted formalin (0.5%) intraplantarly into the rats' right hind paws. After that, the animal was brought back into the room to be observed. To provide for unrestricted viewing of the formalin-injected paw, a mirror was positioned behind the chamber. Rats were given a formalin injection and nociceptive behavior was observed right away. Nociceptive behavior was measured by counting how many times the injected paw flinched during 1-minute intervals spaced five minutes apart, up to sixty minutes after the injection. Flinching could be observed and it was marked by a quick retreat or flexion of the injected paw. The flinching behavior brought on by formalin is biphasic. A brief period of quiescence follows the initial acute phase (0–5 minutes) and a lengthy tonic response (15–60 minutes) follows.[29] A one-way ANOVA and the LSD test were used to statistically assess differences between the treatment groups.

3. Results

The OECD 423 criteria were utilized to determine the LD_{50} values of several extracts. Following is the LD_{50} for each drug (pilot research): An effective dose ratio of three medications is combined to create high and low-dose formulations of both extracts. Four formulations are thus created.

Eddy's Hot Plate Test

Control Group: Initially reaction time was 8.13±0.23seconds, which was increased to 8.83±0.25 seconds after 60 minutes, 8.90±0.28 seconds after 75 minutes, 8.79±0.29 seconds after 90 minutes, 8.78±0.29 seconds after 105 minutes, 8.51±0.21 seconds after 120 minutes, 8.50±0.23 seconds after 135 minutes, 8.83±0.25 seconds after 150 minutes. Standard Group:

Initially, the reaction time was 8.16±0.23 seconds which was increased to 09.90±0.41 seconds after 60 minutes, 10.90±0.47 seconds after 75 minutes, 12.19±0.76 seconds after 90 minutes, 13.20±0.89 seconds after 105 minutes, and 16.17±0.39 seconds after 120 minutes ($p< 0.01$). At 135 minutes, the time was 11.18±0.87 seconds, and at 150 minutes, the time was 07.20±0.55 seconds ($p< 0.05$). LDHA Group: The initial reaction time was 9.65±1.08 seconds, which was increased to 11.35±1.14 seconds after 60 minutes, 11.93±0.95 seconds after 75 minutes, 12.32±1.08 seconds after 90 minutes, 12.83±1.02 seconds after 105 minutes, and 13.82±1.04 seconds after 120 minutes ($p < 0.01$). 11.91±1.27 seconds ($p< 0.05$) were recorded at 135 minutes, and 08.41±0.88 seconds were recorded at 150 minutes. HDHA Group: The initial response time was 8.00±0.41 seconds, however after 60 minutes, it was increased to 9.87±0.41 seconds. After 75 minutes, the average time was 10.67±0.72 seconds, followed by 11.96 ±1.15 seconds ($p< 0.05$) after 90 minutes, 13.52±1.22 seconds ($p< 0.05$) after 105 minutes, and 15.10±1.28 seconds ($p< 0.01$) after 120 minutes. 14.17±1.19 seconds ($p< 0.05$) were reported at 135 minutes and 12.59±1.12 seconds at 150 minutes. LDA Group: The initial response time was 9.85±0.24 seconds, which was increased to 12.68±0.99 seconds after 60 minutes, 16.15±1.40 seconds after 75 minutes, 16.22±1.11 seconds after 90 minutes, 16.42±0.97 seconds after 105 minutes, and 16.68±0.56 seconds ($p<0.05$) after 120 minutes. 12.59±1.51 seconds were recorded at 135 minutes, and 10.40±1.52 seconds were recorded at 150 minutes. HDA Group: The initial response time was 10.57±0.65 seconds, however after 60 minutes, it was increased to 12.51±0.78 seconds. After 75 minutes, the average time was 15.52±0.93 seconds, followed by 16.46±0.78 seconds ($p <0.01$) after 90 minutes, 17.89±0.95 seconds ($p< 0.001$) after 105 minutes, and 20.05±0.66 seconds ($p< 0.001$) after 120 minutes. 17.03±0.98 seconds ($p< 0.001$) were reported at 135 minutes and 13.00±0.89 seconds at 150 minutes.

