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Evaluation Of Analgesic And Anti-Inflammatory Activity Of Herbomineral Formulation Sandhivaataari Gutika

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ABSTRACT

Analgesic and anti- inflammatory activity for herbomineral formulation was evaluated by using hot plate method. Crude extract of herbomineral formulation was prepared by using tween 80.2 % crude drugs which is present in the form of suspension was administered through oral route at a dose of 100 mg/kg, 200 mg/kg in mice weighing between 20-25 g using oral gavage and evaluated for analgesic activity at different time intervals such as 0, 15, 30, 60 and 90 minutes against standard (Tramadol Hydrochloride 22.8 mg/kg i.p) and control group, $P< 0.05$ was considered as significant. At a dose 100 mg/kg herbomineral formulation had shown significant activity10.00 ± 1.10 ($P < 0.001$), 200 mg/kg had shown significant activity at15, 30, 60 and 90 min time interval ($P<0.001$).

Keywords: Analgesic activity, hot plate, herbomineral formulation, Tramadol hydrochloride

Introduction

Analgesics relieve pain as a symptom, without affecting its cause.Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect, new compounds with improved pain management capacity and fewer side effects are being sought with urgency. The long historical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy. Narcotic analgesics are associated with addictive properties and numerous side effects. The search for pharmacological agents to overcome these shortcomings has become a major goal in pain research. For centuries, medicinal plants are the basis for the treatment of various diseases. Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary healthcare and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants. However, the quality control of herbal medicine remains a challenge owing to the fact that there is a high variability in the active constituents involved.

The drug used for the Analgesic, Anti-infamatory activity is the herbo mineral drug named sandhivaataari gutika which is the combination of three different herbomineral substances. So the drug is said to be herbomineral formulation which posses many therapeutic effects on human body, so, it is previously undergone for the test for toxicity.

Preparation of sandhivaataari gutika

The drugs purified Hingula (Cinnabar), Guggulu (commiphoramukul) and Bola (Commiphora mol mol) are made into powder. This powder is pounded in go dugdha (cow's milk) for one day. Afterwards dried and powdered and made into pills in the dose of 125mg[1].

Preliminary phytochemical screening

Table no: 1

Chemical constituent	Observation[2,3,4,5]
Alkaloids	negative
Carbohydrates:	negative
Proteins	negative
Flavanoids	negative
Cardiac glycosides	positive
Anthra quinone Glycosides	positive
Saponin glycosides	positive
Tannins &Phenols	positive

MATERIALS AND METHODS

S.No.	Equipment	Manufacturer
1.	Borosil Soxhlet extractor	Borosil Manufacture
2.	Solvent evaporator	Chemi Tech
3.	Analgesiometer Eddy's hot plate Digital balance Syringes and needles	INCO ELB 300- SHIMADZU Local Market

Chemicals: Tramadol HCl, Diclofenac sodium .

PROCESS

Borosil Soxhlet Extractor

Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration be used to separate the compound from the insoluble substance. Fruit extraction in progress. The sample is placed in the thimble.

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser.

The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Preparation of extracts

First the powered drug was subjected to extraction. The extracts were prepared by using hot air percolation technique using soxhlet apparatus, a process of extraction of a drug with a solvent with several daily shakings. This method was based on the extraction of active constituents by simple hot air percolation using water as solvent.50g of the powdered material was placed inside a thimble supported by cotton pads which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser.

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask.

After 24 hrs, the water extract was filtered and the marc was repeated two more times with the same solvent for effective extraction. Extract was concentrated by open air drying. And the acquired extract was stored in a desiccators[2,3,4].

A Schematic Representation of Extraction

50g of powder was percolated with 500ml water as solvent for several times

↓

Filtered, extract is concentrated by distill

↓

Dried in desiccators

↓

Resulting material was found to weigh as follows

- Water-30gm

Institutional Animal Ethical Committee clearance

Animals: For the experiment Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g, were procured from Mahaveer Enterprises, Hyderabad, India were used in the studies. Animals were maintained under standard environmental conditions(temperature: (24.0±1.0°), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the Institutional Animal Ethical Committee (IAEC, Clearance/2007/1-8) Vishnu institute of pharmaceutical education and research was taken prior to the experiments.

METHODS

Hot plate method

The animal is placed on the hot plate and the time is measured until the animal starts jumping or licking a paw.

