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Research Article

EVALUATION OF CENTRAL AND PERIPHERAL ANALGESIC ACTIVITY OF WHOLE PLANT *GOMPHRENA GLOBOSA* (L) (FAMILY: AMARANTHACEAE)

Hamiduzzaman Md.*
Lecturer, Department of Pharmacy, ASA University Bangladesh
*Corresponding Author Email: hamid018@yahoo.com

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ABSTRACT
Gmophrena globosa (L) including in Amaranthaceae family is an important medicinal plant in our country, especially in hilly tribal people for their folk medicinal practices. The object of my study was to investigate the central and peripheral analgesic activity of the investigated plant based on the use of *Gmophrena globosa* extract for the wound healing to the tribal people in our country. After collection of whole plant it was first of all washed and then sun dried and made powder by grinding machine. The powder was extracted by methanol. Around 5gm of concentrated methanolic plant extract was partitioned by modified Kupchan partitioning protocol into n-hexane soluble fraction, carbon tetrachloride soluble fraction, chloroform soluble fraction and aqueous soluble fraction. All the fractions were subjected to the investigation of central and peripheral analgesic activity. Among all the fractions crude methanolic extract, n-hexane soluble fraction and aqueous soluble fraction at a dose of 400mg/kg had shown significant analgesic activity. They produced an inhibition of writhing 74.6%, 71.4% and 74.5% respectively in comparable with positive control diclofenac. On the other hand central analgesic activity of the test samples were compared with Morphine. The crude methanolic extract and n-hexane soluble fraction have significant central analgesic activity at 400mg/kg dose. The central analgesic activity is highest after 30minutes. As the time progress the analgesic activity decreases. After 60minutes there is almost no central analgesic activity in the plant extracts.
Keywords: *Gomphrena globosa*, Amarantheace, Central analgesic activity, Peripheral analgesic activity.

INTRODUCTION

Medicinal plants are plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, in the world more than 30% of the pharmaceutical preparations are based on plants.[1] Bangladesh is a good repository of medicinal plants comprising of various families including Amaranthaceae.[2] The plants of Amaranthaceae family contain a wide range of pharmacologically active compounds and various types of traditional uses like oliguria, heat and empacho, hypertension[3] and diabetes.[4] *Gmophrena globosa* (L.), (Bengali name Botam phul) is distributed in all over Bangladesh and commonly known as Globe Amaranth which is one of the important medicinal plants in our country. It is cultivated as an ornamental flowering annual herb in garden that bears globe like flowers. Throughout the world there are 176 general and 2400 species available and among them around 30 members are common in Bangladesh.[5-6] This plant has a wide range of folk medicinal uses to the trial people of our country. The object of my investigation is to make a correlation between the scientific bases of folk medicinal uses of *Gmophrena globosa* in our phytochemical laboratory. The present study was designed to investigate the central and peripheral analgesic activity of different fractions of the whole plant of *Gmophrena globosa*.

MATERIAL AND METHODS

Collection of the Plant Sample

Whole plant of *Gmophrena globosa* was collected from Dhaka, Bangladesh in November; 2011. This plant was identified by botanists of the Botany Department of the University of Dhaka. The reference sample for the plant was DUSH, Accession Number 3557 and call number 01.

Preparation of the Plant Material

The whole plant of *Gmophrena globosa* was washed properly, cut into small pieces and then air dried for several days. The pieces were then oven dried for 24hours at considerably low temperature to effect grinding. The pieces were then ground into coarse powder in the laboratory using high capacity grinding machine. About 800gm of the powdered material was taken in a clean, round bottomed flask (5liters) and soaked in 3.5liter of methanol. The container with its content was sealed by foil and kept for a period of 15days accompanying occasional shaking and stirring. The whole mixtures were then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The volume of the filtrate was then reduced using a Buchi Rotavapor at low temperature and pressure. The weight of the crude extract was 29gm. An aliquot (5.0g) of the concentrated aqueous methanol extract was fractionated by the modified Kupchan partitioning protocol into n-hexane, carbon tetrachloride and chloroform.[7] Subsequent evaporation of solvents afforded n-hexane (1.5g), carbon tetrachloride (1.15g), chloroform (0.5g) and aqueous soluble (0.65g) materials. Partitionates obtained by this way were subjected to different biological assays.

