Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

Investigation of *invivo* Anti-inflammatory Potential of Unani Formulation Habb-E-asgand

Ansari Imtiyaz Ahmed TufailAhemad[a*], QaziMajazAhamadAejazuddin[a], G. J. Khan[a]

[a]J.I.I.U's Ali-allana College of Pharmacy, Akkalkuwa, Nandurbar, Maharashtra 425415,India.

Corresponding Author Email: ansari425@gmail.com

Received: 29 Nov 2021 *Revised: 10 Dec 2021* *Accepted & Published: 18 Dec 2021*

Abstract

The goal of this research was to see if Unani Formulation Habb-E-asgand had anti-inflammatory properties *in-vivo*. Unani polyherbal formulation Habb-E-asgand is a blend of many herbal medicinal plants used in arthritis, gout, and joint pain. The current study is an attempt to test and validate its efficacy through scientific means. *In-vivo* anti-inflammatory potential includingcarrageenan induced rat paw edema, von frey test, tail immersion test and other methods were all evaluated for the current Unani formulation. In Carrageenan-induced rat Paw edema, In HEA treated group, paw thickness was 3.025±0.074 at t=0, but it showed increase in paw inflammation after one hour i.e. 3.201±0.072 cms which again showed decrease in paw thickness up to 4thhr i.e. 3.021±0.077, 3.011±0.038, 2.998±0.071 at t=2, 3, and 4 respectively. It showed again getting back to the normal thickness of paw at t=24 hrs i.e. 3.027±0.117 cms. In Diclofenac sodium treated groups, it displayed same pattern as demonstrated by HES treated group. All the results obtained were significant with the control group. Thus HEA and the standard drug showed complete blockade of carrageenan-induced hypersensitivity.The *in-vivo*formulation is shown to be sufficiently powerful and effective in the treatment of inflammation.

Keywords: Habb-e-asgand, anti-inflammatory, DPPH scavenging activity, Polyherbal, Phytochemical screening, Unani.

1. Introduction

Unani medicine is a traditional medical system that started in Greece and evolved during the Arab civilization. The theoretical underpinning of medicine is based on the teachings of Hippocrates, a Greek philosopher, and physician. Following him, several other Greek intellectuals greatly expanded the theory. Galen stands out among them as the one who

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

solidified the foundation upon which Arab physicians such as Razi and Avicenna built the towering structure [1]. According to Unani medicine's basic beliefs, an individual's defense constitution, or power of self-preservation and/or adjustment, is harmed in diseased situations and requires restoration through the application of various therapies indicated in the system [2].

Unani medicine is to discover the most effective techniques for a person to live a healthy life. In Unani literature, there are four sorts of remedies for sustaining health and treating disease. *Ilaj bit-tadbeer* (Regional therapy), *Ilaj bil-ghiza* (Dietotherapy), *Ilaj bid-dawa* (Pharmacotherapy), and *Ilaj bil-jarahat* (Surgery) are the four types of Ilaj [3]. *Riyazat* (exercise), *Dalak* (massage), *Hammam* (Turkish bath), *Taa-leeq* (leeching), *Hijamah* (cupping), and other non-pharmacological methods make up the majority of *Ilaj bit-tadbeer*. *Ilaj bil-ghiza* is based on the recommendation/restriction of various diets that are appropriate for a certain ailment. The delivery of medications to treat a condition is known as pharmacotherapy [4]. Herbal medicine is the use of herbs to promote healing and health maintenance [5-8]. Only approximately a quarter of the estimated 800,000 plant species on the planet have been classified, and only a small percentage of these have been tested for pharmacological efficacy. More plant-based drugs were sought to aid in the treatment of the various ailments that still afflict society [9-10].

The Unani system of medicine lays a major emphasis on lifestyle management for the development and preservation of health. Diet, lifestyle, emotions, interactions with the environment, and even spiritual factors are all considered [11]. It considers both environmental factors like seasons, air quality, food and beverages, as well as internal factors like sleep and wakefulness, activity and rest, evacuation and retention, and so on. Because of its ability to promote health and disease prevention through non-drug lifestyle variables, the Unani system of medicine is immensely relevant to current healthcare [12-15].

Habb-e-Asgand is a popular herbal preparation prescribed for arthritis, gout, and joint pain [16]. It is also known to have aphrodisiac properties [16]. Habb-e-Asgand contains Withania somnifera (L) Dunal (Solanaceae) (English name: winter cherry, Hindi name: ashwagandha) as the main constituent known for its various medicinal uses [17]. Briefly, Habb-e-Asgand Therapy comes in small, round, uniformly shaped pills made with the ingredients of the formulation composition. The blend included Ajwain Desi [Ptychotis ajowan DC (Apiaceae) seeds], Asgand Nagauri (W. somnifera), Chob Bidhara [wood of Gmelina Asiatica L. (Lamiaceae)], Pippl Kalan-Desi [Fruit of Piper longum L. (Piperaceae),

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

dried immature], Pipla Mool (the root of P longum) Moosli Siyah [Curculigo orchioides Gaertn. (Hypoxidaceae) stem], Satawar [Asparagus racemosus Willd. (Asparagaceae)), Zanjabeel-Khushk [rhizome of Zingiber officinalis Roscoe (Zingiberaceae)) and Qand Siyah Kohna [Saccharum officinarum L. (Poaceae)] as basic ingredients [16]. The high content of Qand Siyah Kohna is attributed to its use as a coating material in the preparation of pills.

