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PHYTOCHEMICAL SCREENING & ANIMAL STUDY OF PANCH PHORON AQUEOUS EXTRACT ON MICE MODEL FOR EVALUATING ANTI-NOCICEPTIVE ACTIVITY

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ABSTRACT

Background: The aim of this investigation to assess if the blend of spice contain bioactive molecules and determine the anti-nociceptive activity of Panch Phoron in mice model. **Methods**: Phytochemical screening by Wagner test, Fehling's test, Benedict's test, foam test, Libermann-Burchard's test, saponification test, lead acetate test, alkaline test, gum and mucilage test of panch phoron performed for the detection of phytochemicals. Inflammation was instigated by intraperitoneal injection of acetic acid to perform the writhing test for assessing the peripheral analgesic activity, and tail flick test was performed to measure the central analgesic activity. **Results:** In the results, panch phoron was found to have bioactive molecules: alkaloids, reducing sugar (ketones), saponins, phytosterols, fixed oils & fats, tannins, flavonoids, gum & mucilage. The analgesic effect of Panch phoron was as good as the standard Diclofenac sodium. Panch phoron (250 mg/kg; p.o.) demonstrated activity of 39.55% when used as aqueous extract while the standard drug Diclofenac Na had an event of 64.91% through the onset of action was slow. **Conclusion**: The presence of bioactive molecules in panch phoron ensures the presence of pharmacological activity. Tail flick and acetic acid-induced writhing test conclude, panch phoron to have both central and peripheral analgesic activity.

KEYWORDS: Panch Phoron, phytochemical, nociceptive activity, aqueous extract.

INTRODUCTION

In the early ages, only traditional medicines were the only remedies consisting of only herbal products. The primary medical system in Europe was Traditional Medication Systems or TMS for centuries.[1] Natural medicinal products referred to as products containing medicinal properties which generally obtained from dried plants, parts of plants and extracts of the plant. According to the definition of Danish law "medicinal products whose active substances are only naturally occurring substances in concentrations that are not significantly higher than those found in the environment.[2] Many drugs existence was made possible due to the presence of natural products — namely, anti-cancer drugs from plants, antiviral medicines from plants, anti-malarial drugs from plants. Morphine a crystalline substance was isolated from opium in 1803/04. Later, cocaine, codeine, digitoxin, quinine, and pilocarpine were isolated from natural sources.[3]

In the early 1900s, roots, barks, and leaves were the sources of 80% medicine available then and 25% medicine in the present day are form the same. 300,000-400,000 or higher species are an excellent source for new chemical entities [NES]. In recent times especially since from late 1990 and early 2000, there has been a decline in deriving medicinal products from plants mostly due to the increase in automated high throughput screening (HTS) programs.[4] This increases the speed of screening molecules for bioactivity and HTS could not screen most natural products. Advancement in molecular biology, cellular biology, and genomics.[4]

Panch Phoron is the mix of five spices: the green of fennel seed, black mustard and nigella seeds, golden fenugreek and cumin seeds. It used in South Asian cuisine for flavoring; however, in recent years many health benefits of Panch Phoron has been identified such as anti-diabetes, anti-hypertensive, antimicrobial, anticancer, antioxidant activity.[5]

To our best knowledge, the pharmacological properties have not been studied extensively so far.[6] Therefore this study was conceptualized to test their combined analgesic activity and phytochemical investigation of Panch Phoron. The objectives of the present research are The phytochemical properties of aqueous extract of Panch Phoron and to evaluate the analgesic or anti-nociceptive activities of aqueous extract of Panch Phoron using experimental animal study.

METHODOLOGY

The spices selected for our work and various chemical investigation are: Nigella Sativa; Brassica Nigra; Cuminum Cyminum; Trigonella Foenum-Graecum; Foeniculum Vulgare.[7-11] All the spices were collected from a local market of Mymensingh district, Bangladesh in October 2018. Spices with good quality were collected.

Distilled water, acetic acid, standard diclofenac sodium powder obtained from the chemical storehouse of the department of pharmaceutical sciences, north south university. All the reagents used were analytical grade. The acetic acid solution according to the protocol were freshly prepared for every acetic acid-induced study.

