

Analgesic, anti-inflammatory and CNS depressant activities of *Pandanus foetidus* Roxb. leave.

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ABSTRACT

In this study crude methanol and crude aqueous extracts (CME and CAE) of *Pandanus foetidus* Roxb leaves were used to evaluate the in vivo analgesic, anti-inflammatory and CNS depressant activities in mice. The plant materials were thoroughly extracted with methanol and water to afford a crude mass. Analgesic activity was done by acetic acid induced writhing test and anti-inflammatory activity study was carried out by using carrageenan induced paw edema test. Hole cross and open field tests were considered to evaluate CNS depression activity of the extracts. In writhing test, CAE showed, significant ($p < 0.01$) inhibition of writhing reflex. From the carrageenan induced paw edema test data, CME was found to be moderate but CAE showed higher anti-inflammatory activity in mice as compare to standard. The CNS depressant activity of CME and CAE was 11.5 ± 4.20 and 10 ± 3.15 which is quite comparable with standard drug Diazepam that was 12 ± 2.50 at 90 min after extracts administration. Our findings suggest that, *Pandanus foetidus* leaves can be use as herbal remedy in pain, inflammatory and CNS disorder. In addition, this is first time when anti-inflammatory activity of this plant species has been carried out.

Keywords: *Pandanus foetidus*, Analgesic, Anti-inflammatory, CNS depressant.

I. INTRODUCTION

When body tissue is injured by any infections, chemicals, thermal and mechanical injuries then inflammation is supposed to be occurs [1]. Different types of intracellular proteins such as histamine, serotonin, prostaglandins, etc. are mostly found in the inflammatory cells in which prostaglandin serve as a mediator of inflammation [2]. Actually in little amounts, these biological modulators can elicit pain response in both the

central and peripheral sensory systems. Subsequently, inflammation is frequently associated with pain; indeed it is a complex process [3]. Pain is an upsetting physiological experience associated with actual or potential tissue harm [4]. Neuropathic pain is because of direct injury on dysfunction of peripheral or central nervous system [5]. CNS depressant suppresses or slows the activity of central nervous system to create a calming or sedating effect and to allay anxiety.

Different classes of synthetic drugs are available for the treatment of physiological disorder but they are not equally effective in every cases because of having various and severe adverse effects like gastrointestinal discomfort, liver and kidney damage, adverse cardiovascular effect, mutagenesis and other complicacy. Therefore, the phytochemists in all over the world are looking for new drugs from plant origin as alternatives to synthetic analgesic, anti-inflammatory agent because prolong use of synthetic drugs are not only harmful but also very expensive to develop.

Medicinal plants possess extensive medicinal properties which are natural treasured to phytochemists [6]. Rural people of Bangladesh greatly depend on different medicinal plants for primary health care. In Bangladesh many plant species have medicinal value and different parts of plants are used for several purpose since ancient time. For primary health care herbal medicines are very much demandable worldwide due to their wide biological and medicinal values, higher safety edges, and lesser expenses [7]. Several phytochemical have been isolated from different plant species; these components have received considerable attention due to their therapeutic potential as analgesic, anti-inflammatory, anticancer and antioxidant activities [8]. Like other medicinal plant *Pandanus foetidus* has been used as a folk medicine in Bangladesh for the treatment of

different disease conditions such as infection, cancer, inflammation or other complications [9].

The plant *Pandanus foetidus* Roxb. belongs to the Pandanaceae family. The genus *pandanus* comprises approximately 600 species. *Pandanus foetidus* locally known as Kewa kata, Keora, Keurikanta or Kewakanta. It is found in different areas of Bangladesh, mostly in Sundarban [10] and Chittagong. For the treatment of various diseases such as leprosy, small pox, syphilis, scabies, heart and brain diseases leaves of this plant are used [11, 12]. In diabetes leaves and spadix of *Pandanus foetidus* plant are also reported to use [12]. Essential oil of *P. foetidus* is also used as perfumery as well as medicinal sources [13]. The plant *P. foetidus* possess significant bioactive principles [14] and phytochemical screening of the plant indicate the presence of carbohydrates, saponins, tannins, glycosides, steroids, alkaloids, polyphenol and flavonoids that are responsible for antioxidant activity of this plant species [15,16].

In this study we reported the analgesic, anti-inflammatory and CNS depressant activities of methanol and aqueous extracts of *P. foetidus* leaves in Swiss albino mice that represent the plant *Pandanus foetidus* Roxb. as a potential candidate of herbal medicine.

II. MATERIALS AND METHODS

Collection and preparation of Plant materials

The leaves of *Pandanus foetidus* were collected from University of Rajshahi, Bangladesh, in December 2012. The plant was verified from the department of Botany, University of Rajshahi and a voucher specimen no. 34912 was retained in our lab for future reference.

The fresh leaves were extensively washed with water and subjected in shade drying. For grinding the fresh leaves were cut into small pieces and then dried in an oven at 40-45°C for 36 hrs. With the help of a grinder the dried plant leaves were grinded into powder materials and keep in an air tight container for future use.

