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Investigation of analgesic and anti-inflammatory activities of poly herbal formulation

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ABSTRACT

Hibiscus tiliaceus Linn. (Malvaceae) is a fast growing tree commonly grows along the seashore and back mangroves of China, Iran, India, Malaysia and South Africa. H. tiliaceus leaves are used to treat fever cough and the fresh roots are used to treat dysentery, microbial infection, skin boils and chest congestion. The present study was aimed to investigate the analgesic and anti-inflammatory activities of poly herbal formulation. The plants leaves were collected and extracted with petroleum ether using soxhlet apparatus, the extracts were tested for its phyto constituents. There different ointments were prepared using the extracts and tested for the physico-chemical evaluation. The analgesic activity was tested by using the Tail immersion Method and anti-inflammatory activity was tested by using the phlogistic agents-induced paw edema. The result showed that Tail immersion Method-F3 formulation showed good results for analgesic anti-inflammatory action compared to the standard. In the anti-inflammatory method the F3 formulation showed good anti-inflammatory activity.

Keywords: Hibiscus tiliaceus Linn, H. tiliaceus, Phlogistic agents, Edema, Analgesic.

INTRODUCTION

There are many opportunities existing in the global market for Ayurveda. India with its enriched traditional knowledge, biodiversity and the manpower has the potential required for globalization of Ayurveda. Knowledge of Ayurveda and experimental database can provide new functional leads to reduce time, toxicity and money as the three main problems in drug development process. Knowledge about the herbal drugs and their experimental database can provide researcher with new lead molecules that reduces time, toxicity and expenditure in drug development.

In present worldwide scenario, natural medicines are gaining prominence, as they are more economical, easily available and relatively free from most of the side effects. The increased demand of the polyherbal formulation and neutraceuticals is reflective of positive impact of consolidated efforts which aimed at reviving science of phytopharmacy. A large number of Indian medicinal plants are attributed with various therapeutic activities, because it contains diversified class of chemical constituents. It is believed that current analgesia-inducing drug such as opiates and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their

side effects and lower potency**.** For example in case of morphine acute morphine poisoning, hypotension and drug dependence is more prevalent part. As a result, a search for different alternatives seems to be necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel analgesic agents could therefore be found out [1].

Traditionally herbal medicines provide an interesting, largely unexplored range of potential new drugs. It is of great interest to know whether plants used in folk medicines have potential effect in curing human ailments. Since, inflammation is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells. If they are not controlled, may lead to development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and so forth. Hibiscus tiliaceus Linn. (Malvaceae) is a fast growing tree commonly grows along the seashore and back mangroves of China, Iran, India, Malaysia and South Africa. H. tiliaceus leaves are used to treat fever cough and the fresh roots are used to treat dysentery, microbial infection, skin boils and chest congestion. Fresh flower boils in milk is used to treat ear infections. It is also used as anticonvulsive, anti phlogistic, diuretic, antioxidants, hepatoprotective and hypoglycaemic [2]. The present study was planned to evaluate the analgesic and anti-inflammatory activities of poly herbal formulation.

MATERIALS AND METHODS

Collection & extraction of plant material

The leaves of Hibiscus tiliaceus Linn. & Hibiscus cannabinus L were collected; plant material was dried in shade, powdered, and stored suitably. By the Continuous hot extraction (Soxhalation) - Successive solvent extraction of, Hibiscus tiliaceus, and Hibiscus cannabinus leaves powder was carried out using petroleum ether as a solvent. Extracts were tested for it phyto constituents.

Acute toxicity testing

The Acute toxicity evaluation of Polyherbal extract was performed on the principle of OECD guidelines 423 by using rat and fixed dose studies were selected where the limit dose is 2000 mg/kg. In Acute toxicity study, suspensions of petroleum ether extracts of Hibiscus leaves were made by utilizing 2% acacia solution. Female wistar rats of albino strain 180-220 g were utilized for the current research. Four sets of animals (3 rats in individual set) have been availed in the research. For a period of 18 hrs the rats were starved & body weights were measured and water was withdrawn prior to 4 hrs of dosing. The rats were administered 5 mg/kg dose of PHE and if no mortality is seen within 24 hrs, another group of rats were served with 50 mg/kg. In the related way if mortality is not observed with 50 mg/kg. Further 300 mg/kg is administered to third set of rats. In case of absence of mortality after one day, further the dosing is increased to an extent of 2000 mg/kg to various sets of rats. Individual rats were monitored for 4 hrs for behaviour, autonomic and neurological symptoms or mortality. Body weights were registered 6 hrs post dosing. Following next day, every 1 hr (each Day), behavioural difference, toxic signs or death rate was monitored in the same animals for 1 week and on 8^{th} and 14^{th} day of dosage b.w's were noted. If fatality is not observed, $1/5^{th}$ and $1/10^{th}$ of higher dose was selected as therapeutic dose [3].