Analgesiometer Test

Control Group: The initial response time was 4.21±0.18 seconds, however after 60 minutes, it was increased to 4.31±0.24 seconds. After 75 minutes, the time was 4.32±0.25 seconds followed by 4.19±0.18 seconds after 90 minutes, 4.23±0.19 seconds after 105 minutes, and 4.22±0.21 seconds after 120 minutes. Standard Group: The initial response time was 4.52±0.19 seconds, however after 60 minutes, it was increased to 4.85±0.12 seconds ($p < 0.05$). After 75 minutes, the time was 4.88±0.15 seconds ($p < 0.001$) followed by 4.80±0.18 seconds ($p <0.01$) after 90 minutes, 4.63±0.19 seconds after 105 minutes, and 4.51±0.21 seconds ($p< 0.001$) after 120 minutes. LDHA Group: The initial response time was 4.20±0.13 seconds, however after 60 minutes, it was increased to 4.45±0.14 seconds ($p < 0.05$). After 75 minutes, the time was 4.48±0.09 seconds ($p < 0.001$) followed by 4.39±0.10 seconds ($p <0.01$) after 90 minutes, 4.31±0.10 seconds after 105 minutes and 4.23±0.10seconds ($p< 0.001$) after 120 minutes. HDHA Group: The initial response time was 4.09±0.04 seconds, however after 60 minutes, it was increased to 4.25±0.03 seconds ($p< 0.01$). After 75 minutes, the time was 4.97±0.16 seconds ($p < 0.001$) followed by 4.36±0.02 seconds after 90 minutes, 4.29±0.03 seconds after 105 minutes and 4.06±0.15 seconds after 120 minutes. LDA Group: The initial response time was 4.32±0.25 seconds, however after 60 minutes, it was increased to 4.37±0.25 seconds. After 75 minutes, the time was 4.37±0.25 seconds ($p < 0.001$) followed by 4.37±0.25 seconds after 90 minutes, 4.32±0.26 seconds after 105 minutes and 4.21±0.24 seconds after 120 minutes. HDA Group: The initial response time was 3.39±0.12 seconds, however after 60 minutes, it was increased to 3.46±0.11 seconds ($p< 0.001$). After 75 minutes, the time was 3.47±0.10 seconds ($p <0.001$) followed by 3.4±0.11 seconds after 90 minutes, 3.47±0.12 seconds ($p< 0.01$) after 105 minutes, and 3.32±0.11 seconds after 120 minutes.

Formalin Test

A characteristic pattern of flinching behavior was produced by low doses of formalin treatment. Following the formalin administration, the first phase began right away and progressively subsided after about ten minutes. The second part began after fifteen minutes and went on for

an hour. First Phase: In the standard group, there were 28±1.15 flinches, compared to 25.16±1.93 in the control group. 29.66±1.52 and 31.16±1.64 finches were the results for the LDHA and HDHA groups respectively. The number of finches was found to be 25.83±2.71 in the LDA group and it was substantially lower at 22.83±2.03 (P<0.01) in the HDA group. Second Phase: The number of flinches in the control group was found to be 27.98±1.60, whereas in the standard group, it was dramatically reduced compared to the control group and found to be 15.86±1.89 (P<0.001). It was also considerably lower in the LDHA and HDHA compared to the control group, measuring 15.91±1.77 (P<0.001) and 14.93±1.56 (P<0.001) respectively. It was also considerably lower in the LDA and HDA respectively, measuring 15.98±1.55 (P<0.001) and 14.81±1.18 (P<0.001).

The reaction time was recorded in Eddy's hot plate test at 15-minute intervals for 75 minutes, both before and after the treatment for 60 minutes. At 120 minutes, the reaction times of all the groups given the standard or test medication increased the most. The significance threshold was p<0.001 when the aqueous extract dose was higher (HDA), p<0.01 when the standard drug and both hydro-alcoholic extract doses were used, and p<0.05 when the aqueous extract dose was lower (LDA) reported in Table 4 and Fig. 1.

Table 4. Effect of Formulation in Eddy's Hot Plate Test.

