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Original article

Evaluation of Antinociceptive And Anti-Inflammatory Effect of Aqueous Seed Extract of *Carica Papaya* Linn In Albino Rats

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ABSTRACT

Objective: To evaluate the antinociceptive (analgesic activity) and anti-inflammatory effect of *Carica Papaya (cp) linn* (Aqueous seed extract) in albino rats. **Material and Methods:** To evaluate antinociceptive effect of Carica Papaya Aqueous seed extract (3 doses of 5mg/kg, 10mg/kg, 20mg/kg) in albino rats by Comparing their effect with pethidine by using Radiant heat method & Contact heat method. Anti-inflammatory effect of Carica Papaya Aqueous seed extract (3 doses of 5mg/kg, 10mg/kg, 20mg/kg) was assessed comparing their effect with Aspirin in albino rats by inducing acute Inflammation – by Carrageenan-induced rat paw edema method. **Results:** ***Analgesic models:*** Carica Papaya Aqueous seed extract treated groups with 20mg/kg has showed significant increase in reaction time at 90 minutes when compared to control group in all the two analgesic models of experiments. These findings indicate that Carica Papaya Aqueous seed extract has a promising analgesic activity in comparison with pethidine (standard). **Anti-inflammatory model :** Carica Papaya Aqueous seed extract treated groups with 10mg/kg and 20mg/kg has showed significant reduction in paw edema volume at 5 hours when compared to control group in acute inflammation method. These findings indicate that Carica Papaya Aqueous seed extract was exhibiting more anti-inflammatory effect in comparison with aspirin (standard). **Conclusion:** Carica Papya seed extract had shown significant analgesic effect with high dose and significant anti-inflammatory effect with low dose (combination with Aspirin) , moderate dose and high dose.

KEYWORDS: Antinociceptive , Anti-inflammatory effect , *Carica papaya* Linn.

INTRODUCTION

Carica Papaya Linn. is a widely grown perennial tropical tree, the different parts of the plant are attributed with different medicinal values. The green leaves are used for treatment of malaria, gonorrhea, syphilis, amoebic dysentery[1]. Milky juice of unripe fruit is powerful abortifacient, vermifuge, stomach disorders & for enlargement of liver & spleen[1]. Sukka indigenes chew the dry seed of *C. papaya* to alleviate nagging headache and in reducing swollen wounds[2]. Anti-inflammatory activity of ethanolic extract of C. Papaya leaves was demonstrated by Owoyele et.al[3].

The studies evaluating the antinociceptive and anti-inflammatory activity of C. Papaya seeds are limited. It is believed that current analgesia inducing drugs such as opioids and Non-Steroidal Anti-Inflammatory drugs (NSAIDs) are not useful in all cases because of their side effects and low potency. Morphine causes acute morphine poisoning, hypotension, dependence etc, while the NSAIDs are associated with gastric irritation, bleeding, ulcers and perforation[4],[5].

Carica papaya is a medicinal plant that originated from central America which has spread to different parts of the world including African and Nigerian. It is known for its plethora of folkloric uses. It contains two major bioactive compounds, namely papain and chymopapain which are used in brewing, wine –making, textile and tanning industries[6]. As a result search for other alternative analgesic drug has become necessary and beneficial.

The main purpose of the study is to evaluate the antinociceptive and anti-inflammatory activity of Carica papaya aqueous seed extract.

MATERIALS AND METHODS

All the experimental procedures used in this study were reviewed and approved by Institutional Animal Ethical Committee of Kamineni Institute of Medical Sciences, Narketpally, Andhra Pradesh, India[7]. Sixty six adult Wistar albino rats (150- 200g) were obtained from National Institute of Nutrition, Hyderabad were used. Animals were acclimatized to the laboratory environment for 5 to 7 days before being used in the study.

Animals were housed 6 per cage in a temperature and humidity controlled environment under a 12-hour light/dark cycle (lights on at 7 pm). Food and water was available for all animals for their access.

Instruments and Apparatus: The standard methods for measuring analgesic models like tail flick method is analgesiometer, tail immersion method by hot water bath , as described by M.N.Ghosh and Gerhard Vogel[8],[9]. The standard method for measuring acute anti-inflammatory effect is by Plethysmometer[10].