The animals were divided into four groups with six mice in each group. Group I animals received vehicle (normal saline), animals of group II were administered a 22.8mg/kg (body weight i. p. route) dose of standard drug (Tramadol HCl).While animals of Group III and Group IV were treated with 100 and 200 mg/ kg body weight (oral) of the crude extract of herbo mineral formulation (HMF) respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5°C. A cut off period of 15s, was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 15, 30, 60 and 90 min after administration of the samples.

Tail Flick method

The animals were divided into four groups with six mice in each group. Group I animals received vehicle (normal saline), animals of group II were administered a 9mg/kg (body weight i. p. route) dose of standard drug (Diclofenac sodium).While an Group IV were treated with 100 and 200 mg/ kg body weight (oral) of the crude extract of herbomineral formulation (HMF) respectively. The tail flick test is the water immersion method. In that test, approximately one third of the rodent's tail from the tip is immersed into a $52^{O}C$±0.2 water bath and the amount of time until the rodent flicks or removes its tail is recorded at different time intervals of 0, 15, 30, 60, 90 min[5-15].

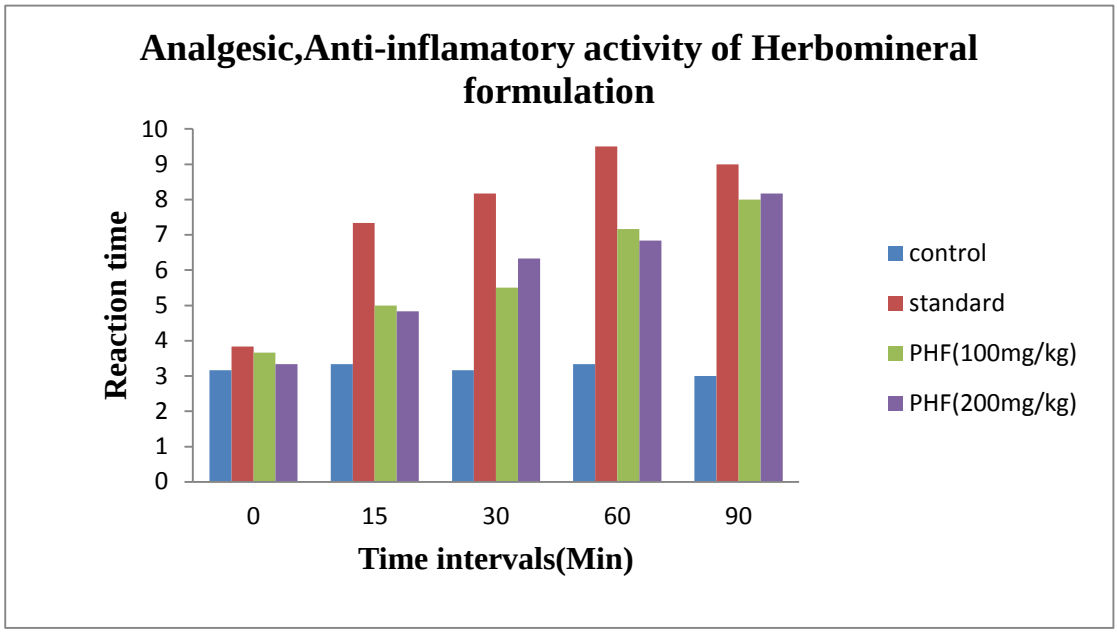
Statistical analysis

All the values expressed as Mean ± SD, n = 6, one way analysis of variance (ANOVA) followed by Dunnet's test. The minimum value of $p < 0.05$ was considered as significant.*$p<0.05$, **$p<0.01$ and ***$p<0.001$ as compared with control group both standard and herbomineral extract treated groups.