PREPARATION OF OINTMENTS

The ointment base was prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointments as F1 to F3 was done by incorporating the active extracts in the base by trituration using mortar and pestle to obtain 100 gm of herbal ointments containing crude extracts. The prepared ointments were filled in tubes, labeled and were stored at room temperature. The most potent extracts for both plants were selected for preparation of ointment. Extracts in different concentrations were used as below [3].

Table 1: Formula for Ointment

S. No	Extract used	Quantity of extract in gm for given Formulation		
		F1	F2	F3
1	Petroleum Ether Extract	0.15 g	-	-
2	Petroleum Ether Extract	-	0.3 g	-
3	Petroleum Ether Extract	-	-	0.45 g
4	Ointment base	100g	100g	100g

PHYSICO-CHEMICAL EVALUATION OF OINTMENT

Accelerated stability study

Accelerated stability study was carried out of ointment formulations at 8°c and 45°c and stability was carried for one month. The different parameter such as colour, odor, texture, traces of gritty particles, skin irritation test were studied for all formulation at 1st month.

Extrudability

A closed collapsible tube containing ointment was pressed firmly at the crimped end. When the cap was removed, ointment extruded until pressure dissipated. Weight in grams required to extrude 0.5 cm ribbon of ointment in 10 seconds was determined

Spreadability test

Spreadability is expressed in terms of time in seconds taken by two slides to flip off from cream when placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the Spreadability. It is calculated by using the formula: S=M x L/T, where M=Weight tied to upper slide, S= Spreadability of formulation, L=length of glass slides, T=time taken to separate the slides.

Viscosity

Viscosity of ointments was measured by using Brookfield viscometer with spindle #7.

Colour and odour

Colour and odour of all ointments was examined by visual examination.

PH

The pH of ointment formulations was determined by using Digital pH meter. One gram of ointment was dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were depicted.

Screening of ointment for analgesic anti-inflammatory action

Ointment formulated was evaluated for its analgesic anti-inflammatory action using various screening models as given below. In all the models the ointment shows significant effects for the activity compared to the standard drugs available in market.

ANALGESIC METHOD

Tail immersion Method

Thirty albino rats were randomly divided into five groups with six rats each, all rats were fasted for 12 hours with clean drinking water provided ad libitum. The animals were pre-treated 60 minutes before tail immersion with 10ml/kg 2% acacia for group A (control), 400 mg/kg acetylsalicylate acid (aspirin) for group B (Standard) and 150, 300 and 450 mg/kg of F1, F2, F3 for groups C, and D & E respectively. Then about 2-3cm of the tail of each of the rat was dipped into a water bath containing warm water maintained at a temperature of 50 ± 1°C and the time taken for the mice to flick its tail or withdraw it from the warm water known as the pain reaction time (PRT) was recorded for all the rats. The cut off time was put at 15 seconds [5].

ANTI-INFLAMMATORY ACTION

Phlogistic agents-induced paw edema

The rats were divided into five groups of six each. Acute inflammation was induced by intraplantar administration of 0.1 ml of carrageenan (1%). Rats were treated with either vehicle or

ointment preparations form F1 to F3 one hr before administration of phlogistic agent was applied to the plantar surface of the hind paw by gentle rubbing 50 times with the index finger. The paw volume was measured prior to injection of phlogistic agent (0 h) and then at predetermined interval for each agent. For carrageenan interval was of 3h. Paw volume was measured using digital plethysmometer. Change in the paw volume was measured and anti-inflammatory activity was calculated by using the formula

% Inhibition of inflammation = 1-(Vt/Vc) ×100

Where, Vt represents the change in the paw volume in ointment treated group and Vc represents the change in the paw volume in the corresponding vehicle treated control group [6].

RESULT

Preliminary phytochemical screening

The preliminary phyto constituents study revealed the presence of different phyto constituents as mentioned in the table: 2.

Table 2: Preliminary Phytochemical screening of pet Ether extract of leaves of Hibiscus tiliaceus & Hibiscus cannabinus

Test for constituent	Hibiscus tiliaceus	Hibiscus cannabinus
Alkaloids	++	++
Tannins	++	--
Flavonoids	++	++
Glycosides	--	++
Carbohydrates	++	++
Steroids	--	++
Saponins	++	--
Proteins	++	++

++ = Presence of constituent; -- = Absence of constituent

ACUTE TOXICITY STUDY

The Polyherbal extract (PHE) was administered to female rats at doses 5, 50, 300 & 2000 mg/kg with oral syringe did not display any symptoms of toxicity. The rats were examined for two weeks, twice in a day has not exhibited toxic signs. Hence oral LD50 of PHE was found to exceed 2000 mg/kg. Therefore 2000 mg/kg was considered as safest higher dose for Polyherbal extract and 1/10th i.e 200 mg/kg (lower dose) and 1/5th of 2000 mg/kg i.e 400 mg/kg (higher dose) was selected for the Pharmacological studies.