Most Unani drugs are herbal or mineral or a combinationof the two. The majority of these drugs are developed forspecific indications but some are meant for a general tonic andrejuvenating properties [18]. In the absence of effective cures for liver disorders,Unani drugs have been studied extensively to develop innovative therapies for liver ailments and drug-inducedliver toxicity. Most Unani drugs aresafe, but toxicity including liver injury has also beenobserved.Moreover, there are very few scientific attempts attesting to andvalidating the safety of Unani drugs. Therefore, the present study evaluates thehepatoprotective and antioxidative potential ofUnani medicine, known as Habb-e-Asgand. Specifically,we evaluated the protective effect of Habb-e-Asgand againstparacetamol-induced liver toxicity. At high doses, paracetamolis known to cause oxidative liver injurythrough the generation of ROS[19, 20].

A review of the literature revealed that, the *invivo* anti-inflammatory potential activity of Unani formulation *Habb-E-asgand*, was not previously conducted to evaluate the formulation's traditional claim as anti-inflammatory and antioxidant. The main objective of the present research work is to study phytochemical screening and *in-vivo*anti-inflammatory potential activity of Unani formulation *Habb-E-asgand*.

2. Material and Methods

2.1 Drugs and Chemicals Used

Habb-e-Asgand (HEA) was purchased from the local Unani medical store of Nandurbar, Maharashtra, India.Oxycodone (Sigma Aldrich, St. Louis, MO, USA), and 0.9% sodium chloride (Hospira, Lake Forest, IL, USA),diclofenac sodium, Oxycodone and Dextropropoxyphenewere purchased and procured from Lab trading laboratory, Aurangabad.All other chemicals and solvents used were of analytical grade available commercially.

2.2 Physicochemical Analysis and Preliminary Phytochemical Screeningof Habb-E-asgand

The specification of the Habb-e-Asgand mixture was evaluated by performing physicochemical analyses such asappearance, color, taste, odor, pH value, friability,

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

hardness, weight change, disintegration time, etc.The powdered drug of Habb-E-asgandpills was subjected to preliminary phytochemical examination for the presence of various phytoconstituents using reported methods[21].

2.3 Animals Used and Ethical Approval

36 male Wister rats and 30 male mice weighing about 150-200 g and 25-30 g each were used for the study. They were fed with a standard pellet diet and were supplied with water ad libitum, housed less than12 h light/dark cycles, with controlled temperature (22-25^{0}C). Animals were acclimatized for atleast one week before the start of the experiment. Care of the animals and experimental procedures were done according to the guidelines of the Institutional Animal Ethics Committee (IAEC) having approval number CPCSEA/IAEC/JLS/16/07/21/11.

2.4*In vivo* Anti-inflammatory activity

Experimental Method (Carrageenan-induced Rat Paw Edema)

The anti-inflammatory activity of Habb-e-Asgand was evaluated by the carrageenan-induced rat paw edema method in rat models.36 rats were divided into six groups of six animals each. Group 1 (control group) was injected with saline and provided with the vehicle, group 2 (Carrageenan control) was injected with carrageenan and was orally treated with the vehicle, group 3 (Treatment 1) was treated with Habb-E-asgand(HEA) 100 mg/kg p.o., group 4 (standard group) treated with diclofenac sodium 100 mg/kg.The inflammation-inducing agent carrageenan, standard, and test drugs were administered in solution form using normal saline water as a vehicle.

In this study, initially, animals were treated with drugs (vehicle, standard, and three treatments) as per the groups mentioned above. Subsequently, 1 h after the above treatment, 0.1 ml of 1% solution of carrageenan was injected subcutaneously into the subplantar region of the right hind paw to induce edema. The edema was expressed as the increment in paw volume due to carrageenan administration. The paw thickness was measured at 0, 1, 2, 3, 4, and 24 h after carrageenan injection using Plethysmometer. Increase in paw thickness was measured as the difference inpaw thickness at “0 hour” and paw thickness at respective hours[22-26].

Paw withdrawal threshold (Von Frey Test)

Mechanical Allodynia: The rat was placed individually on an acrylic cage elevated maze and adopted for the test environment for a minimum of 15 min. From the base of the mesh floor,

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

Von Frey filament was applied to the plantar aspect of the hind paw of the rat. Enough force of the filament was applied to the paw thus causing slight bending and holding for a sec. Paw withdrawal was considered a positive response [27, 28].Oxycodone was used as a standard drug; it was dissolved in 0.9% sodium chloride and administered at a dose of 150 µg/0.1 ml/kg.

Acetic acid-induced vascular permeability

Mice were treated with either HEA or diclofenac at 100 mg/kg dose p.o. or with the vehicle. 1 h after the treatments, the individual mouse was injected i.v. with 2% Evan's blue solution at 10ml/kg body weight through the tail vein. 10 mins later each mouse was injected with 06% acetic acid solution (in saline) i.p. at 10 ml/kg body weight. After 30 min of acetic acid injection, the mice were sacrificed, and the peritoneal cavity was washed with saline (10 ml) three times. The saline washes were subjected to centrifuge for 5 min at 3500 rpm. The supernatant was collected and the absorbance was measured at 590 nm with a plate reader. Evan's blue extravasation was enumerated from a standard curve and was expressed in micrograms [29, 30].

Tail immersion test

Mice were divided into 6 animals in each group. The lower part of the tail (5 cm) was immersed in a beaker containing water maintained at 55 ± 0.5^{0}C. The time taken for the withdrawal of the tail from the water was recorded as a reaction time, with 10 sec as a cut-off time. The reaction time was noted one hour before the administration of drugs and as well one hour after the administration. The Control group was provided with saline, whereas treatment groups were provided withHEA (100 mg/kg) p.o, Dextropropoxyphene (65 mg/kg) was administered as a standard drug subcutaneously, 30 min before the test [31].