Group	**Reaction time in Seconds (Mean ± SEM)**							
		60 min.	**75 min.**	**90 min.**	**105 min.**	**120 min.**	**135 min.**	**150 min**
Control	8.13 ± 0.23	8.83 ± 0.25	8.90 ± 0.28	8.79 ± 0.29	8.78 ± 0.29	8.51 ± 0.21	8.50 ± 0.23	8.83 ± 0.25
Standard	8.16 ± 0.23	9.90 ± 0.41	10.90 ± 0.47	12.19 ± 0.76	13.20 ± 0.89	16.17 ± $0.39x^2a^2b^3$	11.18 ± $0.87x^3$	7.20 ± 0.55
LDHA	9.65 ± 1.08	11.35 ±1.4	11.93 ± 0.95	12.32 ± 1.08	12.83 ± 1.02	13.82 ± $1.04x^2a^3$	11.91 ± 1.27 x^3	8.41 ± 0.88
HDHA	8.00 ± 0.41	9.87± 0.41	10.67 ± 0.72	11.96 ± $1.15x^3$	13.52± $1.22x^3$	15.10± $1.28x^2$	14.17 ± $1.19x^3$	12.59 ± 1.12
LDA	9.85 ± 0.24	12.68 ±0.99	16.15 ± 1.4	16.22 ± 1.11	16.42 ± 0.97	16.68 ± $0.56x^3$	12.59 ± 1.51	10.40 ± 1.52
HDA	10.57 ± 0.65	12.51 ± 0.78	15.52 ± 0.93	16.46 ± 0.78 x^2a^3	17.89 ± $0.95x^1a^1b^2$	20.05 ± 0.66 $x^1a^2b^3c^2d^2$	17.03 ± 0.98 x^1 a^1 b^2	13 ± 0.89

n=6, x = Against Control, y = Against Standard, a = Against LDHA, b = Against HDHA, c = Against LDA, d = Against HAD, 1 = p <0.05, 2 = p < 0.01, 3 = p < 0.001

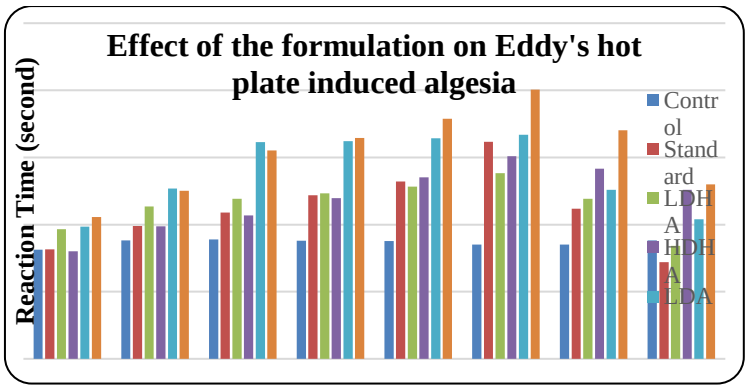

Figure 1. Effect of Formulation in Eddy's Hot Plate Test.
LDHA = Low dose hydro-alcoholic extract, HDHA = High dose hydro-alcoholic extract, LDA = Low dose aqueous extract, HDA = High dose aqueous extract

In all groups, the increase in reaction time in the Analgesiometer test was greatest at 75 minutes. At 75 minutes, all groups had much more time. The HDHA group had the longest reaction time (4.97 sec.). The results are presented in Table 5 and Fig. 2.

Table 5. Effect of Formulation in Analgesiometer Test.

