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An Assessment of Analgesic & Anti-inflammatory Activity *Spondias mombin* and Mapping of Variation of Potency under the Light Geographical Contrast Based Phenomenon

Tahmina Sharif Nila [a], Maliha Rownak Ara Khan [a], Najibah Nasrin [b], Isratul Jannat Mim [a], Farhana Yesmin Peya [a], Amatun Noor Prapty [a], Lia Rose Merry D. Cruze [c], Md. Ashikur Rahman [a], Ishrat Jahan [a], Mafruha Kumkum [d], Raisa Sharmili Habib [b], Md. Rafat Tahsin [c*], Fahima Aktar [d], Tahmina Aktar [e], Jakir Ahmed Chowdhury [f], Shaila Kabir [d], Abu Asad Chowdhury [d] and Md. Shah Amran [d]

[a] Department of Pharmacy, University of Asia Pacific, Farmgate, Dhaka, Bangladesh.
[b] Department of Pharmacy, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.
[c] Department of Pharmaceutical Sciences, North South University, Plot # 15, Block # B, Bashundhara R/A, Dhaka 1229, Bangladesh.
[d] Molecular Pharmacology and Herbal Drug Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.
[e] Department of Physiology, Dhaka Medical College, Dhaka, Bangladesh.
[f] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: Email: whitefang229@gmail.com;*

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ABSTRACT

Because of the short comings of conventional anti-inflammatory drugs, the researchers have driven their attention towards natural products. Medicinal plants have got acknowledged for greatly impacting on the health of individuals and communities. *Spondias mombin* is a great species, traditionally used for several medicative objectives from ancient times. It has also been popular for it's analgesic and anti-inflammatory effect. In this study, we an effort was made to evaluate the analgesic and anti-inflammatory effect of *Spondias mombin* against the conventional drugs. the plant extract from different region was induced through intraperitoneal route and the efficiency was compared with paracetamol and ibuprofen. It was seen that both the conventional drug and extract of *Spondias mombin* significantly reduce the inflammation. The plant extract has also shown effects irrespective of geographical interest. Hence it might be assumed that *Spondias mombin* could be a suitable alternative therapy in reducing pain and inflammation and to surpass the side effects of ordinary conventional medicines.

Keywords: Anti-inflammatory; Spondias mombin; conventional; medicinal plants; analgesic.

1. INTRODUCTION

Pain is a sensation providing unpleasant feeling that something in the body may be wrong, while Inflammation is a natural response of the body triggered by the exposure and removal of threats to the hostile agents [1,2]. However, If not treated on time, this may lead to multiple pathological condition. [2].

Conventionally, some pharmacological approaches are available to treat pain and inflammation such as Nonsteroidal anti-inflammatory drugs (NSAIDs), opioids analgesics corticosteroids and some combination therapy to enhance efficacy [3].

Due to chance of some side effects such as pervasiveness in the rate and severity of nephrotoxicity with NSAIDs [4] and cautionary use of opioids, safety and efficacy of these available conventional drugs in the treatment of pain and inflammation remain questionable [3]. This leads to search for Plant derived natural compound [1]. Magnificent of natural compound can be assessed with three logical criteria: (1) plant contains cluster of compounds which can exert different types of pharmacological activity [5], (2) the prevalence of introducing new chemical entities of wide structural diversity as well as templates for semisynthetic and synthetic modification with improved pharmacologic action [6], (3) selective extraction of bioactive compound from plant to remove unwanted entities [7].

Traditionally, *Spondias mombin* plant has been reported to have significant medicinal value. It falls under the family Anacardiacae and mostly found in the rain forest and in the coastal area [8]. The plant contains variety of phytoconstituents such as tannins, saponins and anthraquinone glycosides [9]. Traditionally, Extract of young leaves is used to treat diarrhea and dysentery. Juice of crushed leaves and powder of dry leaves also heal wounds and inflammations [8]. Its other pharmacological properties include abortifacient [10], anti-microbial [11], anti-diarrhoea [12], anti-viral [13], possess sufficient Vitamin C [14] and Wound-healer [15].

In our study design, Paw edema was analyzed for assessment of anti-inflammatory property and through Acetic acid-induced writhing test and Tail flick method, we measured analgesic property of *Spondias mombin*. We also analyzed if geographical variation having impact on analgesic and anti-inflammatory effect of *Spondias mombin* It is expected that in future, it will open a new doorway in disease management system.