Adjuvant-induced Arthritis (AIA) in Rats

Inoculation of Freund’s complete adjuvant (CFA) in rats caused the induction of arthritis. On day 0, the rats were anesthetized with ketamine and xylazine mixture (80:10 mg/kg, i.p.), and then the rats were injected with 0.1 ml CFA 1 mg/ml of heat-inactivated Mycobacterium tuberculosis in 85% paraffin oil and 15% mannide monooleate (Sigma Aldrich, St. Louis, MO, USA) intradermally at the base of the tail. Control group rats were injected with an equal volume of saline. Grouping was done as follows: Control (no adjuvant, saline), AIA (adjuvant, no treatment), HEA (adjuvant, 100 mg/kg HEA), 0.1 mg/kg methotrexate (MTX,

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

Sigma Aldrich, St. Louis, MO, USA), it is the most utilized anti-rheumatic drug and hence was used as a standard drug administered p.o. Treatments were given daily from the first injection for 27 days. For the determination of hematological parameters, blood samples were collected from the retro-orbital plexus for laboratory tests. Hematological parameters determined include Red Blood Cell (RBC), White Blood Cell (WBC), Platelet count, and Erythrocyte Sedimentation Rate (ESR) [32-35].

3. Results and Discussion

3.1 Physicochemical Analysis and Preliminary Phytochemical Screening of Habb-E-asgand

The Habb-E-asgandpills were light brown and round (solid) with a distinctive agreeable taste and pungentsmell. The pills were stored in a cool, dark place in tightly closed containers, protected from moisture, light, and temperature. Physico-chemical analysis showed a slightly basic pH of 6.5 as shown in **Tables 1 and 2.**

Table 1. Organoleptic Character

Sr. No	Parameters	Observation
1	Size	Round
2	Color	Light Brown
3	Taste	Agreeable
4	Odor	Pungent
5	Appearance	Habb (Pills)

Table 2. Physiochemical Character

Sr. No	Parameters	Habb-e-Asgand
1	Friability Test	0.219%
2	Hardness Test	12.5 kg/cm 2 (Monsanto)
3	Weight Variation	0.0668
4	Disintegration time	55 min
5	a pH of 1% Solution	6.5
6	10% Solution	5.2

The presence of numerous secondary metabolites such as alkaloids, tannins, flavonoids, proteins, and mucilages may explain the therapeutic benefits of Habb-e-Asgand tablets. As a result, preliminary screening assays can aid in the detection of bioactive components, which can lead to medication development and discovery. Furthermore, these tests make it easier to estimate the quantity of pharmacologically active chemical substances and to separate them qualitatively. In the Habb-e-Asgand extract, preliminary phytochemical screening with several qualitative chemical assays revealed the presence of carbohydrates, reducing sugars,

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

protein, amino acid, alkaloids, glycosides, and tannins, but flavonoids, saponins, fats & oils, and steroids were lacking.

3.2 *In-vivo* Anti-inflammatory Activity

Experimental Method (Carrageenan Induced Rat Paw Edema)

Subplantar injection of carrageenan in rats in carrageenan-induced paw edema test showed a time-dependent increase in paw edema. In normal control group at t=0, paw thickness was observed 3.026±0.89cms which was remained same after 24 hrs. In carrageenan control group paw thickness showed exponential increased and displayed significant at $P< 0.001$. It was 3.031±0.076cms at t=0, which was increased after 24 hrs to 4.261±0.123cms. In Carrageenan-induced rat Paw edema, In HEA treated group, paw thickness was 3.025±0.074 at t=0, but it showed increase in paw inflammation after one hour i.e. 3.201±0.072 cms which again showed decrease in paw thickness up to 4thhr i.e. 3.021±0.077, 3.011±0.038, 2.998±0.071 at t=2, 3, and 4 respectively. It showed again getting back to the normal thickness of paw at t=24 hrs i.e. 3.027±0.117 cms. In Diclofenac sodium treated groups, it displayed same pattern as demonstrated by HES treated group. All the results obtained were significant with the control group. The values are tabulated in Table 3 and the graph paw thickness Vs time is illustrated in Fig. 1.

Table 3. Effect of HEA on carrageenan-induced paw edema

Treatment	Paw thickness of rats (cms)					
	0 hr	1 hr	2 hr	3 hr	4 hr	24 hrs
Group-I: Normal control	3.026±0.89	3.026±0.073	3.026±0.082	3.026±0.092	3.026±0.074	3.026±0.109
Group-II: Carrageenan control	3.031±0.076	3.189±0.069	3.392±0.087	3.628±0.093	3.732±0.061	4.261±0.123
Group-III: HEA	3.025±0.074 ***	3.201±0.072 ***	3.021±0.077 ***	3.011±0.038 ***	2.998±0.071 ***	3.027±0.177 ***
Group-IV: Diclofenac sodium (standard)	3.035±0.086 ***	3.098±0.087 ***	2.961±0.069 ***	2.92±0.077 ***	2.899±0.088 ***	3.034±0.139 ***

Values are expressed as mean ± SEM $^{*}p < 0.001$, compared to control group, $^{***}p < 0.001$, compared to carrageenan control group. The difference between the groups was analysed by one-way analysis of variance (ANOVA) followed by Tukey's test.