Evaluation of Analgesic Activity

Drugs and Chemicals

Diclofenac and Loperamide were obtained from ACI pharmaceuticals, Morphine was obtained from Gonoshastho Pharmaceuticals Ltd., Dhaka, Bangladesh and acetic acid was obtained from Merck, Germany.

Table 1: % inhibition of writhing of the different test samples and standard

Group	Number of Writhing (Mean ± SEM)	% of Inhibition of Writhing
1. Negative Control	24.4 ± 0.98	-
2. Positive control	6.4 ± 0.51	77.8
3. Crude Methanolic Exxtract (CME), 200mg/kg	8.6 ± 0.51	64.8
4. Crude Methanolic Exxtract (CME), 400mg/kg	6.2 ± 0.37	74.6
5. n-hexane Soluble Fraction (NHSF), 200mg/kg	12 ± 0.84	50.9
6. n-hexane Soluble Fraction (NHSF), 400mg/kg	7 ± 0.45	71.4
7. Carbon tetrachloride Soluble Fraction(CTF), 200mg/kg	14.2 ± 0.73	41.8
8. Carbon tetrachloride Soluble Fraction(CTF), 400mg/kg	12.4 ± 0.81	49.2
9. Aqueous Soluble Fraction(ASF), 200mg/kg	8.2 ± 0.56	66.4
10. Aqueous Soluble Fraction(ASF), 400mg/kg	6.2 ± 0.45	74.5

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): *P<0.05,
All values are means of individual data obtained from five rats (n = 5)

Table 2: Statistical evaluation of peripheral analgesic tests for different test samples and standard

Group	T- test value	P value	Level of significance
1. Positive control	16.2964	Less than 0.0001	Extremely significant
2. Crude Methanolic Exxtract, 200mg/kg	14.3046	Less than 0.0001	Extremely significant
3. Crude Methanolic Exxtract , 400mg/kg	17.353	Less than 0.0001	Extremely significant
4. n-hexane Soluble Fraction , 200mg/kg	9.6243	0/0017	Very significant
5. n-hexane Soluble Fraction , 400mg/kg	16.1555	Less than 0.0001	Extremely significant
6. Carbon tetrachloride Soluble Fraction, 200mg/kg	9.4281	0.0015	Very significant
7. Carbon tetrachloride Soluble Fraction, 400mg/kg	13.2274	Less than 0.0001	Extremely significant
8. Aqueous Soluble Fraction, 200mg/kg	13,2272	0.0015	Very significant
9. Aqueous Soluble Fraction, 400mg/kg	15.9625	Less than 0.0001	Extremely significant

Table 3: % increase in tail flicking time for the different samples and standard at different time interval

Sample	% increase in tail flicking time	
	30 minutes	60 minutes
1. Negative Control	-	-
2. Positive control	144.4	119.04
3. Crude Methanolic Extract (CME), 400mg/kg	71.25	34.69
4. n-hexane Soluble Fraction (NHSF), 400mg/kg	66.77	32.65
5. Carbon tetrachloride Soluble Fraction(CTF), 400mg/kg	9.58	6.9
6. Aqueous Soluble Fraction(ASF), 400mg/kg	63.57	11.22

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): *P<0.05,
All values are means of individual data obtained from five rats (n = 5)

Table 4: Statistical evaluation of central analgesic tests for different test samples and standard

Sample	Time after	T- test value	P value	Level of significance
1. Positive control	30 minutes	11.5405	Less than 0.0001	Extremely significant
	60 minutes	5.9348	0.0002	Significant
2. Crude Methanolic Extract, 400mg/kg	30 minutes	6.6485	0.0002	Significant
	60 minutes	2.7314	0.0258	Statistically significant
3. n-hexane Soluble Fraction, 400mg/kg	30 minutes	5.0460	Less than 0.0001	Extremely significant
	60 minutes	2.6117	0.031	Statistically significant
4. Carbon tetrachloride Soluble Fraction, 400mg/kg	30 minutes	0.9496	0.3701	Not significant
	60 minutes	3.3267	0.0104	Statistically significant
5. Aqueous Soluble Fraction, 400mg/kg	30 minutes	5.0867	0.0009	Significant
	60 minutes	1.0555	0.322	Not significant