Phytochemical tests for finding alkaloids, flavonoids, saponins, tannin, gums, reducing sugar and terpenoids were carried out for all the extracts by the method explained by Harborne and Sazada.[12] All the phytochemical screening of the extract was performed using the suggested protocols, reagents and chemicals in previous study. Dragendroff's test used for alkaloids detection. Aqeous extract of the Panch Phoron (2 ml) and dilute hydrochloric acid (0.2 ml) were taken in a test tube. After adding 1 ml of Dragendroff's reagent, orange brown precipitate ensured the presence of alkaloids. Flavonoids were detected by adding a few drops of concentrated HCL to a small amount of extract solution. Appearance of a red color indicated the presence of flavonoids. Tannins were detected using the ferric chloride test. Aqueous extract (0.5 g) was dissolved in distilled water (5 to 10 ml) and filtered. Very few drops of 5% ferric chloride solution were added to the filtrate. Presence of tannins was detected and confirmed by a greenish black precipitate during the test. Dilute the plant extract (1ml) with distilled water (20ml) and shake in a graduated cylinder for 15 minutes to identify saponins. The foam layer (about 1 cm) indicated the saponin presence. To detect terpenoids, the Salkow ski test was used. Extract (5ml) was mixed with chloroform (2ml) and was carefully added to form a layer of concentrated sulphuric acid (3 ml). For the nearness of terpenoids, a ruddy dark colored shading of the bury face was shaped to indicate positive outcomes. To identify the presence of gum in the example, Molisch test was performed. Extract (5 ml) was blended with the sulphuric corrosive and the reagent of Molisch. At the intersection of two fluids, the presence of red violet ring showed the nearness of gums. The trial of Fehling was utilized to identify the nearness of sugar decrease. In a test tube were included 1 ml of Fehling's an answer and 1 ml of Fehling's ' B solution. For a moment these blended arrangements were cooked. At that point there was included a similar sum (2 ml) of

the test arrangement. It was seen that block red encourage affirmed the nearness of reducing sugar.[13]

ANIMAL MODEL

Studies were carried out using 40 male Swiss Albino mice weighing 20-30g, 4 to 6 weeks old. They were obtained from Animal Production Unit of Animal House at the Department of Pharmaceutical Sciences, North South University, and kept in individual cages at room temperature of 25±3° Celsius with 12 hours dark/light cycles. They have free access to standard laboratory feed (pellet food crushed to a coarse powder) and water, according to study protocol approved by the Ethical Committee of Department of Pharmaceutical Sciences, North South University, for animal care and experimentation.

Mice were equally divided into control group, positive control, and two different diseases + treatment (extract) groups (10 mice in each Group) : Group I (n=10) serving as a disease + treatment (extract) group with 100 mg/kg body weight per dose of Panch Phoron aqueous extract; Group II (n=10) serving as a disease + treatment (extract) group with 250 mg/kg body weight per dose of Panch Phoron aqueous extract. Every individual group studies were repeated 3 times at 3 days interval. The animal was checked for the weight, food and water intake on a daily basis. Daily body weights were documented regularly. Beds of each mice cages were cleaned at 2 days interval.

The antinociceptive or analgesic properties of Panch Phoron extract was assessed by the tail flick method and the in mice.[3] Control group treated with distilled water (1ml each), the positive control group was treated with standard diclofenac solution (25mg/kg, p.o), the group-I treated with extract (100mg/kg, p.o), and group II treated with extract (250mg/kg, p.o). The pain inhibition percentage (PIP)[4] calculated as follows: Pain inhibition percentage or (PIP) = ((T1-T0)/T0) x 100, Where T1 is post-drug latency and T0 is pre-drug latency. The maximum possible analgesia (MPA) calculated as: (The reaction time - water reaction time) ÷ (10 - water reaction time).

Acetic acid (3%, v/v) was administered intraperitoneally to all the groups at the dose of 0.2 ml 60 min after the oral administration of test (aqueous solution, diclofenac sodium solution, and extract solution) solution. Anti-nociception recorded by counting the number of writhes after the injection of acetic acid for 10 min. Writhe indicated by abdominal constriction and full extension of the hind limb.

RESULT

The data were expressed as mean ± SEM of 10 animals. Results were analyzed statistically by One-way ANOVA test. The difference was considered significant if $p<0.05$.

Table 1: Phytochemical screening of aqueous extract of panch phoron revealed the presence of the various chemical components were the most prominent and the result of phytochemical tests have been summarized below.

Phytochemicals	Presence in Aqueous Extract
Alkaloids	++
Reducing Sugar (Aldehydes)	-
Reducing Sugar (Ketones)	+
Saponins	++
Phytosterols	+
Fixed oils & Fats	++
Tannins	++
Flavonoids	++
Gum & Mucilage	++

Symbol (++) indicates the presence in high concentration; Symbol (+) indicates the presence in moderate or low concentration; Symbol (-) indicates the absence of the constituents.