RESULTS

Table no: 2 Tail Flick Method

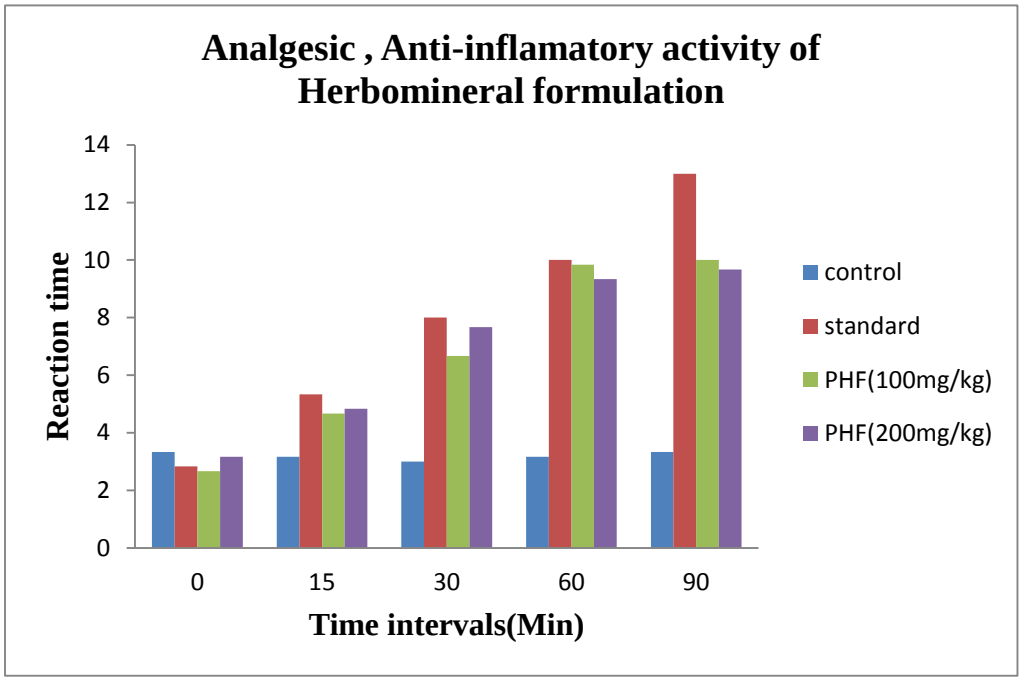
Group	DOSE Mg/Kg	Post Reaction Time (Sec)				
		0 min	15 min	30 min	60 min	90 min
CONTROL		3.17±0.75	3.33±0.52	3.17±0.75	3.33±0.52	3.00±0.89
STD (Diclofenac)	9	3.83±0.75	7.33 ± 1.03 ***	8.17 ± 0.75 ***	9.50 ± 1.05***	9.00± 0.89***
HMF 1	100	3.67±1.21	5.00 ± 0.89*	5.50 ± 1.05**	7.17 ± 0.75***	8.00 ± 2.61***
HMF 2	200	3.33±0.82	4.83±1.47	6.33 ± 1.63***	6.83 ± 0.98***	8.17 ± 1.72***



Effect of sandhivaataari gutica extract on latency to Eddy's hot plate test

Table no: 3

Group	DOSE Mg/Kg	Post Drug Reaction Time (Sec)				
		0 min	15 min	30 min	60 min	90 min
CONTROL		3.33 ± 0.82	3.17 ± 0.75	3.00 ± 0.89	3.17 ± 0.75	3.33 ± 0.82
STD (Tramadol)	22.8	2.83 ± 0.75	5.33 ± 1.03**	8.00 ± 0.89***	10.00 ± 0.89***	13.00 ± 0.89***
HMF 1	100	2.67 ± 0.82	4.67± 0.82*	6.67 ± 0.82***	9.83 ± 0.98***	10.00 ± 1.10***
HMF 2	200	3.17 ± 0.75	4.83± 0.98*	7.67 ± 1.21***	9.33 ± 0.82***	9.67 ± 1.21***



DISCUSSION

The property of Sandhivaataari gutika aqueous extract can also probably due to the blockade of the effects or the synthesis and /or release of PGs and /or other endogenous substances that excite pain nerve endings. The hot plate method test is considered to be selective to examine compounds acting through opoid receptor; the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. Based on the results of the present study, we conclude that the plant extract possesses strong analgesic and Anti -inflammatory potential.

However, further studies are necessary to examine underlying mechanisms of analgesic and anti-inflammatory effects and to isolate the active compounds responsible for these pharmacological activities.

CONCLUSION

Aqueous extract of Sandhivaataari gutika showed significant increase in activity, when compared to standard drug (Tramadol HCL, Diclofenac sodium). Further Studies required identifying the active phytochemical constituents and evaluating their effectiveness in vivo so that they can be synthesized and commercial production begins in earnest.

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