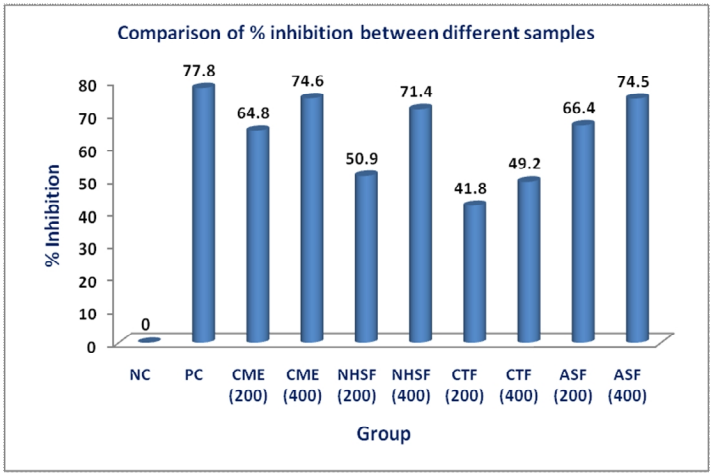

Figure 1: Comparison of % Inhibition of Writhing of Different test samples and Standard
NC= Negative control group, PC= Positive control, CME= Crude methanolic Extract, NHSF= n-hexane soluble fraction, CTF= Carbon tetrachloride soluble fraction and ASF= Aqueous soluble fraction

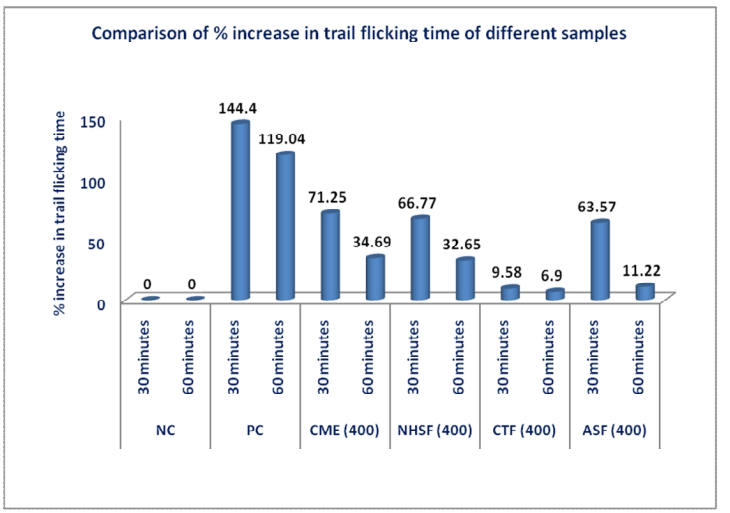

Figure 2: Comparison of % Increase in Trail Flicking Time of Different Test Samples and Standard
NC= Negative control group, PC= Positive control, CME= Crude methanolic Extract, NHSF= n-hexane soluble fraction, CTF= Carbon tetrachloride soluble fraction and ASF= Aqueous soluble fraction

Design of Experimental Animal

Swiss albino mice (25-30g) were obtained from the Animal Research Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR B). The animals were housed in polyvinyl cages and received feed formulated by ICDDR, B. To keep the hydration rate constant, food and water were stopped 12hours before the experiments. The ethics for use of experimental animals were followed carefully. Fifty experimental animals were randomly selected and divided into ten groups denoted as group-I, group-II, group-III(A-B), group-IV(A-B), group-V(A-B) and group-VI(A-B) consisting of 5 mice in each group. The animals were individualized by marking as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3, M-4=Mice 4 and M-5=Mice 5.

Acetic Acid Induced Writhing Test

The peripheral analgesic activity of whole plant *Gmophrena globosa* was measured by the acetic acid induced writhing test as described earlier.[8] briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Diclofenac at oral dose of 100mg/kg was used as standard analgesic agent. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1ml/10g of body weight was used to create pain sensation. In order to administer the crude extract at doses of 400mg/kg body wt and 200mg/kg body wt of mice, 100 and 50mg of the extract were measured respectively and triturated in unidirectional way by adding of small amount of Tween-80 (a suspending agent) and the final volume of the suspension up to 3.0ml. The number of writhing was calculated for 10minutes, 5minutes after the application of acetic acid.