Anti-nociceptive activity of aqueous extract of Panch phoron revealed the presence of analgesic activity was prominent and the result of anti-nociceptive tests have been summarized in the following tables:

Table 2: Effect of Panch Phoron aqueous extract in mice observed in the hot Tail flick test.

Treatment	Dose	Response Time (sec)		
		0 min	30 min	60 min
Control (water)	1 ml	2.522±0.105	2.208±0.106	1.878±0.159
Positive Control	25	3.353±0.118	4.328±0.098	4.372±0.112
(Diclofenac Na)	mg/kg	(MPA 0.111)	(MPA 0.272)	(MPA 0.307)
	100	2.598±0.103	3.082±0.096	4.085±0.145
Panch Phoron Extract	250	2.629±0.196	3.883±0.082	5.087±0.06
	mg/kg	(MPA 0.014)	(MPA 0.215)	(MPA 0.395)

Each data represents the latency of nociceptive response (sec) ± SEM (n = 10). $p < 0.05$ compared with the control group (ANOVA Test).

Table 3: Pain inhibition percentage (PIP) = ((T1-T0)/T0) x100 T1 is post-drug latency and T0 is pre-drug latency.

PIP of Positive Control	00 min	= ((3.353-2.522)/2.522) × 100 = 32.95%
	30 min	= ((4.328-2.208)/2.408) × 100 = 79.73%
	60 min	= ((4.372-1.878)/2.878) × 100 = 51.91%
PIP of Extract (100 mg/kg)	00 min	= ((2.598-2.522)/2.522) × 100 = 3.01%
	30 min	= ((3.082-2.208)/2.208) × 100 = 39.58%
	60 min	= ((4.085-1.878)/2.878) × 100 = 41.93%
PIP of Extract (250 mg/kg)	00 min	= ((2.629-2.522)/2.629) × 100 = 4.24%
	30 min	= ((3.883-2.208)/2.208) × 100 = 75.86%
	60 min	= ((5.087-1.878)/2.878) × 100 = 76.75%

Administered 0.2 ml 3% acetic acid intraperitoneally in 60 min after oral administration. Writhings were counted for 20 min, starting in 5 min after acetic acid administration. $p < 0.05$ versus control, ANNOVA Test, values are means ± SEM (n=10).

Table 4: Effect of Panch Phoron aqueous extract in mice observed in the acetic acid-induced writhing test.

Treatment	Dose	Writhings	% of Writhings	% of inhibition
Control (water)	1 ml	39.7±1.248	100±3.143	0±3.143
Positive Control (Diclofenac Na)	25 mg/kg	13.931±0.577	35.091±1.452	64.909±1.452
Panch Phoron Extract	100 mg/kg	30.52±0.485	76.877±1.222	23.123±1.222
	250 mg/kg	24±0.707	60.453±1.781	39.547±1.781

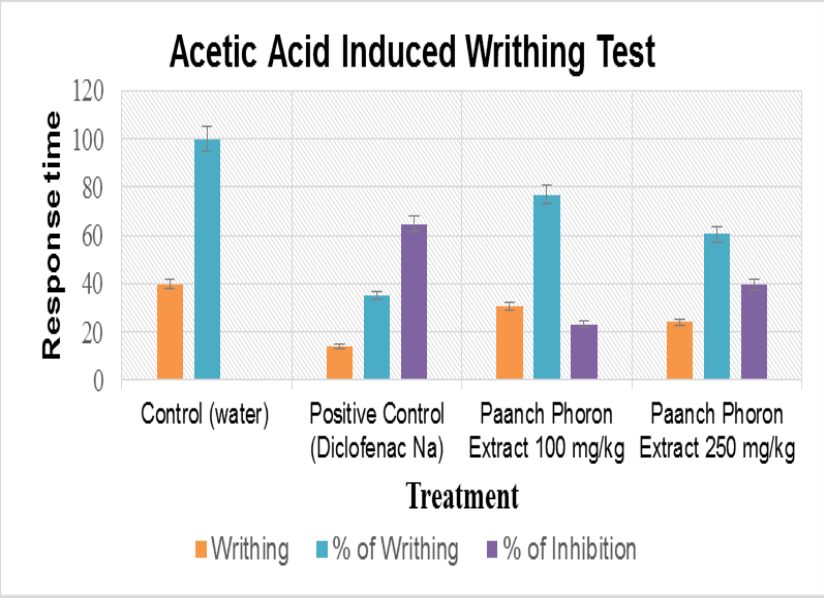

Figure 1: Acetic acid-induced writhing test showing comparative response time among different group.