Drugs used in the experiment:

1.Normal saline (control) 2. Pethidine (standard for analgesic drugs) 3.Carica Papaya Aqueous seed extract (test) 4.Carrageenan (to induce inflammation) 5.Aspirin (standard for anti-inflammatory drugs)

Carica papaya seed extract preparation:

Six mature, unripe fruits of *Carica papaya* were collected from a cultivated garden & identified by a qualified botanist. The raw fruits were cut into pieces and the wet seeds are separated out gently and thoroughly rinsed in tap water two times and air-dried at room temperature for 4 weeks. The dried seeds are pulverized into fine powder using a new domestic mixer grinder. 40 g of the powdered *Carica papaya* seeds are boiled in 500 mL of distilled water for 30 minutes after which they were filtered using a piece of clean white cotton gauze. The filtrate was then evaporated to complete dryness at 40 ºC, producing a fine sweet smelling and chocolate color solid residue. The solid residue was pooled together in an air and water-proof container kept in a refrigerator at 4 ºC. From this stock, fresh preparations was made when ever required[1].

Analgesic model: Thirty wistar albino rats weighing 150-200 gms of either sex has been selected for the study which were categorized in five groups as shown in the table-1,with six animals in each group .

Table 1:Grouping of animals, Dose and Route of Administration

Group (n=6)	Drug	Dose	Route
1	Normal saline(NS)	10 ml/kg	I.P
2	Pethidine	9.1 mg/kg	IP
3	C.P	5 mg/kg	Oral
4	C.P	10 mg/kg	Oral
5	C.P	20 mg/kg	Oral

NS=control, Pethidine=standard, IP= Intra peritoneal, CP = *Carica papaya* & n = number of rats in each group.

Tail flick latency: Tail flick latency was assessed by using analgesiometer (heated nichrome wire). Antinociceptive effect was determined according to the time taken for withdrawal of the tail to thermal stimulation. Cut off period of 10sec was taken to prevent damage to the tail. The pain threshold was tested on day 0,15,30, 60 and 90 minutes.

Tail immersion latency: Tail immersion latency was assessed by using hot water bath(55^{o}c) . Antinociceptive effect was determined by the time taken to withdraw the tail clearly outof water as the reaction time . Cut off period of 15sec was taken to prevent damage to the tail.The pain threshold was tested on day 0,15,30, 60 and 90 minutes.

Anti-inflammatory model: Thirty six wistar albino rats weighing 150-200 gms of either sex has been selected for the study which were categorized in six groups as shown in the table-2 , with six animals in each group.

Material: Chemicals used are carrageenan (1%) – phlogistic agent (carrageenan for inducing inflammation), diethyl ether- anaesthetizing agent . Drugs used are standard Aspirin (acetyl salicylic acid 50mg/kg) given orally & carica papaya aqueous seed extract (5,10, 20mg/kg) orally. Instruments used are : Plethysmometer , precision balance & hot air oven.

Methods: Carrageenan induced paw edema [11]: Paw edema volume is measured in ml for control (normal saline 10ml), reference drug Aspirin (50mg/kg), carica papaya three dose (5mg/kg, 10mg/kg,20mg/kg) before carrageenan was injected in to sub plantar region of the left hind paw. Paw volume was measured by plethysmometer at 1hr, 3 hr and 5hr after subplantar injection of phlogistic agent, carrageenan.

Table-2 Grouping of animals, Dose and Route of Administration

Group (n=6)	Drug	Dose	Route
1	Normal saline(NS)	10 ml/kg	I.P
2	Aspirin	50 mg/kg	Oral
3	C.P	5 mg/kg	Oral
4	C.P	10 mg/kg	Oral
5	C.P	20 mg/kg	Oral
6	Aspirin+C.P	50 mg/kg + 5 mg/kg	Oral

NS=control, Aspirin=standard, IP= Intra peritoneal, CP = *Carica papaya* & n = number of rats in each group.

Acute inflammatory model: Thirty minutes after oral drug administration of carica papaya, acute inflammation was induced by injecting 0.05ml of 1% carrageenan in normal saline into the subplantar region of the left hind paw. The paw oedema volume was measured by mercury displacement with the help of a Plethysmometer at 0 ,1, 3 and 5 hr after injecting carrageenan[11]. The difference of paw volume between '0' hour and subsequent readings was considered as edema volume .The % inhibition of edema in various groups was calculated using the formula

$$\% \text{ of oedema inhibition} = 1 - \frac{\text{Paw edema volume in treated groups}}{\text{Paw edema volume in control group}} \times 100$$

Statistical Analysis: All data analysis was completed using SPSS software. Data are expressed as Mean ± SD. One way analysis of variance (ANOVA) followed by Least significant difference (LSD) test for post-hoc analysis was used. P values less than 0.05 was considered statistically significant.