Even though popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent

regulation and quick effects, but their safety and efficacy was always remained questionable, resulting in the dependence on the natural products by more than 80% of the total population in the developing world, because of its time tested safety and efficacy [16]. A huge number of natural product-derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidate Even though popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects, but their safety and efficacy was always remained questionable, resulting in the dependence on the natural products by more than 80% of the total population in the developing world, because of its time tested safety and efficacy. A huge number of natural product-derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidate Even though popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects, but their safety and efficacy was always remained questionable, resulting in the dependence on the natural products by more than 80% of the total population in the developing world, because of its time tested safety and efficacy. A huge number of natural product-derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidate Even though popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects, but their safety and efficacy was always remained questionable, resulting in the dependence on the natural products by more than 80% of the total population in the developing world, because of its time tested safety and efficacy. A huge number of natural product-derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidate.

1.1 Study Design

Healthy adult male Wistar rats with bodyweight 130-150 gram were collected from the Department of Pharmacy of Jahangirnagar University, Savar, Dhaka, Bangladesh and the rats were kept under controlled temperature with 12±1h light/dark cycle at the Institute of Nutrition & Food Science, University of Dhaka. The animals were fed with standard pellet diet and water ad libitum. Before initiating the study, the rats were kept there for acclimatization. After that bodyweight of each rat was measured and were divided into following groups where an even distribution of rodent as per their body weight has been taken place and each group contained 5 rats.

Table 1.To initiate the study, the rodents are arranged into the following group

Carrageenan-Induced			***Acetic acid-induced***			***Tail flick***		
Group Number	**Group Specification**	**Dose**	**Group Number**	**Group Specification**	**Dose**	**Group Number**	**Group Specification**	**Dose**
1	Negative Control (N.C)	N/A	1	Acetic Acid	N/A	1	(Tail flick Test) TFT	N/A
2	Carrageenan (Car)	N/A	2	Paracetamol(P100)+Acetic Acid	100	2	P_{100}+TFT	100
3	Car+ Ibuprofen$_{20}$ (Ib$_{20)}$	20	3	SM500 of HT + Acetic Acid	500	3	SM_{500} of HT + TFT	500
4	Car+ *Spondias mombin*$_{500}$ (SM$_{500)}$ of Hill Track (HT)	500	4	SM500 of LL + Acetic Acid	500	4	SM_{500} of LL + TFT	500
5	Car+ SM_{500} of Low Land(LL)	500	5	SM500 of NB + Acetic Acid	500	5	SM_{500} of NB + TFT	500
6	Car+ SM_{500} of North Bengal (NB)	500						

2. METHODS AND MATERIALS

Paw edema measurement test, the acetic acid-induced writhing test and tail-flick method are used to stimulate the rodent with pain.

2.1 Carrageenan-Induced Acute Inflammatory Model

The effectiveness of anti-inflammatory agent is estimated with the widely recognized Carrageenan prompted rodent paw edema method. The method was applied with a special kind of instrument called plethysmometer to carry out the anti-inflammatory test. The Paw volume of each rodent was recorded initially. 30 minutes after the given doses of the test drugs and extracts, 1% of the freshly prepared carrageenan solution was injected into the sub plantar tissue of the left rear paw. It was given at a dose of 0.1 mL per 100 g body weight to incited edema. At 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 minutes, estimation of the paw volume was carried out after injecting the Carrageenan utilizing a plethysmometer., the rate of the hindrance of edema was then calculated by using Eq. 2

% Inhibition of paw edema = (Vc – Vt / Vc) X 100 [17,18].

2.2 Acetic Acid-induced Writhing Test

The acetic acid-induced writhing test was utilized to detect peripheral analgesic activity [19]. Plant extract and test drug (Ibuprofen) was given 30 minutes prior to the intraperitoneal transportation of acidic acid. The 1% acidic acid (10ml/kg) intraperitoneal injection was administered to elevate the writhes in rats. Starting from 15minutes after infusion of acidic acid, the number of writhes (muscular contraction ions) was quantified for around a period of 60 minutes. To compare and contrast, the number of writhes of each group with the control group, the percent downturn of writhes counts was reconciled to demonstrate as follows:

Percentage of writhes which is one of the parameters to establish analgesic activity was computed by following Eq. 1

$$\left\{\frac{A.\ Control\ mean - Treatment\ mean}{A\ Control\ mean}\right\} \times 100 \quad \text{Eq. 1 [2]}$$

Where $T\ Control$ = the mean number of the writhing of each test group.

$A\ Control$ = The mean number of the writhing of acetic acid control group

2.3 Tail Flick Test Method

Another well-known method demonstrated by Love and Smith, 1941 [20] named Tail-flick method, is used to analysis pain-relieving personal behavior patterns along with slight variety. A radiant heat programmed tail-flick analgesia meter has been performed (UGO BASILE®, Germany) to estimate response latencies of the rats. A suitable thermal condition was maintained by warming the nicrome wire of the device keeping by the assistance of heat controller and a uniform 4 Amps voltage is maintained through the exposed nicrome. In accordance, radiant heat is employed to the tail of the mice 5cm away from the tip of the tail to evaluate discomfort. Response time has been preserved for control rats or animals treating with a test medication and plant extract. The study was undertaken at 0, 15, 30, 45 and 60 minutes after the use of the test substances.