DISCUSSION

There have been less research which included the evaluation of different medicinal activity of Panch phoron and its individual spices. There has been interest in its ability to relieve pain. Here Panch phoron aqueous extract was evaluated for anti-nociceptive activity. Phytochemical tests were performed to evaluate the presence of therapeutic activity and nociceptive evaluation was performed in mice model to evaluate analgesic activity. Acetic acid writhing test and tail flick test were performed to detect peripheral and central analgesia. Both peripheral and central analgesia was evaluated by acetic acid test, on the other hand, tail flick test only responds to centrally acting analgesics. PGE2 and PGF2α and prostaglandins are released when acetic acid is administered by intraperitoneal route.[14]

Table 5.

Treatment	Dose	Response Time (sec)		
		0 min	30 min	60 min
Control (water)	1 ml	2.522±0.105	2.208±0.106	1.878±0.159
Positive Control (Diclofenac Na)	25 mg/kg	3.353±0.118 (MPA 0.111)	4.328±0.098 (MPA 0.272)	4.372±0.112 (MPA 0.307)
Panch Phoron Extract	100 mg/kg	2.598±0.103 (MPA 0.011)	3.082±0.096 (MPA 0.112)	4.085±0.145 (MPA 0.271)
	250 mg/kg	2.629±0.196 (MPA 0.014)	3.883±0.082 (MPA 0.215)	5.087±0.06 (MPA 0.395)

Tail flick method of analgesia is effective in estimating the efficacy and potency of centrally acting analgesics. This was evident in this study wherein the pain threshold increased significantly during the period of observation in all the four groups except the control group (Table 5). The pain threshold of extract increased in a dose-dependent manner. The extract shows comparable threshold to diclofenac sodium at 60 min indicating a slow onset of action of the drug (Table 5). If analgesia is centrally acting then thermal pain threshold increases. In a dose-dependent manner thermal reaction time increased significantly ($P < 0.001$) in two doses of aqueous extract of Panch phoron. PIP effect of 75.68 and 170.87% were observed in the second dose of Panch phoron aqueous extract (Table 1). Diclofenac sodium showed low activity than the Panch phoron extract toward thermal pain.

In acetic acid-induced writhing model, compounds with percentage analgesia of less than 70% are considered to have minimal analgesic activity.[15] Percentage analgesia with both extracts was less than 70% Percentage inhibition of writhing is an indication of the effectiveness of analgesic. Higher the percentage of inhibition more the analgesic activity. A

lower percentage of inhibition indicates low analgesic activity. The writhing response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. Acetic acid injection induced hind limb stretching and abdominal constrictions were found to be reduced significantly ($P < 0.001$) in two doses in a dose-dependent manner (Table 4). It was seen that 23.13% writhing was inhibited in 1st dose and, 39.55% writhing was inhibited in 2nd dose. In the results, the effect of Panch phoron was less than good as the standard Diclofenac sodium indicating very low analgesic activity. Panch phoron (250 mg/kg; p.o.) demonstrated an activity 39.55% of when used as aqueous extract while the standard drug Diclofenac Na had an activity of 64.91% indicating comparable but very low analgesic activity. So we can say that both central and peripheral analgesic activity is demonstrated by the extract.

In the study, we did not evaluate the underlying mechanism by which the Panch phoron extract shows analgesic activity. Due to the sensitization of the nociceptive receptors to prostaglandins, abdominal constrictions were noticed. So, we may assume the effect seen is due to the inhibition of the prostaglandins.[15] Cumin essential oil was investigated for the anti-inflammatory effects in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells and the underlying mechanisms. In studies, it has been found that the oil of Cumin a spice of Panch phoron exerted anti-inflammatory effects in LPS-stimulated RAW cells through inhibiting NF-κB and mitogen-activated protein kinases. These are the mediators of pain suggesting the possible mechanism through which Panch phoron exerts its analgesic activity.[16]

CONCLUSION

Phytochemical screening of the panch phoron components has shown bioactive compounds. The main objective of the experiment was phytochemical screening and demonstration of the analgesic effect of the ingredients together.[17] In our screening we found panch phoron to contain bioactive molecules which may have a wide range of therapeutic benefits. Also, the test for analgesic activity results demonstrates it to have slight analgesic activity. Further studies can be performed such as carrageenin-induced hind paw edema test to evaluate the analgesic effect of panch phoron. The mechanism through which it exerts the analgesic activity has not been evaluated in this study. A more in-depth investigation can be conducted to evaluate the mechanism of how panch phoron exerts this analgesic activity.

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