PHYSICO-CHEMICAL EVALUATION PARAMETERS FOR OINTMENTS

Accelerated stability study- The ointment was found to be physically stable at different temperatures. There were no changes in the Spreadability, diffusion and irritant effect even after the exposure to different temperatures. For 01 month stability study at different temperature conditions viz. room temperature, 8°c and 45°c, different parameters were studied and results observed as below. [Table 3].

Table 3: Evaluation of Physico-chemical parameters for different Ointment Formulations

S. No.	Parameter	Ointment Formulations		
		F1	F2	F3
1	Nature	Semisolid	Semisolid	Semisolid
2	Color	Green	Green	Green
3	Odor	Characteristic	Characteristic	Characteristic
4	Texture	Gummy	Gummy	Gummy
5	Trace of gritty particles	No	No	No
6	Skin irritation	No	No	No

Extrudability- The ointment showed good extrudability. Spreadability test- The ointment readily spread when applied on the skin topically and rubbed gently. Viscosity- Soft semisolid. pH - 6.2

SCREENING OF OINTMENT FOR ANALGESIC AND ANTI-INFLAMMATORY ACTION

Analgesic activity

Tail Immersion Method

All the test and standard drugs significantly ($p<0.001$) reduce the pain as compare to the control group. By applying Student Newman-Keuls test, it was shown that there is significant ($p<0.01$) effect of F-1, F-2 & F3 as compare to the Standard at 15 minutes and there is significant ($p<0.05$) effect of F-3 and standard group. Tail immersion method-F3 formulation showed good results for analgesic anti-inflammatory action compared to the standard.

Table 4: Effect of various ointment formulations on thermic stimulus-induced pain in rat (Tail immersion method)

Group	Treatment	Dose (mg/kg)	Mean Pre drug reaction time	Mean Post drug reaction time
A (Control)	2% acacia	10ml/kg	-	-
B (Standard)	Aspirin	400 mg/kg	2.57±0.54	4.07±0.38***
C	F1	150 mg/kg	2.31±0.60	3.21±0.51**
D	F2	300 mg/kg	1.38±0.16	3.53±1.16**
E	F3	450 mg/kg	1.66±0.12	3.89±0.28***

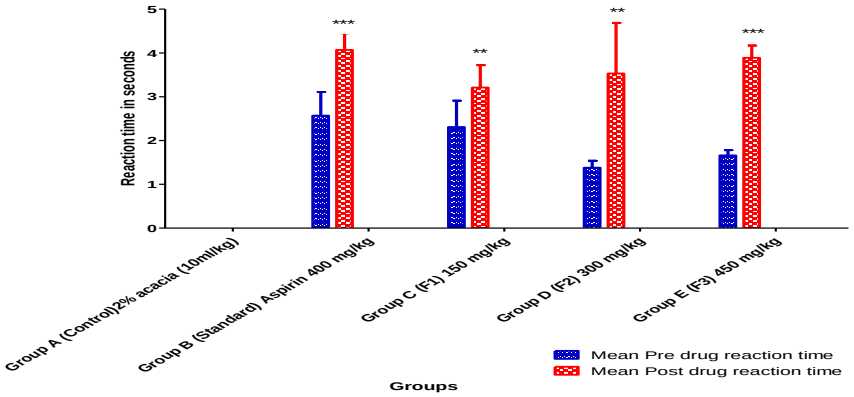


Figure 1: Effect of various ointment formulations on thermic stimulus-induced pain in rat (Tail immersion method)