Preparation of crude extracts

The leaves of *Pandanus foetidus* were dried under shade and pulverized in a mechanical grinder. The coarse powder (3.6 kg) of leave was extracted with methanol and water respectively using cold extraction process. The liquid extract was then filtered off through cotton and then finally through filter paper (Whatman No.1). The filtrate was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to obtain

crude methanol (2.1 gm) and crude aqueous (2.8 gm) extract respectively.

Experimental animals

The experiment was conducted on Swiss albino mice of both sexes, aged 4-5 weeks and weighting was about 20-25gm. The mice were purchased from the international center for diarrheal disease and research, Bangladesh (ICDDR) and were kept in standard environmental conditions (temperature: (23.0 ± 2.0°C), relative humidity: 55-65% and 12 h light/12 h dark cycle) before initiating the experiment. All protocols for animal experiment were approved by the institutional animal ethical committee of Rajshahi University.

Grouping of animals

Randomly selected of both sexed animals were divided into four groups contained five mice in each group for control, standard and two test samples group respectively. Each group received a particular treatment i.e. control, standard (10 mg/kg) and the test sample (CME and CAE of 10 mg/kg). Each mouse was weighed properly and the doses of the test samples and standard materials were adjusted according to their body weight.

Study of analgesic activity

Acetic acid-induced writhing test

Acetic acid-induced writhing method was used to evaluate the analgesic activity of plant extracts which is described by Sharma et. al.[17]. The control group was received 0.7% v/v acetic acid and Diclofenac-Na was used as standard.

Study of Anti-inflammatory activity

Carrageenan-induced paw edema test

Anti-inflammatory activity of test sample was determined by method namely-Carrageenan induced paw edema in Swiss albino mice illustrate by Elisabesky et al 1995 [18].

The increase in paw volume with time was calculated and compared with control group. Percent inhibition was calculated using the formula-

$$\% \text{ Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_c and V_t represent average paw volume of control and treated animal respectively.

Study of CNS depressant activity

Hole cross method

The experiment was carried out according to Takagi et al 1971 [19]. The aim of this study was to characterize the emotional behavior of mice. The

test groups were received the leave extracts of Pandanus foetidus whereas control and standard group were given vehicle (1% Tween 80 in water) and Diazepam orally with the help of a feeding needle. After administration of test sample and standard drug the number of passages of mice through the hole was counted for a period of 3 minute.

Open field method

This experiment was conducted as described by Gupta et al 1999 [20]. This study was carried out by using a wooden board of open field whose floor was divided into a series of alternatively colored black and white. The number of squares accessed by the animals was counted for 3 minute after administration of test and standard sample.

III. STATISTICAL ANALYSIS

The data were expressed as the mean \pm SEM of three replicate experiments. The analysis was done using Statistical Package for the Social Sciences (SPSS) in the version of 15.0; SPSS Inc, Chicago. All the values are subjected to ANOVA followed by Dunnett's test and $p < 0.01$ were considered to be statistically significant.

IV. RESULTS

Analgesic activity

Acetic acid-induced writhing test

The significant suppression of writhing was observed by the extracts of Pandanus foetidus leaves in writhing test. Compared to standard drug Diclofenac sodium, CME and CAE showed 30.76% and 28.92% writhing inhibition respectively in which the analgesic activity of methanol extract was quite medium. Analgesic activity of standard and two extracts of Pandanus foetidus leaves shown in Table 1.

Anti-inflammatory activity

Carrageenan induced paw edema method

The CME and CAE also produced anti-inflammatory effect of 53.33% and 60% respectively in Carrageenan induced paw edema method shown in table-2. This is also the first time in which anti-inflammatory effect of CAE and CME of Pandanus foetidus was carried out.

Central nervous system (CNS) depressant activity

Hole cross test

The effect of plant extracts on CNS was also assessed by hole cross method. A reduction of locomotors activity indicates the sedative as well as

depression of the central nervous system. The result indicated that both the extracts significantly decreased the locomotors activity which attributes CNS depression effect by the extracts as shown in the table -3.

Open field test

In open field method, both the extracts showed a notable decreased in locomotors activity with time. The extracts were showing significant decreased in number of movement (CME: 11.5 ± 4.20 and CAE: 10 ± 3.15) as compared to standard drug Diazepam (12 ± 2.50) in the test and standard group of animals shown in table-4.

V. DISCUSSION

Our study purpose was design to explore the analgesic, anti-inflammatory and CNS depressant activity of the methanol and aqueous extracts of Pandanus foetidus Roxb. leaves.

Acetic acid induces writhing method was used to studied the analgesic activity in which pain is supposed to be sensed by stimulating the inflammatory cell to release free arachidonic acid [21]. Peritoneal mast cells [22], acid sensing ion channels [23] and the prostaglandin pathways [24] act as mediators of peripheral analgesic action which can be evaluated by writhing method. Therefore the significant analgesic action of P. foetidus leave extract was based on the inhibition of writhing.

