IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON ANALGESIC ACTIVITY OF POLYHERBAL FORMULATION IN EXPERIMENTAL MICE.

[1]Ms. Salve Jagruti R., [2]Mr. Gaikar Mayur T.
[1]S.Y.M.Pharm Student Dept. Pharmacology, [2]Assistant Professor of Dept. of Pharmacology
Pravara Rural Education Society's College of Pharmacy (For Women) Chincholi, Sinnar, Nashikh -422102, Maharashtra, India.

ABSTRACT
To evaluate analgesic activity of a polyherbal formulation-PHF [hydro- alcoholic extract of Hibacus rose-amensts (50mg), Funnel seeds (50mg) Prosopir cinerario (Sung), & Fleus racemosa (Sting)] compare it with Diclofenac Na hy using writhing test inmale or female mice were divided into four groups of 6 each for both tests. PHF (250, 300 mg/kg, po body weight) and Diclofenac Na 150 mg/kg po.) Made as suspensions prepared in 15% carboxy methyl cellulose (control) and fed to rats orally. The physicochemical evaluations carried out in terms of loss un drying, ash value, extractive values and acid insoluble ash value etc. Qualitative analysts of various phytochemical constituents were determined by the well-known test protocol available in the literature. Phytochemical analysis revealed th the presences of phenols, flavonoids, tannins, saponins, alkaloids. Analgesic activity was assessed by counting the number of writhes induced by 0.7% acetic acid (10 ml/kg) in the 30 min. Number of writhing and percentage protection against writhing was evaluated. The results indicated that the poly-herbal formulation possesses good analgesic activity in the experimental animal models.
Keyword: Analgesic activity, Physicochemical evaluations, Phytochemical analysis, Hibiscus rosa-sinensis Fennel seeds, Prosopis cineraria, Ficus racemosa

INTRODUCTION

International association for the study of pain defines pain as - Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1]. The therapeutic agents currently available for the treatment of pain usually have limited effectiveness and safety [2]. Analgesics relieve pain, without affecting its cause. Analgesics are divided into two groups, opioid analgesic and non-opioid analgesic [3]. None of the currently used analgesic agents i.e., opioid & nonopioids fulfills the criteria for ideal analgesics. Repeated use of non-steroidal anti- inflammatory drugs (NSAIDs) may induce several adverse effects, such as gastrointestinal lesions or renal and liver failure [4]. NSAIDs may cause or exacerbate gastrointestinal upsets, peptic ulcers, platelet dysfunction. It may cause bronchospasm resulting in exacerbation of bronchial asthma. Opioids are reserved for severe pain. Adverse effects of opioids include sedation, nausea, vomiting, constipation, physical dependence, tolerance, and respirator depression & urinary retention. The use of conventional drugs for the treatment of pain and inflammation has largely resulted in various side effects. These challenges have triggered scientific researchers all over the world in search of alternative therapy [5]. Research into new effective and safe analgesic agents with satisfactory tolerability and proven efficacy is urgently needed [6]. Hence, analgesic drugs lacking these adverse effects are being searched all over the world as an alternative to NSAIDs and opiates.

During this process, the investigation of the efficacy of plant-based drugs used in traditional medicine has been paid great attention because they are cheap, have little side effects, and according to WHO, still about 80% of the world population rely mainly on plant-based drugs [7].

