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Analgesic activity of poly herbal formulation in experimental rats by acetic acid induced writhing test model and Hot plate model

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ABSTRACT

To evaluate analgesic activity of a polyherbal formulation-PHF [hydro-alcoholic extract of *Hibiscus rosa-sinensis (50mg), Fennel seeds (50mg), Prosopis cineraria (50mg), & Ficus racemosa (50mg)*] compare it with Diclofenac Na by using Eddys hot plate and writhing test in Adult Wistar rats. Rats were divided into four groups of 6 each for both tests. PHF (250, 300 mg/kg, p.o. body weight) and Diclofenac Na (50 mg/kg, p.o.) made as suspensions prepared in 1% carboxy methyl cellulose (control) and were fed to rats orally. The physicochemical evaluations carried out in terms of loss on drying, ash value, extractive values and acid insoluble ash value ect. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence 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. In Eddys hot plate method, they were placed individually on hot plate maintained at a temperature of 55 ± 0.5 ºC. The latency to lick the paw (reaction time) was noted at 0, 30, 60, 90 and 120 min. The cut off time was set at 20 sec to avoid damage to the skin. In acetic acid writhing method, PHF (250, 300 mg/kg, p. o.) significantly ($p < 0.001$) decreased the number of writhing 39±1.55*, 29.0±0.43*resp. Maximum percentage of inhibition of writhing response shown by Diclofenac Na was 73.03 %. In hot plate method, PHF showed a significant increase in the elevated basal reaction time at 30, 60, 90 and 120 min. 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*

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INTRODUCTION

Current management of fever, pain and inflammatory diseases is limited to the use of nonsteroidal and steroidal 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. One of the basic tenets of herbal medicine is that interactions between different constituents occur, enhancing activity or reducing the likelihood of adverse effects. Such interactions may be additive or truly synergistic in that compounds interact to produce an effect greater than the sum of the individual contribution of each. Although difficult to establish, true synergy between herbal constituents has been documented experimentally [1]. 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 commonly 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 'mouri' 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]. Antiinflammatory, 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 bark 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, prosogerin 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 the traditional system of medicine in India. Apart from the usage in traditional medicine, scientific studies indicate *F. racemosa* to possess various biological effects such as hepatoprotective, chemopreventive, antidiabetic, antiinflammatory, antipyretic, antitussive and antidiuretic [16-22]. The objective of our study was to evaluate the efficacy of poly-herbal 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 HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich 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 40^0C 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 rats of weighing 150-200g 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 rat's 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 on 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 Rats.

Acute Toxicity Studies

PHF was studied for acute oral toxicity as per revised OECD guidelines No. 423 [26]. The extract was devoid of any toxicity in rats when given in doses up to 5000 mg/kg by oral route. Hence 250 and 300 mg/kg doses of extract were used for the study.

Analgesic Activity

Acetic Acid Induced Writhing Method

In this method, rats in groups of four each were treated with vehicle, PHF (250 and 300 mg/kg, p.o.) and Diclofenac Na (50 mg/kg, P. o.). Analgesic activity of PHF was assessed by counting the number of writhes induced by 0.7% acetic acid (10 ml/kg i.p.) Number of writhes per animal was counted in the following 30min. Percentage protection against writhing was taken as an index of analgesia [27,28].

Hot Plate Method

Rats in groups of four each were treated with vehicle, Diclofenac Na (50 mg/kg, p.o.), PHF (250 and 300 mg/kg, p.o.). They were placed individually on hot plate maintained at a temperature of 55 ± 0.5 ºC .The latency to the paws was the reaction time. The reaction time was noted at 0, 30, 45, 60, 90 and 120 min. The cut off time was set at 20 sec to avoid damage to the skin [29,30].

RESULTS

The crude extracts so obtained after soxhlet extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. 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 plants extracts.

S. No	Extracts	Yield (gm)	Perceentage Yield
1	***(T1)***	16.801	15.05%
2	***(T2)***	12.502	10.30%
3	***(T3)***	14.200	13.25%
4	***(T4)***	15.020	14.65%

Where is:- (T1)= Hibiscus rosa-sinensis, (T2)= Fennel seeds , (T3) = Prosopis cineraria, (T4)= Ficus racemosa

The physical constituent's 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.