3. RESULTS AND DISCUSSION

3.1 Anti-inflammatory Response of Carrageenan-Induced Acute Inflammatory Model

A statistically significant difference from Table 2 was observed in edema conditions between the negative control group and treatment groups. Like the standard drug, *Spondias mombin* extract significantly reduces edema formation irrespective of geographical variation.

3.2 Analgesic Activity by Acetic acid-induced Writhing Test

It was observed from the Table 3 that *Spondias mombin* extract reduced the abdominal contraction induced by acetic acid writhing test but in a dose-dependent manner. Paracetamol reduced the pain significantly in standard dose. Likewise, *Spondias mombin* extract significantly reduced pain irrespective of geographical variation.

Table 2. Evaluation of anti-inflammatory response of Spondias mombin leaf extract and Ibuprofen through paw edema test in a rat model (*presents the level of significance of result), Here, the percentage of inhibition of inflammatory mediation was calculated by collecting rat´s paw before and after injection shown in Eq. 1

Group	Time				
	0 Minute(Just before carrageenan injection)	1 hour (just before treatment	2 Hours	3 hours	4 hours
N.C	103.77±2.82	103.56±211	103.74±2.68	103.45±2.19	103.44±2.08
Car	101.28±2.39	126.51±3.82	140.47±4.58	145.73±4.43	160.63±4.79
Car+Ib_{20}	102.48±2.28	128.48±4.13	120.33±5.51** 14.34%)	112.08±3.42** (23.09%)	108.65±3.10** (32.36%)
Car+ SM_{500} of HT	100.45±3.47	131.66±5.16	124.59±5.62** (11.30%)	119.24±4.85** (18.18%)	113.16±3.91** (29.55%)
Car+ SM_{500} of LL	104.82±4.38	135.56±4.96	128.37±6.69** (8.61%)	122.45±5.66** (15.97%)	116.89±5.02** 27.23%
Car+ SM_{500} of NB	103.36±4.59	133.62±5.21	127.90±5.17** (8.95%)	121.44±5.11** (16.67%)	116.48±4.47** 27.49%)

Table 3. An evaluation of the Analgesic effect of different doses of Spondias mombin and Paracetamol by acetic acid writhing test (*presents the level of significance of result). Here, the percent of writhes, one of the parameter to demonstrate analgesic activity was calculated by following Eq. 2

Group specification	Dose	Number of writhing	% Inhibition
Acetic Acid		99.73	
P_{100}+Acetic Acid	100	55.89*	43.96%
SM_{500} of HT + Acetic Acid	500	66.15*	33.67%
SM_{500} of LL + Acetic Acid	500	64.37*	35.46%
SM_{500} of NB + Acetic Acid	500	68.51*	31.30%

Table 4. An evaluation of the analgesic activity of Spondias mombin and Paracetamol by the tail-flick test method

Group No	Group Specification	Basal Reaction	Reaction time in second			
			After 15 minutes	After 30 minutes	After 45 minutes	After 60 Minutes
1		2.49±0.66	2.31±1.02	2.49±1.38	3.19±1.79	2.39±0.75
2	P_{100}+TFT	2.85±1.77	3.61±2.19	4.84±2.83	5.31±3.33*	5.78±3.24*
3	SM_{500} of HT + TFT	2.45±1.91	3.52±2.29	3.97±2.66	4.63±3.08	5.10±4.32**
4	SM_{500} of LL + TFT	2.39±2.31	3.17±2.62	3.82±5.17	4.50±4.78**	4.88±5.13**
5	SM_{500} of NB + TFT	2.28±2.13	3.31±1.76	3.69±4.58	4.37±5.13	5.15±4.35

3.3 Analgesic Activity by Tail Flick Test Method

From the data of Table 4 it is clearly stated that the response time of rodents by Tail flick test method using paracetamol and *Spondias mombin* extracts were significantly analogous no matter from which geographical region it has been collected.

4. CONCLUSION

Given the findings above, conjecture about *Spondias mombin* leaf extracts possess significant anti-inflammatory and analgesic properties. The extracts considerably lower the pain. Besides using Carrageenan-Induced Acute Inflammatory Model, Acetic acid-induced writhing test, Tail flick test method, *Spondias mombin* leaf extract's anti-inflammatory and analgesic

properties are traced analogous to that conventional drugs with identical doses irrespective of geographical variation. We, consequently, assume that this plant medicament can be a better way in pain and inflammation management.

5. LIMITATIONS

Only the analgesic and anti-inflammatory effects of *Spondias mombin* are observed. Other effects such as diabetes are not analysed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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