Recently, alternative agents, such as natural products, have been shown to contain richly diverse compounds, leading to the discovery of compounds with medical applications, particularly in the treatment of pain [8]. The use of herbal medicines worldwide has provided an excellent opportunity for India to look for therapeutic lead compounds from our ancient system of therapy, i.e. Ayurveda, which can be utilized for development of new drug [9]. In extensive literature search we came to know that in many pharmaceutical institutes much animal experimentation established analgesic, anti-inflammatory & anti- pyretic properties of different herbs [10-16]. Hibiscus rosa sinensis is one of the most common garden shrubs used for hedges [2]. The herb Hibiscus belonging to the family Malvacecae and is commanly known as Jasvand [3], Flowers are used in all kinds of inflammation; internally they are prescribed in the form of decoction of bronchial catarrh, as a becenic and sudorific roots are mucilaginous and demulcent, valuable in cough [2]. The buds have cooling and astringent effect and it removes burning sensation of the body [3]. The extract of the leaves is used to relieve pain. Foeniculum vulgare Mill. [Apiaceae), known in English as sweet fennel and in Bengali as is an aromatic plant commonly grown in Bangladesh primarily for its seeds. Which are used for both culinary and medicinal purposes. The plant belongs to the carrot family of plants. Antioxidant properties have been reported for various parts of the plant [4, 5]. Antidiabetic antihyperlipidemic and hepatoprotective. Effect has been reported for a polyherbal formulation containing the plant [6]. Anti-inflammatory, analgesic and antioxidant activities have been reported for fruits of the plant [7]. Seeds of the plant have been found to be effective in relieving pain during dysmenorrheal [8]. The fruits and their constituents have been shown to inhibit 5-lipoxygenase activity [9]. Prosopis cineraria are a small to moderate sized tree belongs to the family mimosaceae. It is found distributed in the regions of Arabia and various parts of India like Rajasthan, Gujarat, Haryana, Uttarpradesh and Tamilnadu. The hark is used as a remedy for rheumatism, cough, common cold, asthma and scorpion strings (10, 11). It was reported to possess new piperidine alkaloid spicigerin, prusogeria E along with gallic acid, pautelin, luteolin and rutin [12]. Prosogerin A and B were isolated from its flowers. [13]. various pharmacological activities like analgesic and antipyretic activities have been reported for different extracts of this plant [14]. Ficus racemosa Linn (Moraceae) is an evergreen, moderate to large sized spreading, lactiferous deciduous tree, without much prominent aerial roots found throughout greater part of India in moist localities and is often cultivated in villages for its edible fruit [15]. All parts of this plant (leaves, fruits, bark, latex, and sap of the root) are medicinally important in traditional system of medicine in India. Apart from the usage in traditional medicine, scientific studies indicate F. racemosa to possess various hiological effects such hepatoprotective, chemopreventive, antidiabetic, antiinflammatory, antipyretic, antitussive and antidiuretic [16-22), The objective of our study was to evaluate the efficacy of polyherbal formulation by virtue of their analgesic potential in laboratory animals using various animal models.

MATERIALS AND METHODS

Plants material

The leaves of plant Hibiscus rosa-sinensis, Prosopis cineraria, Ficus racemosa and seed of Fennel, was collected from various places from Bhel area Govindpura Bhopal (M.P.) during the month of May 2018. The plant has been identified and authentication by Head of the Department Botany at the S. S. L. Jain P.G. College, Vidisha (M.P.). The plant part specimens were submitted as herbarium with voucher specimen no. 2018/48.

Chemical reagents

All the chemicals used in this study were obtained from HI Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine- Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Physicochemical study

Loss on Drying

About 10 gm. of the powdered drug was weighed in a Petri dish. It was dried at 105°C for 1 hour in hot air oven and then reweighed. Loss on drying was determined from calculating the initial and final weight.

Total Ash Value

About 7 gm. accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle furnace. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated.

Acid Insoluble Ash Value

Accurately weighed 1 gm. ash was boiled for 5 minute with 25ml hydrochloric acid by covering the crucible with a watch-glass on water bath then cooled. The watch glass was rinsed with 5 ml of hydrochloric acid and this liquid was added in to the crucible. Then the content was filtered on a previously weighed Whatsman filter paper and

filtrate was dried and weighed. Acid insoluble ash value was determined by calculating the % content remaining after deducting the weight of filter paper.

Water Soluble Ash Value

Accurately weighed 1 gm. ash was boiled for 5 minute with 25ml distilled water by covering the crucible with a watch- glass on water bath then cooled. The watch- glass was rinsed with 5 ml of distilled water and this liquid was added in to the crucible. The % of remaining content was deducted from initial % of ash taken (i.e. 100%) to determine the water soluble ash value.

