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EVALUATION OF LAXATIVE EFFECT OF ETHANOL EXTRACT OF LEAVES OF *ANNONA MURICATA L.*

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ABSTRACT

This study was aimed to assess the possible laxative effect ofethanol extract of leaves of *Annona muricata L.*in albino's wistar rats. The laxative activity was determined based on the weight of the faeces matter. The effects of ethanol extract of leaves of *Annona muricata L.* and reference standard on the gastro intestinal motility rate were also evaluated. The ethanol extract of leaves of *Annona muricata L.* administered orally at two different doses produced significant laxative activity and reduced loperamide induced constipation in dose dependent manner. The effect of the extract at 200 and 400 mg/kg (p.o.) was similar to that of reference drug sodium picosulfate (5 mg/kg, p.o). The same doses of the extract (200 and 400 mg/kg, p.o.) produced a significant increase ($p < 0.01$) of intestinal transit in comparison with castor oil (2 ml) ($p < 0.01$). The results showed that the ethanol extract of leaves of *Annona muricata L.* has a significant laxative activity.

KEY WORDS: Laxative, Loperamide, Constipation, Gastro intestinal motility, Intestinal transit.

INTRODUCTION

Constipation also known as costiveness refers to bowel movements that are infrequent and/or hard to pass. Constipation is a symptom with many causes. These causes are of two types: obstructed defecation and colonic hypomobility. About 50% of patients evaluated for constipation at tertiary referral hospitals have obstructed defecation. This type of constipation has mechanical and functional causes. Causes of colonic slow transit constipation include diet, hormones, side effects of medications, and heavy metal toxicity. Laxatives are among the most widely used drugs. However, their consumption is limited due to insufficient efficacy or the side effects, especially when used continuously or with contraindications. Bloating, cramping, diarrhea, and metabolic disturbances such as hypercalcemia, hyperphosphatemia, hyponatremia, and hypokalemia are among the most common side effects [1,2].

*Annona muricata*L. (Family:Annonaceae) is a small tree to 7 m tall. Leaves alternate, petiolate, theblades leathery and oblong-lanceolate. Flowers 3-merous, sepals andpetals fleshy, and greenish in colour. Fruit a fleshy syncarp with a greenexocarp covered with conspicous long pseudo-spines, mesocarp asomewhat fibrous juicy sweet-sour flesh surrounding several largesmooth black seeds. Flowers and fruit are usually available throughoutthe year.Habitat: Cultivated at lower elevations.It is distributed in native to tropical America and introduced to the South Pacific as a fruit tree within the last 100 years. It contains many Constituents such as Annomonicin, annomontacin, annomuricins, annonacins and derivatives, annonacinone, anomuricine, anomurine, anonaine, anoniine. atherospermine, atherosperminine, corepoxylone, corossolone, coclaurine, coreximine, epomuricenins, gigantetrocins, gigantetronenin, deacetyluvaricin, gomothalamicin, howiicins, montanacin, muricatacin, muricatetrocins, muricatocins, murihexocins, murisolin, rolliniastatin, solamin, muricine, muricinine, reticuline, lipids, monotetrahydro-furan acetogenins, beta-sitosterol, stigmasterol, andtannins. It is reported as Antimalarial, smooth muscle relaxant, uterine stimulant, anticrustacean, antiparasitic, cytotoxic (acetogenins), cardiac depressant, antiamoebic, antibacterial, antifungal, hypertensive,

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spasmogenic,vasodilator, insecticide, smooth muscle relaxant. It is traditionally used in Tonga, an infusion of the leaves is used in treating stomach ailments [3-8].Therefore, the present was evaluating the laxative activity of ethanol extract of *Annona muricata*L. to prove the traditional claim of this plant.

MATERIALS AND METHODS

Collection and authentication of plant material

The Plant material of *Annona muricata L.*leaveswas collected from Tirunelveli District, in the Month of December 2012. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The leaves of the *Annona muricata L.* are properly washed in tap water and then rinsed in distilled water. The rinsed leaves are dried in an oven at 35^{o}C for 4 days. The dried leaves of *Annona muricata L.* was crushed to obtain powder. These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The ethanol extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of ethanol extract of leaves of *Annona muricata L.* was found to be 8.5 % w/w.The ethanol extract was administered to the animals by suspending each time in 1%w/v SCMC.

Experimental animals

Adult Wistar rats of either sex weighing 180-250 gms were used in pharmacological and toxicological studies. The inbred animals were taken from the animal house and maintained in a well-ventilated room with at 12:12 hr light, dark cycle in polypropylene cages and maintained at 22±1˚C with humidity at 55±5%. They were fed balanced rodent pellet diet from Poultry Research station, Nandanam, Chennai-35 and tap water ad *libitum* throughout the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).