Radiant Heat Tail-flick Method

Evaluation of central analgesic activity of *Gmophrena globosa* was carried by tail flicking method using Morphine as a positive control[9]. The changes in sensitivity of test animal due to analgesic activity of drugs are measured in this method. A constant heat stress is applied to rat tail, which acts as pain stimulus. When the stimulus exceeds the threshold, rat show a quick withdrawal of its tail. Time taken by the rat to withdraw the tail is termed as tail flicking time. Analgesic compound elongates this responding time. By this test discrimination was done between centrally acting

morphine like analgesics and non-opiate analgesics. Analgesics of only central (narcotic) type, e.g. morphine, pethidine, pentazocine etc can increase the tail flick latency period indicating analgesia[10].

The test rats were orally fed with test materials whereas the positive control received morphine subcutaneously. After an interval of forty minutes, rats were kept into cages leaving the proximal third of their tail exposed over a holder having a thin wire. In order to make the wire hot, current was allowed to pass through the wire at a low intensity (3amperes). The animals flicks the tail aside or tries to escape. The time required to withdraw the tail was recorded.

Statistical Analysis

Values for analgesic activity were expressed as "mean increase in latency after drug administration ±SEM" in terms of seconds. The significance of difference between means was determined by t-test values of $p<0.05$ were considered significant.

RESULTS

Acetic Acid Induced Writhing Test

Methanolic crude extract and its different fractions of *Gmophrena globosa* were subjected to screening for analgesic activity by acetic acid induced writhing inhibition method. The test was performed by taking samples at doses of 200 and 400mg/kg body weight. The result was evaluated statistically. Statistical evaluation of the data confirmed that the crude methanolic extract, n-hexane soluble fraction and aqueous soluble fraction at a dose of 400mg/kg had shown significant analgesic activity. They produced an inhibition of writhing 74.6%, 71.4% and 74.5% respectively. All other fractions also tested and revealed mild analgesic activity. The data obtained from the experiment were proven to be statistically significant for the effective fractions. All the data were shown in Table 1, Table 2 and comparison of % of inhibition of writhing of different samples and standard was shown in Figure 1.

Radiant Heat Tail-flick Method

After administration of the samples, tail flicking time was tested after 30 and 60minutes. Time required for tail flicking by each mouse was recorded and the average flicking time of each group was calculated. The % of time elongation of tail flicking was calculated in respect to the control. The higher the elongation percentage of the group the greater is the groups' central analgesic activity. The central analgesic activity of the test samples were compared with Morphine. The analysis and statistical evaluation of the data leads to the following important conclusions

a) The crude methanolic extract and n-hexane soluble fractions had significant central analgesic activity at 400mg/kg dose.

b) The central analgesic activity was highest after 30minutes.

c) As the time progressed the analgesic activity decreased. After 60minutes there was almost no central analgesic activity.

All the data were shown in Table 3, Table 4 and comparison of % of increase in trail flicking time of different samples and standard was shown in Figure 2.

DISCUSSION

The analgesic activity of the whole plant *Gmophrena globosa* was studied for central (narcotic) and peripheral (nonnarcotic) activities. We know that intraperitoneal administration of acetic acid (1%) causes localized inflammation in mice. Following inflammation, there is biogenesis of prostaglandins (from cyclooxygenase pathway) and leukotrienes (lipooxygenase pathway). The released prostaglandins, mainly prostacyclin (PGI2) and prostaglandin-E has been reported responsible for pain sensation. The test samples has revealed significant pain reliving capacity i.e. cause blockade of prostaglandin synthesis those are responsible for pain sensation. Different fractions of *Gmophrena globosa* had produced significant analgesic activity which is statistically proved. Among all the fractions crude methanolic extract, n-hexane soluble fraction and aqueous soluble fraction at a dose of 400mg/kg had shown significant analgesic activity in comparable to standard Diclofenac oral suspension 100mg/kg body weight. On the other hand the crude methanolic extract and n-hexane soluble fractions reveled significant central analgesic activity at 400mg/kg dose in comparable to Morphine as a standard. So it could be concluded that *Gmophrena globosa* has important secondary metabolites which are important for novel pharmacological activity.

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