Foaming Index

About 1 gm. coarse powder was weighted and transferred to a 500 ml conical flask containing 100 ml of water. It was maintained at moderate boiling for 30 minute on water bath. It was cool and filtered in to a 100 ml volumetric flask. Volume was diluted by adding sufficient amount of water. The decoction was poured in test tube, and then shaken in a lengthwise motion for 15 seconds. They were allowed stand for 15 minutes and the height of foam was measured to determine the foaming index.

Extraction Procedure

500 gm. of dried powdered of leaves/seeds of plant has been extracted with hydroalcoholic solvent (1:1) using hot continuous percolation process for 48 hrs. and dried using vacuum evaporator at 40°C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [23].

Qualitative Phytochemical Analysis of Plant Extract

The extract obtained from all plants was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [24, 25). The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils. Protein, amino acid and tannins

Animals

Male and Female Albino Mice of weighing 25-30gm were used for the study. The animals were housed in solid-bottomed polypropelene cages and acclimatized to animal house conditions. The rats were fed with commercial rats' diet and water ad libitium. The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments Animals (CPSCEA) and Institutional Animal Ethical Committee (IAEC) of Sapience Bioanalytical Research Lab Bhopal (Proposal no: SBRL/IAEC/NOV2018/01)

Preparation of poly-herbal formulation

The hydro-alcoholic extract of Hibiscus rosa-sinensis (50mg). Fennel seeds (50mg), Prosopis cineraria (50mg). & Ficus racemosa (50mg) was dissolved in suspending agent (1% CMC aqueous) before orally administered to the Rats. Standard drug was dissolved in suspending agent (1% CMC) before orally administered to the mice.

Grouping of Rats: Animals were equally divided into four groups viz. A, B, C & D (each group had six animals)

Group A: Served as control group received 1⁒ CMC, (p.o).

Group B: Served as a standard group received diclofenac sodium 50 mg/kg body weight.

Group C: Served as test drug group III PHF extract received in a dose of 250 mg/Kg body weight.

Group D: Served as a test drug group IV received PHF extract in a dose of 300 mg/kg body weight.

A] Acute Oral Toxicity Study:

Acute oral toxicity study was done as per OECD 425 guideline [17]. As ingredients of this polyherbal formulation (PHF) are herbal &individually tested for its toxicity, this formulation is supposed to be nontoxic. First, a female Wister rat weighing 200 gm. was given a polyherbal formulation (PHF) in a limited dose of 2000 mg/Kg body weight after overnight fasting. It was observed for any toxic effects like convulsions, tremors, diarrhea, and salivation lethargy. As it didn't die in 24 h. Another 4 rats (2 males & 2 females) were given polyherbal formulation in a same limit dose of 2000 mg/Kg body weight next day. All these animals were observed for two weeks for the incidence of any toxic effect. None of the animals showed any changes in the respiratory, circulatory or nervous system. As none of the animal died LD 50 is considered to be more than 2000 mg/kg body weight.

B] Analgesic Activity Study:

Acetic Acid-Writhing in Mice -

Analgesic activity study of test drug was done by using acetic acid-induced writhing test Acetic Acid-Induced Writhing in mice. This method is useful for the evaluation of the peripheral. Analgesic activity of the drug. All animals fasted overnight. Dose calculation of drug for each animal according to body weight in the respective group was done. One hour before inducing writhes with acetic acid, all animals received their respective drug as per body weight [18]. One hour after dosing, writhing was induced in mice by intraperitoneal injection of 0.7%

acetic acid in a dose of 10 ml/kg body weight [19].Numbers of writhes were counted for 10 min beginning from 5th min. after the acetic acid injection. Writhing is a response consisting of contraction of an abdominal wall, pelvic rotation followed by hind limb extension [20]. Percentage inhibition of writhing response in each group was calculated by using the following formula.

Percentage inhibition N-N. /N x 100

N: Average no. of writhes of the control group

N average no. of writhes of the test group (or a standard group)

OBSERVATION AND RESULTS

The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the leaves of the plants using hydroalcoholic as solvents are depicted in the Table 1.

Table 1: Percentage yield of all plant extracts.