Acute toxicity study

The acute toxicity of ethanol extract of leaves of *Annona muricata L.* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [9].

Method I: Laxative Activity Test

The method of Capasso et al. [10]was followed for this activity. Rats fasted for 12 h before the experiment were placed individually in cages lined with clean filter paper. Group I: received vehicle 1%w/v SCMC (2 ml, p.o.) considered as negative control. Group II: received standard drug sodium picosulfate (5 mg/kg, p.o) considered as positive control. Group III& IV receivedethanol extract of leaves of *Annona muricata L.*, (200& 400 mg/kg) p.o. suspended in 1%w/v SCMC respectively.The administration was done using metal oropharyngeal cannula. The faeces production (total number of normal as well as wet faeces) in all four groups was monitored for 16 h.

Method II: Laxative activity on loperamide induced constipation in rats

This study was carried out, as earlier described by Takahura et al. [11]. Rats were placed individually in cages lined with clean filter paper, allowed to fast for 18 hours and divided into four groups of six animals each. Group I: received vehicle 1%w/v SCMC (2 ml, p.o.) considered as negative control. Group II: received standard drug sodium picosulfate (5 mg/kg, p.o) considered as positive control. Group III& IV receivedethanol extract of leaves of *Annona muricata L.*, (200 & 400 mg/kg) p.o. suspended in 1%w/v SCMC respectively.After 1 h treatment, all the group animals received Loperamide (5 mg/kg, p.o.) by oral gavage. The faeces production (total number of normal as well as wet faeces) in all four groups was monitored for 8 h.

Method III: Gastrointestinal motility tests in rats

The method of Mascolo et al. [12] was used. Rats were divided into different groups of six rats each and fasted for 18 hours before the experiment. Group I: received vehicle 1%w/v SCMC (2 ml, p.o.) considered as negative control.Group II: received castor oil (2 ml/rat, p.o.) considered as positive control positive control.Group III& IV receivedethanol extract of leaves of *Annona muricata L.*, (200 & 400 mg/kg) p.o. suspended in 1%w/v SCMC respectively.

After 30 min, the animals were given 1 mL of freshly prepared charcoal meal (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal). Following 30 min of charcoal administration, the rats were sacrificed by cervical dislocation and the abdomen immediately cut open, to excise the whole small intestine (pylorus region to caecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured for obtaining the charcoal transport ratio or percentage.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test *P* values less than 0.05 were considered as significance.

RESULTS

Effect of the ethanol extract of *Annona muricata L.* on Laxative activity

In this study, the ethanol extract of *Annona muricata L.* showed increase in fecal output of rats when compared to the control group (table 1). The effects of the ethanol extract of *Annona muricata L.* at the doses of 200 & 400mg/kg (p.o.) increased significantly fecal output of rats compared to control group ($p < 0.01$). The effect of the theethanol extract of *Annona muricata L.* at the doses of 400mg/kg (p.o.) was comparable to that of the standard drug sodium picosulfate (5 mg/kg, p.o.).

Effect of the ethanol extract of *Annona muricata L.* on loperamide induced constipation in rats

In the loperamide-induced constipation, the ethanol extract of *Annona muricata L.*at the doses of 200 & 400mg/kg (p.o.), increased the total number of faeces and the results were statistically significant ($p < 0.01$) (Table 2). The dose of 400mg/kg the ethanol extract of *Annona muricata L.*maximum increased the total number of faeces. The reduction of the loperamide induced constipation at 400 mg/kg (p.o.) of the ethanol extract of *Annona muricata L.* treatment was found to be almost comparable with that of treatment by 5 mg/kg of sodium picosulfate.

Effect of the ethanol extract of *Annona muricata L.* on gastrointestinal motility

The results of gastrointestinal motility test are reported in Table 3. Theethanol extract of *Annona muricata L.*increased propulsion of the charcoal meal through the gastrointestinal tract. The dose of 400mg/kg ethanol extract of *Annona muricata L.*significantly increased in the propulsion of charcoal meal compared to control group (1%w/v SCMC, p.o.) ($p< 0.01$). Castor oil (2 ml/rat, p.o.) produced greater gastrointestinal motility effect, similarlyethanol extract of *Annona muricata L.*(400mg/kg) treated animals showed greatly increased the small intestinal transit.