Groups	**Reaction Time in seconds (Mean ± SEM)**								
	Initial	**15 min**	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**	**105 min**	**120 min**
Control	4.21± 0.18	4.18± 0.20	4.30± 0.21	4.28± 0.22	4.31 ± 0.24	4.32± 0.25	4.19± 0.18	4.23± 0.19	4.22± 0.21
Standard	4.52 ± 0.19	4.57 ± 0.19	4.78 ± 0.21	4.82 ± 0.19	4.85 ± 0.12	4.88 ± 0.15	4.80 ± 0.18	4.63 ± 0.19	4.51± 0.21
LDHA	4.20 ± 0.13	4.41 ± 0.15	4.37 ± 0.12	4.37 ± 0.11	4.45 ± 0.14 x^3	4.48 ± 0.09 $x^1a^2b^2c^2d^2$	4.39 ± 0.10 $x^2a^2b^3c^3$	4.31±0.10	4.23±0.10
HDHA	4.09±0.04	4.13±0.03	4.19±0.03	4.23±0.03	4.25 ± 0.03 x^2	4.97±0.16 $x^1a^2b^2c^2d^3$	4.36 ± 0.02	4.29±0.03	4.06±0.15
LDA	4.32 ± 0.25	4.33 ±0.25	4.35 ± 0.24	4.36± 0.24	4.37 ± 0.25	4.37 ± 0.25 $x^1a^2b^2c^3$	4.37 ± 0.25	4.32 ± 0.26	4.21 ± 0.24
HAD	3.39 ± 0.12	3.55 ± 0.22	3.37 ± 0.11	3.39 ± 0.12 $x^2a^2b^2$	3.46 ± 0.11 $x^1a^1b^2$	3.47 ± 0.10 $x^1a^1b^1c^2d^3$	3.47 ± 0.12 $x^2a^2b^3$	3.40 ± 0.11	3.32 ± 0.11

n=6, x: Against Control, y: Against Standard, a: Against LDHA, b: Against HDHA, c: Against LDA, d: Against HAD, 1: $p < 0.05$, 2: $p < 0.01$, 3: $p < 0.001$.

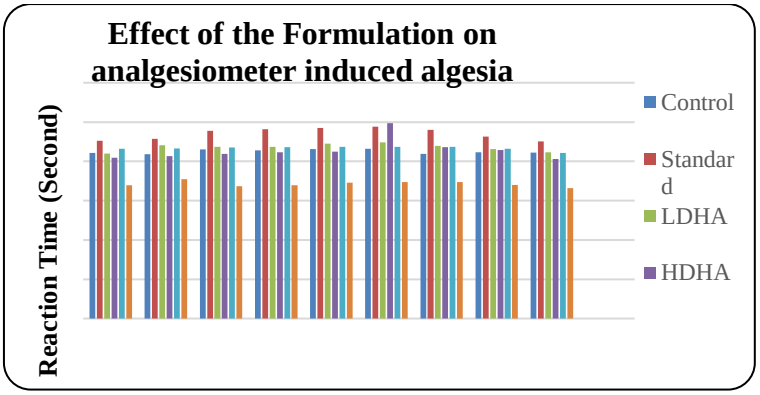

Figure 2. Effect of Formulation in Analgesiometer Test.
LDHA = Low dose hydro-alcoholic extract, HDHA = High dose hydro-alcoholic extract, LDA = Low dose aqueous extract, HDA = High dose aqueous extract

The Formalin test is an antinociceptive test that is done on animals to determine the mechanism of analgesia in addition to its presence. It uses small dosages of formalin. Animals in the treatment groups showed no reaction to formalin-induced pain during the first five minutes of the test. However, during the second phase (15 min^{-1} hr), there was a significant ($P<0.001$) decrease in the number of flinches in all groups treated with the test drug or the standard drug when compared to the control group. The flinches are considerably decreased by the higher dose of the aqueous extract than by the lesser dose of the same ($P<0.05$) reported in Table 6 and Fig. 3.

Table 6. Effect of Formulation on the Biphasic Flinching Behavior in Formalin Test.

Group	First Phase (0-5min) (Mean ± SEM)	Second Phase (15-60min) (Mean ± SEM)
Control	25.16±1.93	27.98±1.60
Standard	28±1.15	15.86±1.89 X^1
LDHA	29.66±1.52	15.91±1.77 X^1
HDHA	31.16±1.64	14.93±1.56 $X^1C^2Y^3$
LDA	25.83±2.71	15.98±1.55 X^1
HAD	22.83±2.03a^2	14.81±1.18 X^1C^3
F Value	1.23	14.70

n=6, x: Against Control, y: Against Standard, a: Against LDHA, b: Against HDHA, c: Against LDA, d: Against HAD, 1: $p<0.05$, 2: $p < 0.01$, 3: $p < 0.001$.