Sr.no.	Extract	Yield	Percentage Yield
1	***T1***	16.801	15.5%
2	***T2***	12.502	10.30%
3	***T3***	14.200	13.25%
4	***T4***	15.020	14.65%

Where is: T1= Habiscus rosa-sinensis, T2= fennel seed, T4= Prosopis cineraria, T4= Ficus racemosa.

The physical constituents' estimation of the drugs is an essential parameter to determine adulteration or inappropriate handling of drugs. The physicochemical characters of powder drug of leaves/seeds of all plant such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, and water soluble ash, loss after drying and foreign substances are given in Table2.

Table no 2: Physiochemical analysis of powder of all plants parts

Sr. no	Parameters	observations			
		T1	***T2***	***T3***	***T4***
1	Loss on drying	1.38	1.85	1.45	1.15
2	Total ash value	4.10	3.75	3.53	4.05
3	Acid insoluble ash value	0.95	1.0	0.75	1.05
4	Water soluble ash value	0.90	0.95	.085	0.93
5	Foaming index	1.05cm	1.15cm	0.82 cm	0.6cm

Where is: T1= Habiscus rosa-sinensis, T2= fennel seed, T4= Prosopis cineraria, T4= Ficus racemosa.

The results of qualitative phytochemical analysis of the crude powder of all plants are shown in Table 3.Hydroalcoholic extract of all plants shown the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins and saponins.

Table no 3: Phytochemical Screening of hydro – alcoholic extracts

Sr. no	Phytocontituents	***T1***	***T2***	***T3***	***T4***
1	Alkaloids	Present	Absent	Absent	Present
2	Glycoside	Absent	Present	Present	Absent
3	Carbohydrate	Present	Absent	Present	Absent
4	Tannin & Phenols	Present	Present	Present	Present
5	Flavonoids	Present	Present	Present	Present
6	Steroids	Absent	Present	Present	Absent
7	Saponins	Present	Present	Absent	Absent
8	Protein	Present	Absent	Present	Present
9	Gums & mucilage	Absent	Absent	Absent	Present

Where is: T1= Habiscus rosa-sinensis, T2= fennel seed, T4= Prosopis cineraria, T4= Ficus racemosa.

Acetic Acid Induced Writhing Method

Acetic Acid Induced Writhing Method In vehicle treated mice 89± 0.5 writhing were observed in observation period of 30 min. PHF (250, and 300 mg/kg, p.o.) decreased the number of writhing and the differences in writhing were statistically significant at p < 0.001 Diclofenac Na (50 mg/kg po.) reduced the number of writhing induced by acetic acid to 24+1.63. The observations are given in Table 4.

Table 4: Effect of PHF (250, and 300 mg/kg, p.o.) on Acetic acid induced writhing test in mice

Group Name	Treatment	Dose	No. of writhes/30mins	Inhibition % writhing
Disease Control	Control	0.7 % acetic acid in volume of 10 mg /kg,i.p.	89± 0.5	.....
Standard	Diclofenac Na + 0.7 % acetic acid in vol. 10 mg /kg i.p. solution	50 mg/kg, p.o.	24±1.63*	73.03 %
Test-1	Poly-herbal formulation Extarct + 0.7 % acetic acid in vol. 10 mg/kg i.p.	250 mg /kg, p.o.	39±1.55*	56.17 %
Test-2	Poly-herbal formulation Extarct + 0.7 % acetic acid in vol. 10 mg/kg i.p.	300 mg/kg p.o.	29.0±0.43*	67.41%

*p < 0.001 compared to control, Values are mean ±SEM, of six animals in each group.