Table 1. Effect of the ethanol extract of *Annona muricata L.* on Laxative activity

Treatment & Dose	Faeces output (g)	
	0-8h	8 h-16 h
Group I Vehicle 1%w/v SCMC, (2 ml, p.o.)	1.88±0.0322	1.24±0.0564
Group II Sodium Picosulfate (5 mg/kg, p.o)	5.64±0.0547**	5.45±0.0208**
Group III Ethanol extract of leaves of *Annona muricata L.*, (200mg/kg) p.o.	2.42±0.0366**	1.72±0.0252**
Group IV Ethanol extract of leaves of *Annona muricata L.*, (400mg/kg) p.o.	4.56±0.0243**	4.22±0.0421**

Values are expressed as mean ± SEM (n=6), *p<0.05; **p<0.01 denotes significance with respect to the control group using one way ANOVA followed by Dunnett's test.

Table 2.Effect of the ethanol extract of *Annona muricata L.* on loperamide induced constipation in rats

Treatment & Dose	Weight of faeces (g)
	0-8h
Group I Vehicle 1%w/v SCMC, (2 ml, p.o.)	1.12±0.026
Group II Sodium Picosulfate (5 mg/kg, p.o)	4.52±0.0322**
Group III Ethanol extract of leaves of *Annona muricata L.*, (200mg/kg) p.o.	1.62±0.0120*
Group IV Ethanol extract of leaves of *Annona muricata L.*, (400mg/kg) p.o.	3.46±0.0354**

Values are expressed as mean ± SEM (n=6), *p<0.05; **p<0.01 denotes significance with respect to the control group using one way ANOVA followed by Dunnett's test.

Table 3. Effect of the ethanol extract of *Annona muricata L.* on gastrointestinal motility

Treatment & Dose	Small intestinal transit		
	Total length of intestine	Distance traveled by marker	% of intestinal transit
Group I (Buttermilk 3 ml, p.o.)	113.64±1.422	29.42±1.740	25.89
Group II Castor oil (2 ml/rat, p.o.)	110.52±1.262	79.62±1.366**	72.04
Group III Ethanol extract of leaves of *Annona muricata L.*, (200mg/kg) p.o.	114.67±1.222	40.29±1.277*	35.14
Group IV Ethanol extract of leaves of *Annona muricata L.*, (400mg/kg) p.o.	112.48±1.152	70.94±1.842**	63.07

Values are expressed as mean ± SEM (n=6), *p<0.05; **p<0.01 denotes significance with respect to the control group using one way ANOVA followed by Dunnett's test.

Figure 1. Effect of the ethanol extract of *Annona muricata L.* on Laxative activity

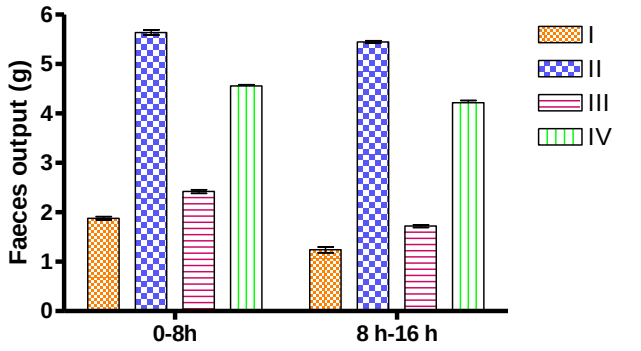


Figure 2.Effect of the ethanol extract of *Annona muricata L.* on loperamide induced constipation in rats

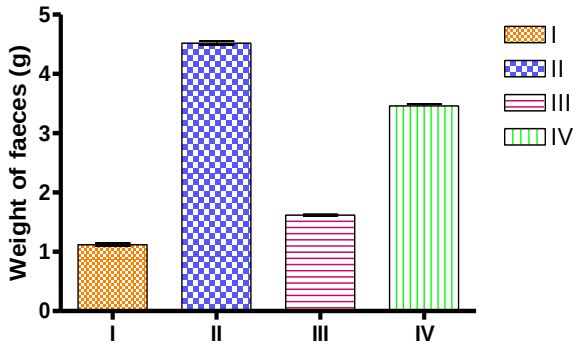
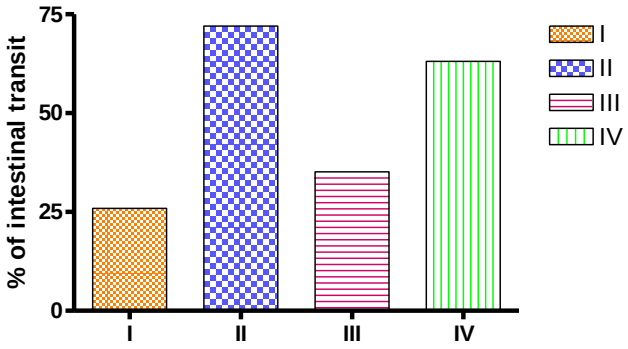


Figure 3. Effect of the ethanol extract of *Annona muricata L.* on gastrointestinal motility



DISCUSSION AND CONCLUSION

The laxative activity of ethanol extract of *Annona muricata L.*was studied in albino wistar rats. The results showed that an oral administration of the ethanol extract of *Annona muricata L.*produced significantly increase in faeces output of rats and the stimulation of gastrointestinal motility. These effects were comparable with that of castor oil (standard drug) at dose of 2ml/rat.