ANTI- INFLAMMATORYACTION

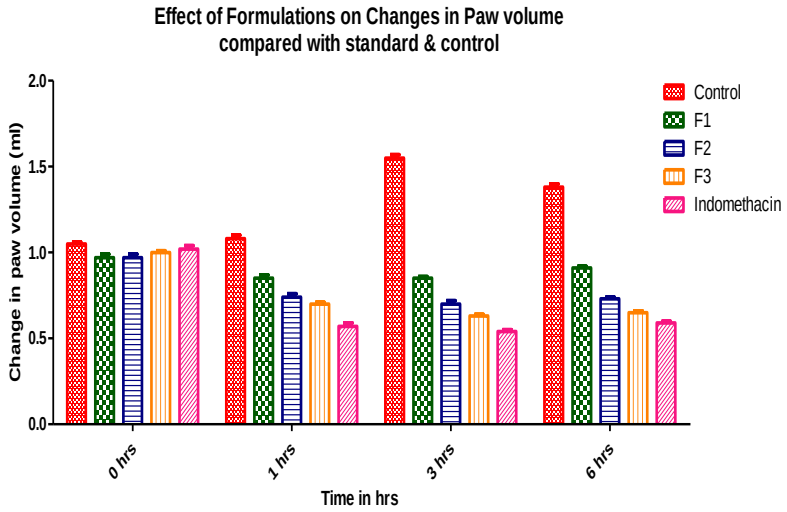
Phlogistic -induced paw edema

At the end of the 6 hour the F3 formulation showed significant reduction in the paw edema 0.65±0.01 ml, which was comparable to the standard 0.59±0.01 (70.31). There by the F3 formulation showed good anti-inflammatory activity.

Table 5 Effect of various ointment formulations on phlogistic agent induced rat paw edema

Groups	Change in paw volume (ml) mean ± SEM and (%inhibition)			
	0 hr	1 hr	3 hr	6hr
Control	1.05 ±0.01	1.08±0.02	1.55±0.02	1.38±0.02
F1	0.97±0.02 (33.00)	0.85±0.02 (33.00)	0.85±0.01 (36.5)	0.91±0.01 (38.06)
F2	0.97 ±0.02 (33.00)	0.74±0.02(33.00)	0.70±0.02 (36.5)	0.73±0.01 (38.06)
F3	1.00±0.01 (29.00)	0.70±0.01(29.00)	0.63±0.01(29.35)	0.65±0.01 (25.00)
Indomethacin	1.02±0.02 (64.00)	0.57±0.02(64.00)	0.54±0.01(66.07)	0.59±0.01 (70.31)

All values are mean ±SEM (n=6); *p< 0.05 when compared to control.



Graph 5.2- Effect of various ointment formulations on phlogistic agent induced rat paw edema

DISCUSSIONS

According to a report by World Health Organization (WHO), the use of herbal medicines is in increasing trend in both developing and industrialized countries. Considering the fact that over one-third of the population in developing countries lack access to essential medicines and the provision of safe and effective traditional therapies could become a critical tool to increase access to health care, WHO launched its first ever comprehensive traditional medicine strategy in 2002 [7].

Botanical authentication of a plant depends entirely on microscopically and microscopically characters. Each crude drug derived from the vegetable kingdom consists of a definite part of the plant.

The extracts obtained after extraction was characterized by preliminary phytochemical test for rough ideas of main constituents present in extracts. Petroleum ether extracts showed presence of flavonoids, alkaloids, tannins, carbohydrates and proteins.

Herbal ointments of extracts of each plant using were prepared using simple ointment base by fusion method. We used the on phlogistic agent induced rat paw edema model of inflammation to study the interaction between cytokines, prostaglandin E2 (PGE2) and cell migration during the various phases of acute local inflammation induced by carrageenan.

In case of analgesia, prostaglandins and bradykinin were suggested to play an important role in the pain process. Some sterols and triterpenes are responsible for analgesic activity. As phytochemical tests showed presence of these constituents in petroleum ether extracts, they may be responsible for the activity.

CONCLUSION

It has concluded that the resulted Phytochemical screening of Hibiscus tiliaceus leaves extract shows presence of Alkaloids, Tannins, Flavonoids,

Carbohydrates, Saponins and Proteins. The resulted Phytochemical screening of Hibiscus cannabinus leaves extract shows presence of Alkaloids, Tannins, Carbohydrates, Sterols, Proteins and Saponins. The standardized extracts of Hibiscus tiliaceus and Hibiscus cannabinus Linn. Plant parts were formulated in ointment. The analytical parameters signified that the ointment is of good quality, purity and safety. The ointment of formulation F3 containing extracts of Hibiscus tiliaceus and Hibiscus cannabinus Linn. A plant part possesses significant analgesic and anti-inflammatory effect in animal models compared to standard formulation. Beta-Sitosterol is present in the leaves of Hibiscus cannabinus Linn and is reported to possess analgesic and anti-inflammatory activity. From the study it is confirmed that Beta-Sitosterol along with other compounds present in the plants are responsible for analgesic and anti-inflammatory activity. The data presented in this study demonstrate that Hibiscus tiliaceus and Hibiscus cannabinus Linn. Plants parts extract in the form of ointment possess significant topical analgesic and anti-inflammatory properties, supporting their traditional use for the treatment.

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