S. No.	Parameters	Observation (%)			
		(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	4.09
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	1.05 cm	1.15 cm	0.82 cm	0.6 cm

Where is:-* (T1)*= Hibiscus rosa-sinensis,* (T2)*= Fennel seeds ,* (T3) *= 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 3: Phytochemical screening of hydro - alcoholic extract of all plants extracts.

S. No	Identification Test	Test name	***(T1)***	***(T2)***	***(T3)***	***(T4)***
1	Alkaloids	Mayer's test	+	-	-	+
		Dragendroff's test	+	-	+	-
		Wagner's test	+	+	+	-
2	Glycosides	Killer-killani test	-	+	+	-
3	Carbohydrates	Molisch's test	+	-	+	-
		Fehling test	-	-	-	+
4	Tannins & Phenols	Gelatin test	+	+	+	+
		Ferric chloride test	-	+	+	-
5	Flavonoids	Shinoda test	+	+	+	+
		Alkaline reagent test	+	+	+	+
6	Steroids	Libermamm-Burchard test	-	+	+	-
		Salkowski test	+	+	-	+
7	Saponins	Foam test	+	+	-	-
8	Protein	Xanthoprotic	+	-	+	+
9	Gums & Musilage	With 95% Elcohol	-	-	-	+

***Where is:- (T1)= Hibiscus rosa-sinensis, (T2)= Fennel seeds , (T3) = Prosopis cineraria, (T4)= Ficus racemosa* , (+) = Present, (–) = Absent**

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 p.o.) 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 / 30 mins	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.

Hot Plate Method

In vehicle treated mice, elevated basal reaction time in hot plate test was 4.89 ± 0.34 sec. Diclofenac Na (50 mg/kg p.o.) increased the elevated basal reaction time to 16.0 ±0.19.The PHF (250, and 300 mg/kg, p.o.) showed a significant increase in the elevated basal reaction time at 30, 60, 90, and 120 min. The observations are given in Table 5.

Table 5 Effect of PHF (250 and 300 mg/kg, p.o.) on reaction time in hot plate test in mice.

			Reaction time in seconds (20 secs)				
Group Name	**Treatment**	**Dose**	0 min	30 min	60 min	90 min	120 min
Disease Control	Control	1% CMC, p.o.	2.14 ± 0.25	3.21 ± 0.04	4.99 ± 0.12	4.51 ± 0.34	4.89 ± 0.34
Standard	Diclofenac Na	50 mg/kg, p.o.	4.05 ±0.33	7.1 ± 0.03*	13.2 ±0.35*	14.9 ±0.36*	16.0 ±0.19*
Test-1	Poly-herbal formulation Extracts	250 mg /kg, p.o.	7.3 ± 0.17*	11.4 ± 0.58	12.9 ± 0.63*	13.43 ± 0.54*	14.9 ± 0.21*
Test-2	Poly-herbal formulation Extracts	300 mg/kg p.o.	8.02 ±0.31	11.6 ±0.61*	14.7 ±0.34*	14.29±0.27*	16.8 ± 0.33*

Values are expressed as mean ± SEM. *P <0.01, *P < 0.001 (N= 6) Values are mean ±SEM, of six animals in each group.

DISCUSSIONS

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 PHF 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. The hot plate method originally described by Woolfe and Mac Donald, 1994 has been found to be suitable for the evaluation of centrally but not peripherally acting analgesics. It involves higher brain functions and consists of responses to nociceptive stimuli organized at a supraspinal level [37].The nociceoptors seem to be sensitized by sensory nerves. In our study, PHF (250 and 300 mg/kg, p.o.) significantly (p<0.001) elevated the mean basal reaction time in hot plate method. All plant may prove superior to Polyherbal formulation for its better standardization, quality control, safety profile, easy availability and low cost.

CONCLUSION

The present study indicates that PHF has significant analgesic properties. Thus, it can be concluded that PHF posse's 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.

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