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

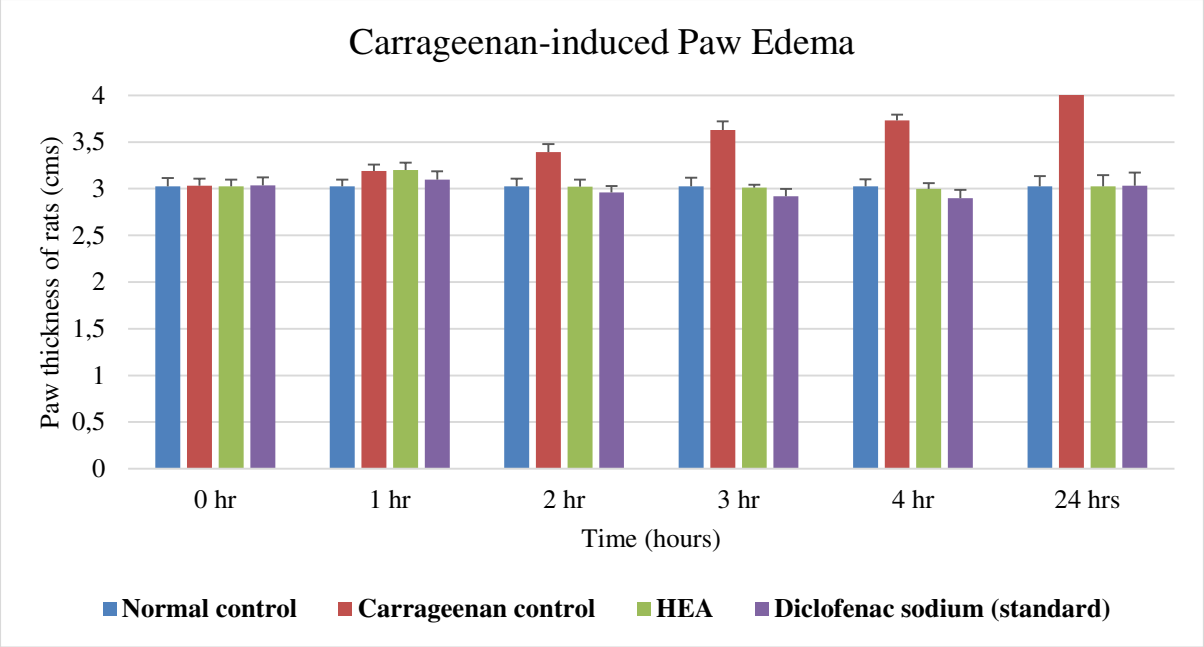

Fig. 1 Effect of HEA on carrageenan-induced paw edema

Paw-withdrawal Threshold (Von Frey Test)

Effect of HEAon Paw Withdrawal Threshold

In fig. 2, table 4, the von Frey test reporting pain threshold measurement is displayed. A decreased withdrawal response was observed through a non-noxious mechanical stimulation of the paw (allodynia-like measure) in carrageenan-treated animals, which maintained a plateau at 2 and 3 hours after treatment. Treatment with HEA ($p<0.001$), showed a significant decrease in pain from 0 to 60 min, similar treatment with standard oxycodone also showed a significant decrease ($p<0.001$) in pain from 0 to 60 min. Thus HEA and the standard drug showed complete blockade of carrageenan-induced hypersensitivity.

Table 4: Effect of HEA on paw withdrawal threshold

Treatment	Paw withdrawal threshold (g)				
	0 min	15 min	30 min	45 min	60 min
Normal control	0.64 ± 0.01	0.65 ± 0.01	0.67±0.01	0.69±0.01	0.66±0.01
Carrageenan control	0.29 ± 0.01	0.23±0.01$^{\alpha}$	0.26±0.01$^{\alpha}$	0.27±0.01$^{\alpha}$	0.26±0.01$^{\alpha}$
HEA	0.31 ± 0.01	0.51 ± 0.03^{a}	0.63±0.01^{a}	0.53±0.02^{a}	0.50±0.02^{a}
Standard	0.38 ± 0.01	0.81 ± 0.02^{a}	0.85±0.02^{a}	0.83±0.03^{a}	0.79±0.03^{a}

Values are expressed as mean ± SEM. $^{\alpha}p < 0.001$, compared to control group, $^{a}p < 0.001$, compared to carrageenan control group. The difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test.