To determine acute inflammation carrageenan induced paw edema test is widely used. This biphasic process is mediated and sustained by histamine, serotonin, bradykinin and synthesis of prostaglandins from the damaged tissue [25, 26]. Therefore the significant inhibitory effects represents by the extracts suggest that there is a possibility of inhibition of cyclooxygenase as well as prostaglandin synthesis and this effect is correlated to the standard drug Indomethacin whose action is mediated by the inhibition of cyclooxygenase enzyme [27]. So, it can be assumed that the anti-inflammatory activity of the extracts may be due to the inhibition of cyclooxygenase enzyme which leads to subsequent inhibition of prostaglandin synthesis.

Sedative effect is produced by decreased locomotors activity. The major inhibitory neurotransmitter in the central nervous system is Gamma-amino-butyric acid (GABA) through which many sedative drugs exert their action. Literature survey suggest that the plant P. foetidus contains flavonoids and tannin [15, 16] which act as ligands for GABA receptors in the central

nervous system, so it is indicate that the extracts of *P. foetidus* may exploit GABA receptor [28]. From the result it is reveal that the extracts remarkably decreased locomotor activity, which suggest that the extracts of *Pandanus foetidus* may act as benzodiazepine-like molecules to produce CNS depression.

VI. CONCLUSION

Our present findings indicate that the plant extracts have moderate CNS depression, anti-inflammatory and analgesic activity and can be use as a traditional medicinal plant to manage inflammation associated conditions. Our previous findings suggest that the methanol and aqueous extract of this plant species contain polyphenol and flavonoids which possess potent analgesic, anti-inflammatory and CNS depressant action. However, the chemical constituents and their exact mechanism for these activities still have not been reported and need to be further investigation in future.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors. Author Nurunnahar designed the study, performed the experimental analysis and managed the analyses of the study. Author AM and RZ performed the statistical analysis, wrote the first draft of the manuscript and managed the final submission of the manuscript. Authors MZ, SA, HU, AR and SK managed the literature searches and help in experimental analysis. All authors read and approved the final manuscript.

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COMPETING INTERESTS

The Authors declared that no competing interest exists.

ETHICS APPROVAL

This study was approved by University Ethics Committee at Institute of Biological Sciences.

ABBREVIATIONS

CAE: Crude Aqueous Extract, CME: Crude Methanol Extract, GABA: Gamma-Amino-Butyric Acid.

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Table 1: Analgesic activity of methanol and aqueous extracts of Pandanus foetidus leaves in writhing method.

Sample	Dose	Writhing number	%Inhibition of Writhing No.
Control	-	39.0±1.0	0
Diclofenac Na	10	5.0±1.0***	87.17
Methanol Extract	10	27.0±2.0**	30.76
Aqueous Extract	10	28.0±2.0**	28.92

Data are presented as Mean ± SD, P**<0.01, P***<0.001 are considered as significant level compared to control group. ANOVA followed by dunnet's test is done in SPSS version 15.0

Table 2: Anti-inflammatory activity of methanol and aqueous extract of Pandanus foetidus leaves in Carrageenan induced paw edema method.

Data are presented as Mean ± SD, P**<0.01, P***<0.001 are considered as statistically significant level

Sample	Dose (mg/kg)	Edema diameter (mm)				% Inhibiti on after 3 hour
		0.5hr	1hr	2hr	3hr	
Control	-	1.4±0.70	1.4±0.42	1.5±0.70	1.5±0.35	-
Indomethacin	10	7.4±0.12	5.34±0.012* *	3.92±0.019**	1.33±0.005***	91.27
Methanol Extract	10	1.3±0.35	1.3±0.35	1.2±0.70**	1.1±0.35***	53.33
Aqueous Extract	10	1.2±0.30	1.2±0.10	1.1±0.35**	1.0±0.1***	60

compared to control group. ANOVA followed by dunnet's test is done in SPSS version 15.0

Table 3: CNS depressant activity of methanol and aqueous extract of Pandanus foetidus leaves in hole cross method.

Sample	Dose (mg/kg)	0 Min	30 Min	60 Min	90 Min
Control	-	9.5±0.70	8.5±2.12	7.0±1.41	6.5±1.5
Diazepam	10	7.0±1.0	4.5±2.12	3.5±3.53	2.0±2.82
Methanol Extract	10	6.5±0.70	4.0±1.41	2.5±2.12	2.0±2.82
Aqueous Extract	10	7.5±0.60	6.0±1.25	4.5±0.75	3.5±1.25

Table 4: CNS depressant activity of methanol and aqueous extract of Pandanus foetidus leaves by open field method.

Sample	Dose (mg/kg)	0 Min	30Min	60 Min	90 Min
Control	-	94.0±5.65	96.5±7.56	43.5±12.02	29.5±3.53
Diazepam	10	52±8.23	29.5±5.33	21±7.12	12±2.50
Methanol Extract	10	46.5±6.36	40.5±8.26	18.5±3.53	11.5±4.20
Aqueous Extract	10	41±11.31	31±3.25	24±8.48	10±3.15