RESULTS

As shown in the (table -3) Carica papaya (20mg/kg) treatment has increased tail flick latency to the radiant heat at 90 minutes compared to zero minute . Further Carica papaya treated animals tail with drawl latency after immersion in hot water (50 ± 5^0c) at 90 minutes has also increased compared to zero minute (table-4). The above observations indicate statistically significant ($P < 0.001$) for analgesic doses. Carica papaya (10 & 20mg/kg) treatment and combination group (Carica papaya +50 mg Aspirin) has showed significant decrease in paw edema volume from 1 hour to 5 hours (table-5) which is more efficacious than Aspirin (50mg/kg) in reducing the paw edema volume from 1 hour to 5 hours . Observations indicate statistically significant ($P < 0.001$) for anti-inflammatory doses.

Table-3 Comparison of tail flick latency in seconds(Mean±SD) in CP treated albino rats:

	GROUP-I	GROUP-II	GROUP-III	GROUP-IV	GROUP-V	ONE WAY ANOVA
Treatment/ Minutes	Normal saline	Pethidine 9.1 mg/kg	*C.P* 5 mg/kg	*C.P* 10 mg/kg	*C.P* 20 mg/kg	P-value
0 min	2.91±0.20	3.08±0.20	2.91±0.20	3.16±0.25	3.08±0.20	0.204
15 min	3.08±0.37	5.5±0.44	3.0±0.31	3.20±0.25	3.22±0.37	0.001
30 min	3.25±0.27	7.41±0.37	3.16±0.25	3.25±0.27	5.91±0.58	0.001
60 min	3.16±0.25	9.66±0.4	3.16±0.25	5.91±0.58	8.0±0.44	0.001
90 min	3.33±0.25	9.83±0.25	3.33±0.25	7.33±0.40	9.5±0.44	0.001

CP = *Carica papaya*

Table-4 Comparison of tail immersion latency in seconds (Mean±SD) in CP treated albino rats:

	GROUP-I	GROUP-II	GROUP-III	GROUP-IV	GROUP-V	ONE WAY ANOVA
Treatment/ Minutes	Normal saline	Pethidine 9.1 mg/kg	*C.P* 5 mg/kg	*C.P* 10 mg/kg	*C.P* 20 mg/kg	P-value
0 min	2.83±0.25	3.08±0.20	3.16±0.25	3.16±0.25	3.00±0.31	0.180
15 min	3.0±0.31	7.66±0.40	3.16±0.25	3.41±0.58	4.83±0.51	0.001
30 min	3.25±0.27	10.66±0.81	3.25±0.27	5.91±0.58	7.25±0.52	0.001
60 min	3.16±0.25	14.00±0.89	3.16±0.25	6.91±0.58	10.08±0.58	0.001
90 min	3.58±0.37	14.6±0.51	3.83±0.6	8.66±0.51	14.2±0.27	0.001

CP = *Carica papaya*

Table-5 Comparison of paw edema volume of different groups

	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	ONE WAY ANOVA
Treatment / hrs	Normal saline	Aspirin 50 mg/kg	*C.P* 5 mg/kg	*C.P* 10 mg/kg	*C.P* 20 mg/kg	*Group-II +group-III*	P-value
1hr	0.5±0.14	0.4±0.06	0.38±0.07	0.4±0.06	0.3±0.09	0.08±0.08	0.06
3hr	0.51±0.19	0.45±0.08	0.45±0.08	0.25±0.10	0.2±0.09	0.05±0.05	0.001
5hr	0.65±0.14	0.4±0.06	0.4±0.06	0.1±0.06	0.1±0.1	0.05±0.05	0.001

DISCUSSION

Medicinal plants are believed to be important source of new chemical substance with potential therapeutic effect and plant species that traditionally have been used as pain killers should be seen as strategy in research for new antinociceptive drugs[12]. Among several plants, the strongest candidate was carica papaya with papain and chymopapain as active ingredients[13],[14],[15]. We therefore have chosen this study the effect of carica papaya seed extract on analgesic and anti-inflammatory effect in carragenan induced inflammation.