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

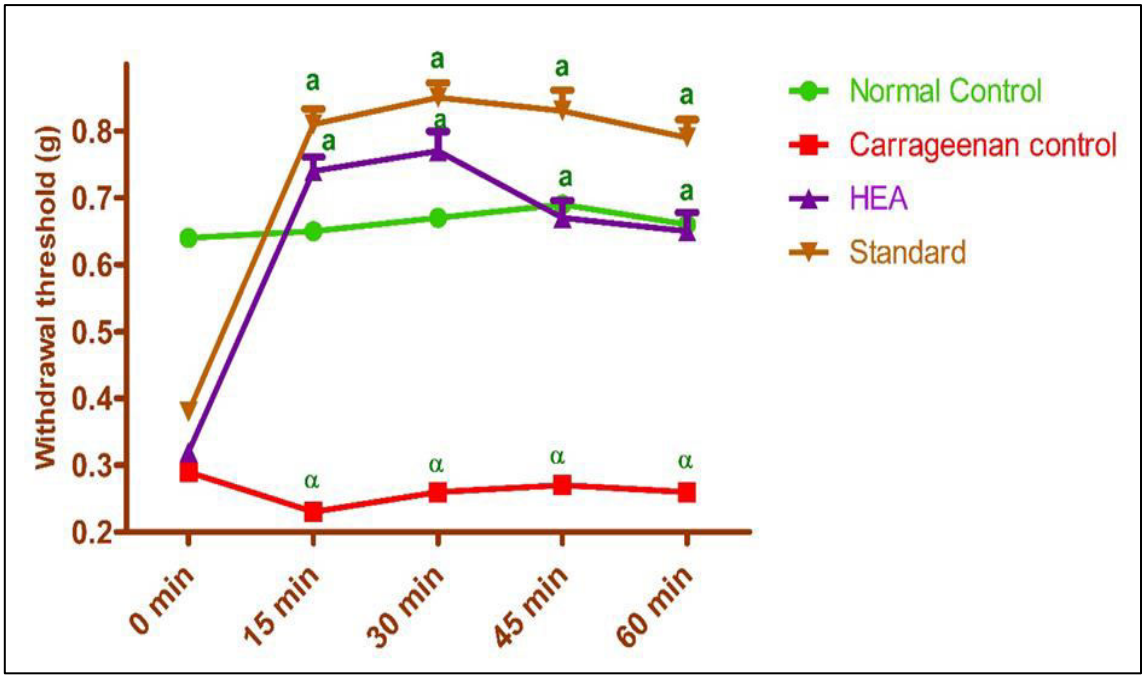

Fig. 2. Effect of HEA on paw withdrawal threshold

Acetic acid-induced Vascular Permeability

Effect of HEA on Evans Blue dye Extravasation into the Peritoneal Cavity of Mice

Standard reduced the dye leakage into the peritoneum more effectively when compared to HES and control. This reduction of dye leakage indicates that HEA possesses anti-inflammatory action due to the reduced permeability of vessels. In the present method, HEA ($p < 0.001$) and metformin ($p < 0.001$) have shown significant inhibition of dye leakage (Fig. 3).

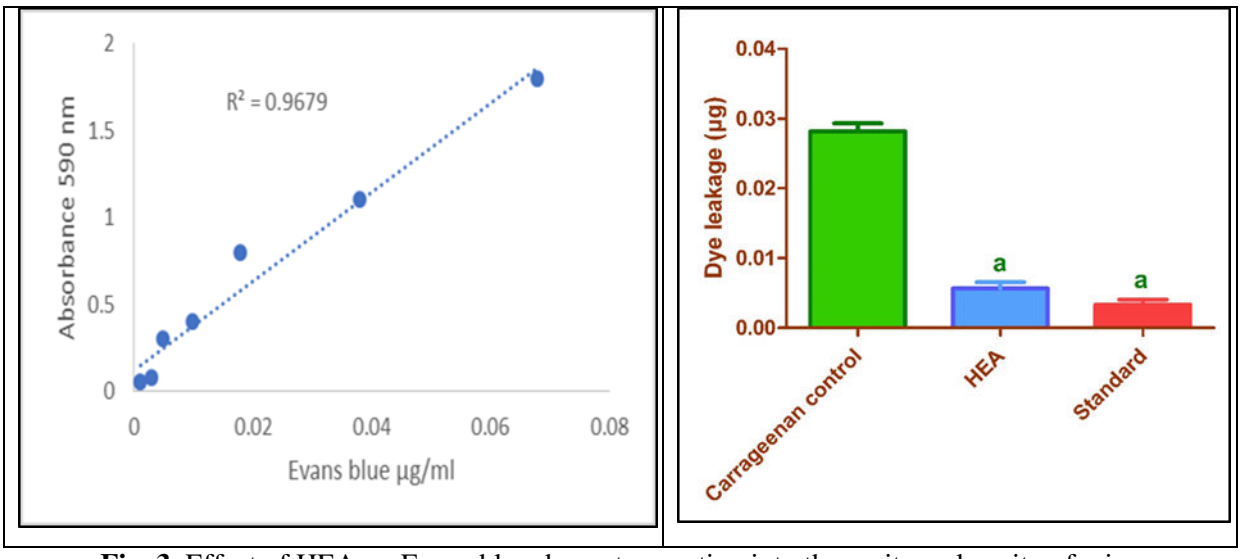

Fig. 3. Effect of HEA on Evans blue dye extravasation into the peritoneal cavity of mice

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

Effect of HEA on Tail Withdrawal Reflexes-induced by Tail immersion Method in Mice

All three samples have shown significant inhibition concerning control (HEA $p<0.001$). From table 5, fig. 4 it is evident that all the extracts have shown significant analgesic activity. This was slightly lower than dextropropoxyphene.

Table 5. Effect of HEA on tail withdrawal reflexes induced by tail immersion method in mice

Drug (dose)	Before treatment (sec)	After treatment (sec)
Control (saline)	5.7 ± 0.082	5.8 ± 0.099
HEA (100 mg/kg)	6.0 ± 0.053	7.3 ± 0.19[a]
Dextropropoxyphene (65 mg/kg)	5.7 ± 0.066	10 ± 0.16[a]