DISCUSSION

A large number of herbal drugs are reputed to have excellent medicinal value and are in use for the treatment of several ailments. In folk medicine, various indigenous drugs are used in single and/or in combined forms for treating different types of inflammatory and arthritic conditions with considerable success. Although the use of these drugs has a sound tradition and their medicinal uses and general safety are well known to native people their use has yet to be rationalized in therapeutics using the current methodology. Scientific studies are therefore required to assess their safety and efficacy [30]. It has become imperative to scrutinize herbal products for evaluating their acclaimed properties as recently numbers of herbal products are being introduced in the market. Keeping this view we have attempted to study the PHF for its analgesic activity in experimental induced animal models of pain. Pain is associated with various clinical conditions like arthritis, cancer and vascular diseases [31, 32]. PHF was evaluated for its analgesic activity in animal models. A significant ($p<0.001$) analgesic activity was observed for PHP in acetic acid induced writhing and hot plate methods. In the present study, PHF demonstrated a significant ($p<0.001$) analgesic activity at different dose levels in various animal models of pain. Acetic acid induced. Writhing is a sensitive method for screening peripheral analgesic effect of compounds. The stimulation of peritoneal nociceptors is indirect and occurs through the release of endogenous substances which stimulate nerve endings [33, 34]. A great increase occurs in concentration of PGE2 and PGF2a in the peritoneal fluid after acetic acid injection and the analgesic effect of substances similar to diclofenac could be due to the blockade of prostaglandin synthesis [35, 36]. In our study, PHF (250 and 300 mg/kg. p.o.) significantly (p 0.001) reduced the number of writhing induced by acetic Acid.

CONCLUSION

The present study indicates that PHF has significant analgesic properties. Thus, it can be concluded that PHF possess analgesic property which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanism and may have a potential benefit for the management of pain disorders**.**

REFERENCES

1. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine, 2001; 8:401-9.
2. Caius,J.F. The medicinal and poisonous plant of India: Scientific publishers. 1992; 457-458.
3. Kirtikar,K.R., Mayor and B.D. Basu. Indian medicinal plants. International book distributor. 1987; 1:335-336,
4. Barros L, Heleno SA, Carvalho AM, Ferreira IC: Systematic evaluation of the antioxidant potential of different parts of Foeniculum vulgare Mill. from Portugal. Food Chem Toxicol. 2009; 47(10):2458-2464.
5. Hilmi Y, Abushama MF, Abdalgadir H, Khalid A, Khalid H: A study of antioxidant activity, enzymatic inhibition and in vitro toxicity of selected traditional Sudanese plants with anti- diabetic potential. BMC Complement Altern Med. 2014; 14:149.
6. Gengiah K, Hari R, Anbu J: Antidiabetic antihyperlipidemic and hepato-protective effect of Gluconorm-5: A polyherbal formulation in steptozotocin induced hyperglycemic rats. Anc Sci Life. 2014; 34(1):23-32.
7. Choi EM, Hwang JK: Antiinflammatory, analgesic and antioxidant activities of the fruit of Foeniculum vulgare. Fitoterapia. 2004; 75(6):557-565.
8. Modaress Nejad V, Asadipour M: Comparison of the effectiveness of fennel and mefenamic acid on pain intensity in dysmenorrhoea. East Mediterr Health J. 2006; 12(3-4):423-427.
9. Lee JH, Lee DU, Kim YS, Kim HP: 5-Lipoxygenase Inhibition of the Fructus of Foeniculum vulgare and Its Constituents. Biomol Ther (Seoul). 2012; 20(1):113-117.
10. Bhattacharjee SK, Handbook of medicinal plants, Pointer publication, Jaipur, 2001; 3rd ed, 284.
11. Rastogi RP, Mehrotra BN, Compendium of Indian Medicinal Plants: A CDRI Series, Lucknow, Publication and information Directorate, New Delhi, 1995; 4th vol, 597.
12. Hussain A, Virmani OP, Dictionary of Indian Medicinal Plants, Central Institute of Medicinal And Aromatic plants, Lucknow, 1992; 1st ed, 376.
13. Kritikar KR, Basu BD, Indian Medicinal Plants, 1987; 2nd ed, 910-912.
14. R.V.M.Manuikandar, V.Rajesh, R.Sambathkumar, P.Perumal,C.Davis raj, Journal of pharmacy research 2009; 2(4):660-662.
15. Anonymous. The wealth of india. Council of scientific and industrial research, New Delhi, India. 1952:35-6.
16. Mandal SC, Tapan K, Maity J, Das M, Pal M, Saha BP. Hepatoprotective activity of Ficus racemosa leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother. Res. 1999; 13(5):430- 2.