Tail flick method and tail immersion method are the most common test for evaluating the analgesic effects of chemicals in rodents and Caragenan induced inflammation is the most common test for evaluating the anti-inflammatory effect of chemicals. Papain and chymopapain is used for pain and swelling (inflammation) as well as fluid retention following trauma and surgery. The mechanism by which pain relief is obtained is not clearly understood[16].

Carica papaya treated group with high doses showed significant analgesic effect and anti-inflammatory effect with all the three dose. In combination with standard Aspirin low dose also showed significant promising anti-inflammatory effect. Hence many more studies are required to confirm the analgesic and anti-inflammatory effect of caricapapya.

CONCLUSION

Carica papaya treated group with high doses showed significant analgesic effect and anti-

inflammatory effect with all the three dose. In combination with standard Aspirin low dose also showed significant promising anti-inflammatory effect. However further cellular, sub cellular and clinical studies are required for definitive antinociceptive and anti-inflammatory action of carica papaya seed extract.

REFERENCES

1. Adeneye AA, Olagunju JA, Banjo AA. The Aqueous Seed Extract Of Carica papaya Linn. Prevents Carbon Tetrachloride Induced Hepatotoxicity In Rats. International Journal of Applied Research in Natural Products. June 2009; Vol. 2(2):19-32.

2.Anaga AO, Onehi EV. Antinociceptive and anti-inflammatory effects of the methanol seed extract of Carica papaya in mice and rats. African Journal of Pharmacy and Pharmacology. April 2010; Vol. 4(4): 140-4.

3.Owoyele BV, Adebukola OM , Funmilayo AA. Anti-inflammatory activity of ethanolic extract of carica papaya.Inflammo pharmacology(2008);16(4): 168-73.

4.Sharma HL,Sharma KK. Non steroidal Anti-inflammatory Agents, Drugs for Gout and Antirheumatic Drugs. In: Sharma HL,Sharma. Principles of Pharmacology11 thed. Hyderabad:Paras Publishers;2011.p.366-372.

5.Tripathi KD.Opiod Analgesics and Antagonists. In:Tripathi KD.Essentials of Medical Pharmacology, 5th ed. New Delhi :2004.p.422-424.

6.Brocklehurst, K. and Salih, E. (1985). Fresh non-fruit latex of Carica papaya contains papain and multiple forms of chymopapain A and papaya proteinase OMEGA. Biochem.J.228(2): 525-527.

7.Yamamura H,Zimmermann M. M.Ethical guidelines for investigators of experimental pain in conscious animals.pain.1983;16:109-110.

8.Ghosh MN.Some common evaluation techniques. In: Fundamentals Of Experimental Pharmacology,5th ed.Kolkata:Hilton and company;2011.p.151-52.

9.Gerhard Vogel H.Drug Discovery and Evaluation,Pharmacological assays ,2nd ed, Radiant heat method 2002. p.694-97.

10.Ghosh MN.Some common evaluation techniques.In:Fundamentals Of Experimental Pharmacology, 5th ed Kolkata: Hilton and company;2011.p.154.

11.Winter CA, Risley EA,Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc.soc.Exp.Biol.Med 1962 ;111: 544 - 47.

12.Hejazian SH,Mosadelegh MH.Antinociceptive effect of caricum copiticum extract in mice using formalin test.World appl.sci.j 2008; 3(2) : 215-19.

13.Holt H.T . Carica papaya as an ancillary therapy for athletic injuries. Current Therapeutic Research 1969;11: 621-24.

14.Starkov GL, Osna AI ,Rossinky VI .Papain as a therapeutic enzyme in medicine. Klinichestkaya Medistsina 1978; 56(8): 119-22.

15.Novey HS, Keenan W J, Fairshter RD.Pulmonary disease in workers exposed to papain.Clinico-physiological and immunological studies .Clinical Allergy 1980;10 (6): 721-31.

16. Jenner JR, Buttle DJ, Dixon AK. Mechanism of action of intradiacal chymopapain in the treatment of sciatica. A clinical , biochemical and radiological study. Annals of the Rheumatic diseases .1986 ; 45(6):441-49.

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