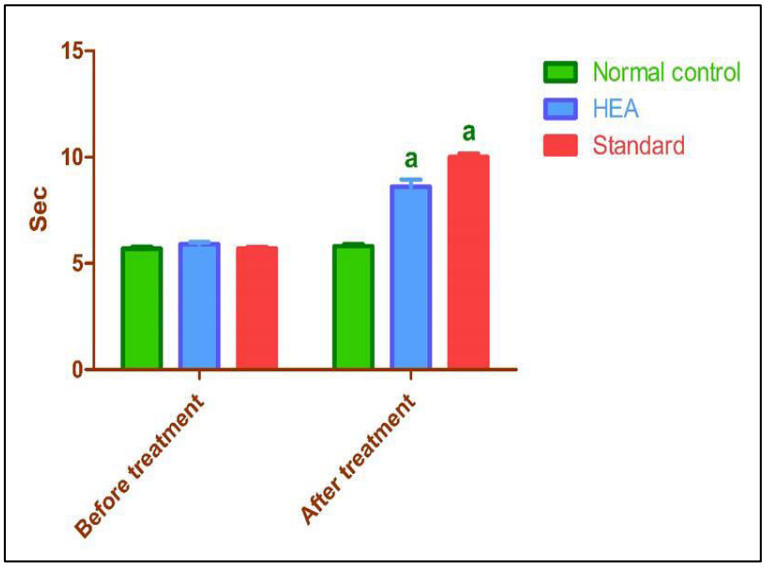

Fig. 4. Effect of HEA on tail withdrawal reflexes induced by tail immersion method in mice

Attenuation of Adjuvant-induced Arthritis in Rats by HEA

Paw volume was increased significantly in the AIA group when compared to the normal control group ($p<0.001$) from day 8 to day 28. Compared with the normal control group, HEA and MTX-treated groups showed an obvious decrease in edema of the paw ($p<0.001$) (Fig. 5).

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

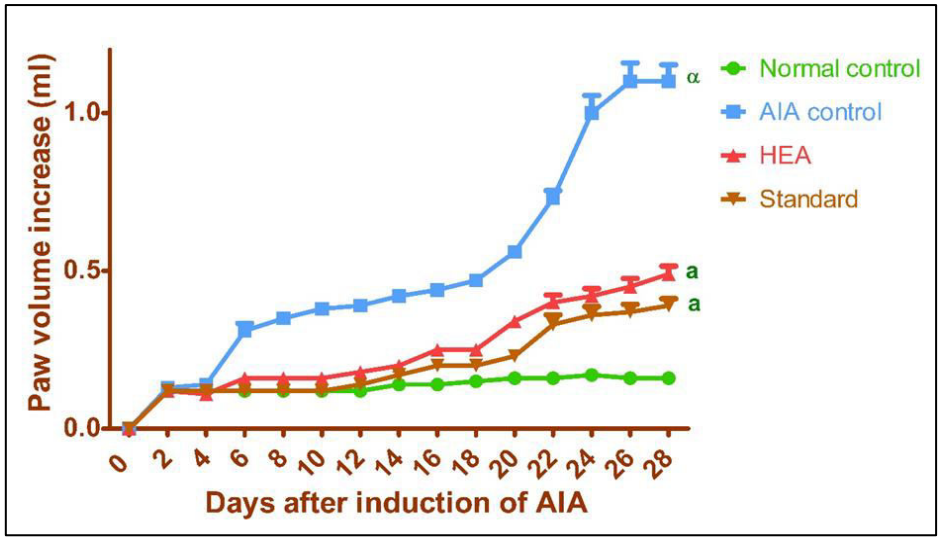

Fig. 5. Anti-inflammatory effect of HEA on AIA in rats

Effect of HEA on the HaematologicalParameter of Rats Treated with Freund's Complete Adjuvant

Table 6 and fig. 6 represent the hematological changes associated with an arthritic condition. Levels of WBC, platelet count, and ESR were increased in arthritic rats, while the level of RBC was decreased. These levels were observed to be near normal on treatment with standard drugs, were as HEA has shown less significant or no significance when compared to the AIA group.

Table 6. Effect of HEA on the haematological parameter of rats treated with Freund's complete adjuvant

Group	WBC count (10^3/mm^3)	RBC (x 10^6/µL)	Platelet count (10^5/mm^3)	ESR (mm/hr)
Normal control	8.7 ± 0.25	6.0 ± 0.15	2.4 ± 0.16	3.7 ± 0.19
AIA control	12 ± 0.31$^{\alpha}$	4.3 ± 0.28$^{\alpha}$	3.5 ± 0.11$^{\alpha}$	5.6 ± 0.15$^{\alpha}$
HEA	10 ± 0.34^{b}	5.5 ± 0.18^{c}	2.9 ± 0.07^{c}	5.2 ± 0.16
Standard	9.5 ± 0.26^{a}	6.0 ± 0.21^{a}	2.5 ± 0.12^{a}	4.4 ± 0.09^{c}