Castor oil causes diarrhea due to its active metabolite ricinoleic acid and its affects electrolyte transport and smooth muscle contractility in the intestine. Its cathartic action is due to water accumulation in the intestine [13,14]. This stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. The observed activities therefore suggest that laxative activity of the ethanol extract of *Annona muricata L.*may be mediated through this mechanism of action of ricinoleic acid.

The results showed that the ethanol extract of *Annona muricata L.*significantly increased the propulsion of charcoal meal. The propulsion of charcoal meal is probably due to the increasing of peristaltic movement in rat gastrointestinal tract resulting from the stimulation of cholinergic receptors by ethanol extract of *Annona muricata L.* [15].The Presence of phytoconstituents like terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids [16] have been previously found to be responsible for laxative activities in plants. Phytochemical screening of the extract of *Annona muricata L.* revealed the presence of flavonoids, polyphenols, and polyterpenes. These constituents may be responsible for the laxative activity of *Annona muricata L.*

The results of this study justify the use of the leaves of *Annona muricata L.* as laxative in traditional medicine. Further studies may be directed at characterizing the bioactive ingredients that are responsible for the observed activity in the plant.

REFERENCES

1. Chatoor D, Emmnauel A. Constipation and evacuation disorders. *Best Pract Res ClinGastroenterol*, 23(4), 2009, 517–30.
2. Washabau RJ. Gastrointestinal motility disorders and gastrointestinal prokinetic therapy. Vet Clin North Amer. *Small AnimPract*, 33, 2003, 1007-1028.
3. Hisham Aet al. Epoxymurins A and B, Two Biogenetic Precursors of Annonaceous Acetogenins from *Annona muricata*. *Tetrahedron*, 49(31), 1993, 6913-6920.

4. Roblot F. et al. Two acetogenins from *Annona muricata* seeds. *Phytochemistry*, 34(1), 1993, 281-285.
5. Zeng Let al. Five new monotetrahydrofuran ring acetogenins from the leaves of Annona muricata. *Tet. Lett.,* 36(30), 1995, 5291-5294.
6. Wu Fe al. Two new cytotoxic monotetrahydrofuran Annonaceous acetogenins, annomuricins A and B, from the leaves of Annona muricata. *J. Nat. Prod*., 58(6), 1995, 830-836; 902-908; 909-915.
7. Sundarrao Ket al. Preliminary screening of antibacterial and antitumor activities of Papua New Guinean native medicinal plants. *Int. J. Pharmacol*., 31(1), 1993, 3-6.
8. Weiner MA. Ethnomedicine in Tonga. *Econ. Bot*., 25, 1971, 423-450.
9. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.
10. Capasso F, Mascolo N, Autore G, Romano V. Laxatives and the production of autacoids by rat colon. *J pharm Pharmacol*, 38, 186, 627-629.
11. Takaharu S, Fujie M, Yuji L, Kouji M, Hideo K. Laxative and Antidiarrheal activity of polycarbophil in mice and rats. *Jpn J Pharmacol*, 89, 2002, 133-141.
12. Mascolo N, Izzo AA, Avtore G, Barboto F, Capasso F. Nitric oxide and castor oil induced diarrhea. *J PharmacolExpTherap*, 1994, 268:291-295.
13. Chitme HR, Chandra R, Kaushik S. Studies on Antidiarrheal activity on calotropisgigantean in experimental animals. *J Pharm PharmaceutSci*, 7, 2004, 70-75.
14. Ammon HV, Thomas PJ, Phillips SF. Effect of the oleic acid and ricinoleic acid net jejunal water and electrolyte movement. *J Clin Invest*, 53, 1974, 374-379.
15. Galvez J, Zavzuelo A, Crespo ME, Lorente MD, Ocete MA, Jimenez J. Anti diarrhoeic activity of Euphorbia hirta extract and isolation of an active flavonoid constituent. *Planta Med*, 59, 1993, 333-336.
16. Longanga-Otshudi A, Vercruysse A, Foriers A. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomela area, Democratic Republic of Congo (DRC). *J Ethnopharmacol*, 71, 2000, 411-423.