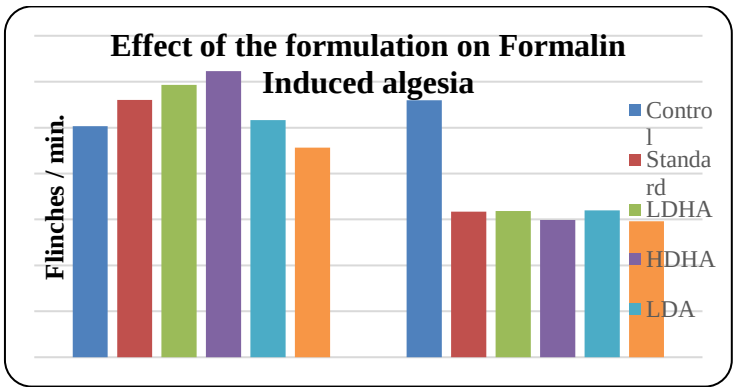

Figure 3. Effect of Formulation on the Biphasic Flinching Behavior in Formalin Test.
LDHA = Low dose hydro-alcoholic extract, HDHA = High dose hydro-alcoholic extract, LDA = Low dose aqueous extract, HDA = High dose aqueous extract

4. Discussion

This suggests that there is a dose-dependent reaction in the aqueous extract formulation. The larger dosage of hydro-alcoholic extract in the test formulation appeared to considerably lower the amount of flinches when compared to the regular medication ($P<0.05$). When comparing the treatments, it was observed that the larger dose of hydro-alcoholic extract (HDHA) significantly reduced the number of flinches compared to the lower dose of aqueous extract (LDA) ($P<0.01$). The primary cause of a plant extract's analgesic effect is the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids.[30-33] Depending on the particular plant and its chemical constituents, these metabolites can interact with the body's multiple pain pathways by blocking the synthesis of inflammatory mediators like prostaglandins, modifying neurotransmitter activity, and directly affecting pain receptors. These effects can be measured using a variety of techniques, including the acetic acid-induced writhing test, the hot plate method, and the formalin test, which all evaluate different facets of pain perception in animal models.[34-37] Prostaglandin inhibition is one of the main modes of action. Many plant chemicals, especially flavonoids, can inhibit the enzyme cyclooxygenase (COX), which is in charge of producing prostaglandins, which are important mediators of inflammation and pain. Opioid receptor interaction: Certain plant alkaloids can attach to central nervous system opioid receptors, imitating the effects of endogenous opioids and causing analgesia. Neurotransmitter modulation: A few plant extracts can affect the release or absorption of neurotransmitters that are involved in pain perception, such as norepinephrine and serotonin. Antioxidant activity: Certain plant chemicals might lessen oxidative stress, which is frequently connected to pain perception.

5. Conclusion

The test formulation drugs were also examined for any potential analgesic impact using Eddy's hot plate test, the analgesiometer test, and the formalin test because the majority of medicines have analgesic activity. These tests were chosen due to several benefits, such as their low tissue damage and susceptibility to potent analgesics. The analgesic property of the test formulation is confirmed by the replication of the analgesic effects demonstrated in Eddy's hot plate test and even in the analgesiometer test. It is well established that tests with excruciating thermal stimuli are opioid medication-selective. Therefore, the analgesiometer test and Eddy's hot plate test are typically employed to investigate opioid analgesia. Given that opioid analgesics yield favorable findings in both approaches, this study suggests that the test formulation's analgesic efficacy is, at least in part, opioid-type-specific. Hydro-alcoholic extract has a strong analgesic effect at large doses for acute, sub-acute, and chronic conditions. By demonstrating that the more practical suspension form is effective and could eventually replace the more costly and inconvenient powder form, the study contributes to an advance in Unani health care. These results support the description found in the Unani literature since this formulation has been shown to have analgesic, anti-inflammatory, and anti-arthritic properties.

Acknowledgments

Prof. Abdul Latif authenticated the crude drugs. Ginger, colchicum, and nux vomica with specimen voucher numbers SC-0226/17, SC-0227/17, and SC-0228/17, respectively. Cruds drugs were submitted to the museum of the Department of Ilmul Advia (Pharmacy), Ajmal Khan Tibbya College, Aligarh Muslim University, Aligarh for future reference. The author is grateful to the Era College of Pharmacy, Era University, Lucknow.

Conflict of Interest

There is no potential conflict of interest.

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