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

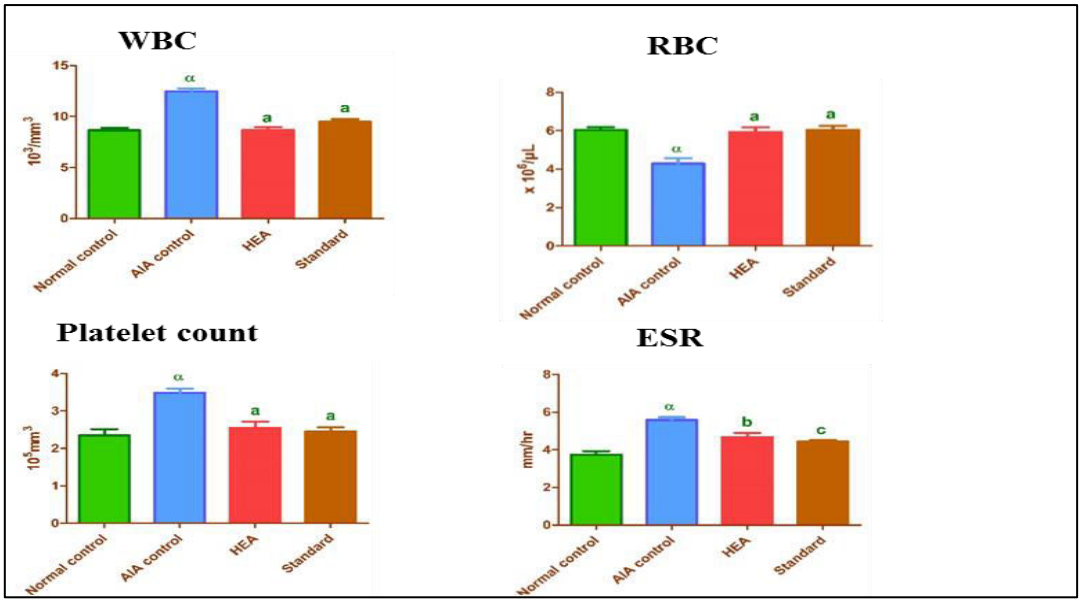

Fig. 6. Effect of HEA on the haematological parameter of rats treated with Freund's complete adjuvant

Conclusion

A series of tests evaluating a range of activities such as carrageenan-induced rat paw edema, von Frey test, adjuvant-induced arthritis (AIA) in rats, *in-vivo*anti-inflammatory activities of the aqueous extract, *in-vivo*Habb-E-asgand'sefficiency could be explained by the inclusion of various polyherbal components, which are known to be powerful antioxidants and anti-inflammatory.Overall, the current study demonstrates that formulation has anti-inflammatory properties, which not only validates some ethnomedicinal claims but also identifies a promising candidate for further investigation, particularly in chronic inflammatory disorders such as rheumatoid arthritis. More research is being done to identify the biomolecules that cause the anti-inflammatory and antioxidant benefits.

References

1. George SK, Rajesh R, Kumar S, Sulekha B, Balaram P. A polyherbal ayurvedic drug–Indukantha Ghritha as an adjuvant to cancer chemotherapy via immunomodulation. Immunobiology. 2008;213(8):641-9.
2. Singh RS, Dhaliwal R, Puri M. Production of high fructose syrup from Asparagus inulin using immobilized exoinulinase from Kluyveromyces marxianus YS-1. J. Ind. Microbiol. Biotechnol. 2007;34(10):649-55.
3. Kar P, Asim M, Sarma MP, Patki PS. HD-03/ES: a promising herbal drug for HBV antiviral therapy. Antivir. Res.2009;84(3):249-53.

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

4. Nwaehujor CO, Udeh NE. Screening of ethyl acetate extract of Bridelia micrantha for hepatoprotective and anti-oxidant activities on Wistar rats. Asian Pac. J. Trop. Med.2011;4(10):796-8.
5. Ghazanfar K, Dar SA, Nazir T, Akbar S. Subchronic oral toxicity study of Habb-e-Suranjan in albino Wistar rats. J. Complement. Integr. 2018;15(3):20170144.
6. Khan SL, Siddiqui FA. Beta-Sitosterol: an immunostimulant, antioxidant, and inhibitor of SARS-CoV-2 spike glycoprotein. Naunyn-Schmiedeb. Arch. Pharmacol. 2020;2(1):12-16.
7. Chaudhari RN, Khan SL, Chaudhary RS, Jain SP, Sidduqui FA. &BETA;-sitosterol: Isolation from muntingia calabura linn. bark extract, structural elucidation, and molecular docking studies as a potential inhibitor of sars-cov-2 mpro (COVID-19). Asian J. Pharm. Clin. Res.2020;1(1):204-9.
8. Khan SL, Siddiqui FA, Jain SP, Sonwane GM. Discovery of potential inhibitors of SARS-CoV-2 (COVID-19) Main Protease (Mpro) from Nigella Sativa (black seed) by molecular docking study. Coronaviruses. 2021;2(3):384-402.
9. Khan SL, Siddiqui FA, Shaikh MS, Nema NV, Shaikh AA. Discovery of potential inhibitors of the receptor-binding domain (RBD) of pandemic disease-causing SARS-CoV-2 Spike glycoprotein from Triphala through molecular docking. Curr. Chinese Chem. 2021;1(1):1-9.
10. Siddiqui FA, Khan SL, Marathe RP, Nema NV. Design, synthesis, and in silico studies of novel N-(2-Aminophenyl)-2, 3-diphenylquinoxaline-6-sulfonamide derivatives targeting receptor-binding domain (RBD) of SARS-CoV-2 spike glycoprotein and their evaluation as antimicrobial and antimalarial agents. Lett Drug Des Discov.2021;18(9):915-31.
11. Khan S, Kale M, Siddiqui F, Nema N. Novel pyrimidine-benzimidazole hybrids with antibacterial and antifungal properties and potential inhibition of SARS-CoV-2 main protease and spike glycoprotein. DCM. 2021;4(2):102-19.
12. Khan A, Unnisa A, Sohel M, Panpaliya N, Saboo SG, Siddiqui F, Khan S. Investigation of phytoconstituents of Enicostemma littorale as potential glucokinase activators through molecular docking for the treatment of type 2 diabetes mellitus. In SilicoPharmacol. 2022;10(1):1-6.
13. Shntaif AH, Khan S, Tapadiya G, Chettupalli A, Saboo S, Shaikh MS, Siddiqui F, Amara RR. Rational drug design, synthesis, and biological evaluation of novel N-(2-arylaminophenyl)-2, 3-diphenylquinoxaline-6-sulfonamides as potential antimalarial, antifungal, and antibacterial agents. DCM. 2021;4(4):290-304.
14. Unnisa, A., Khan, S. L., Sheikh, F. A., Mahefooz, S., Kazi, A. A., Siddiqui, F. A., ... & Saboo, S. G. In-silico Inhibitory Potential of Triphala Constituents Against Cytochrome P450 2E1 for the Prevention of Thioacetamide-induced Hepatotoxicity. JPRI.2021;33(43A): 367-375.
15. Ahmed, N. Z., Ahmad, K., Ezhil, R., Anjum, N., & Khan, A. A. Evaluation of Analgesic Effect of Unani Pharmacopoeial Formulation Habb-E-Suranjan and Raughan-E-Suranjan in Wajaul Mafasil (Joints Pain) Patients: An Open Prospective Clinical Trial. J. drug deliv. ther. 2021;11(2-S):44-9.

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

16. Benabou R, Waters C. Hepatotoxic profile of catecholOmethyltranferase inhibitors in Parkinson's disease. Expert Opin Drug Saf. 2003;2(3):263-7.
17. Bhattacharya A, Muruganandam AV, Kumar V, Bhattacharya SK. Effect of polyherbal formulation, EuMil, on neurochemical perturbations induced by chronic stress. Indian J Exp Biol. 2002;40(1):1161–3.
18. Leone L, Marchitiello M, Natilli M, Romano MF. Measuring the effectiveness of homeopathic care through objective and shared indicators. Homeopathy. 2011;100(04):212-9.
19. Craig DG, Bates CM, Davidson JS, Martin KG, Hayes PC, Simpson KJ. Staggered overdose patterns and delay to hospital presentation are associated with adverse outcomes following paracetamol-induced hepatotoxicity. Br. J. Clin. Pharmacol. 2012;73(2):285-94.
20. Suhail S, Jamil SS, Jilani S, Jahangir U, Qamar MW. Habb-e-Suranjaan: A classical analgesic Unani formulation. Int. J. Adv. Pharm. Med. Bioallied. Sci. 2017;1(1):1-6.
21. Akhtar H, Siddiqi M. The clinical study of primary gout and comparative evaluation of Habb-E-Suranjan with allopurinol in its management. J. pharmacogn. phytochem. 2019;8(4):84-90.
22. Elsakka M, Pavelescu M, Grigorescu E. Withania somnifera, a plant with a great therapeutical future. Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi. 1989;93(2):349-50.
23. Ali M, Khan SA, Chang PS, Haque R, Bhatia K, Ahmad S. Habb-e-Asgand, polyherbal Unani formulation, protects the liver and antioxidative enzymes against paracetamol-induced hepatotoxicity. Pharm. Biol.2014;52(4):506-15.
24. Parmer NS, Prakash S. Evaluation of analgesics, anti-inflammatory, and antipyretic activity. In: Screening Methods in Pharmacology. Narosa Publishing House Pvt. Ltd.; New Delhi, India, 1(1), 211-215.
25. Patil S, Anarthe S, Jadhav R, Surana S. Evaluation of anti-inflammatory activity and in-vitro antioxidant activity of Indian Mistletoe, the Hemiparasite Dendrophthoe falcate LF (Loranthaceae). Iran. J. Pharm. Sci. IJPR. 2011;10(2):253.
26. Karim N, Khan I, Khan W, Khan I, Khan A, Halim SA, Khan H, Hussain J, Al-Harrasi A. Anti-nociceptive and anti-inflammatory activities of asparacosin a involve selective cyclooxygenase 2 and inflammatory cytokines inhibition: an in-vitro, in-vivo, and in-silico approach. Front. immunol. 2019 Mar 26;10:581.
27. Sanklecha D., Upaganlawar A. and Upasani C. Neuroprotective Effects of Protocatechuic 242 Acid in Diabetes Induced Neuropathic Pain. Am J BiochemMol Biol. 2017;7(3):111-117.
28. Micheli L, Vasarri M, Barletta E, Lucarini E, Ghelardini C, Degl'Innocenti D, Di Cesare Mannelli L. Efficacy of Posidonia oceanica extract against inflammatory pain: In vivo studies in mice. Marine drugs. 2021;19(2):48.

ISSN: 0975-3583,0976-2833 VOL12,ISSUE06,2021

29. Ampadu FA, Boakye-Gyasi E, Osafo N, Benneh CK, Ekuadzi E, Woode E. Antipleuritic and vascular permeability inhibition of the ethyl acetate-petroleum ether stem bark extract of Maerua angolensis DC (Capparaceae) in murine. Int J Inflam. 2018;1(1):1-10.
30. Kumar T, Jain V. Antinociceptive and anti-inflammatory activities of Bridelia retusa methanolic fruit extract in experimental animals. Sci. World J. 2014;1(1):1-12.
31. Zhang ZC, Zhang SJ, Jin B, Wu Y, Yang XF, Yu B, Xie QM. Ciclamilast ameliorates adjuvant-induced arthritis in a rat model. BioMed Res. Int. 2015;1(1):1-11.
32. Saha S, Guria T, Singha T, Maity TK. Evaluation of analgesic and anti-inflammatory activity of chloroform and methanol extracts of Centella Asiatica Linn. int. sch. res. Notices.2013;1(1), 1-6.
33. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-200.
34. Desmarchelier C, Novoa Bermudez MJ, Cossio J, Ciccia G, Boveris A. Antioxidant and prooxidant activities in aqueous extracts of Argentine plants. Int. J. Pharmacogn. Phytochem. Res.1997;35(2):116-20.
35. Tatiya AU, Saluja AK, Kalaskar MG, Surana SJ, Patil PH. Evaluation of analgesic and anti-inflammatory activity of Bridelia retusa (Spreng) bark. J. Tradit. Complement. Med. 2017;7(4):441-51.