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Research Paper

Anti-diarrhea Effect of Aqueous Extracts of *Momordica balsamina* and *Stachytarpheta indica* in Rats.

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ABSTRACT

Momordica balsamina and *Stachytarpheta indica* are used in Nigeria for the management of diarrhea. Crude extract of Leaves and stem of *M. balsamina* and whole plant of *S. indica* were evaluated on perfused isolated rabbit jejunum and castor oil-induced diarrhea model in rats. The aqueous crude extract of *M. balsamina* (0.4, 0.8 and 1.6 mg/ml) produced a dose dependent transient contractile response followed by relaxation of isolated rabbit jejunum. *S. indica* (0.2, 0.4 and 0.8 mg/ml) produced dose dependent sustained contraction without relaxation. In the castor oil-induced diarrhea model, 87.5 and 64.5% protections were produced by extracts of *M. balsamina* and S. indica respectively. These results revealed that the aqueous extracts of *M. balsamina* (0.1, 0.25 and 1.0 g/Kg) and *S. indica* (1.5 and 3.0 g/Kg) have antidiarrhea activity, with *M. balsamina* exhibiting greater potency. Their effects on smooth muscle showed that *M. balsamina* will be more useful than *S. indica* in the management of diarrhea. The effect of *M. balsamina* was comparable to that of loperamide, a strong antidiarrhea drug, in castor oil induced diarrhea. It can not be concluded that the extract of *S. indica* has antidiarrhea effect since the results from the two methods used were contradictory. This may partially explain their use in traditional medicine for the treatment of diarrhea. Investigations on the traditional use of *S. indica* revealed that it is used for managing both diarrhea and constipation. This is supported by both results; castor oil induced diarrhea for its antidiarrhea use and smooth muscle contraction for its use in constipation. *M. balsamina* extract was shown from this study to have antidiarrhea properties. These results support their use in folk medicine.

Keywords: Antidiarrhea, Castor oil, Medicinal plants, Aueous extracts, Smooth muscle.

INTRODUCTION

Diarrhea is defined as the passage of abnormal liquid or unformed stool at an increased frequency (Ahlquist, et al., 2001). Causes of Diarrhea include Infectious agents, certain medications, plant and animal toxins, GIT (gastro-intestinal) disorders, and substances that increase gastrointestinal tract secretions. It can also be caused by the ingestion of poorly absorbable materials, or inflammatory and dysmotility problems of the gastro-intestinal tract (Ahlquist, et al., 2001). Traditional herbal practitioners often use medicinal herbs for the management of diarrhea and other related health problems. Some herbal medicines (e.g. *Xylocarpus granatum* and *Guiera senegalensis*) have been reported to be scientifically effective in the management of diarrhea.

In Nigeria, diarrhea resulting from infection is one of the known killer diseases among children under 5 years (Audu, et al., 2000). This may not be unconnected to the fact that toddlers are at times left to play in unhygienic environment. Such exposure can lead to bacterial and viral infections, which can cause diarrhea. Diarrhea is also associated with some terminal illness like AIDS, and if untreated can lead to demise of the patient. Diarrhea accounts for about 4-5 million deaths annually, of these 8% have been reported to be from developing countries, putting a heavy burden on the country's health budget (Syder and Merson, 1982). Patients who cannot afford the cost of treatment with orthodox medicines often resort to the use of herbs for the management of this disorder.

Momordica balsamina Linn is a climber or trailer with stems attaining 4-5mm in length (Burkill, 1985). It is locally known as Balsam apple (English), Garahuni (Hausa), Akbon-ndewe (Igbo) and Ejirin (Yoruba) (Burkill, 1985). The whole plant is used as a bitter stomachic and an infusion is used as a wash in the management of fevers and yaws (Dalziel, 1937). A macerate of the whole plant is also used as a galactogogue and to massage the chest to relieve intercostals pains (Burkill, 1985).

Stachytarpheta indica Vahl, known locally as snake weed (English), Tsarkiyar kusu (Hausa); Írù amure (Yoruba), Brazilian tea and devil's couch (Burkill, 1985). The plant has been used locally as an Abortifacient, and in the management of Asthma, Headache, Alopecia, Bronchitis, Bruise, Chest Cold, Constipation, Itch, Diarrhea, Skin Sore, Vermifuge, Dysentery, Dysmenorrhea, Erysipelas, Fever, Inflammation, Liver Disease, Poisoning, Tumor, Venereal Disease, Cataract, Sedative, Anti-Fertility, Rheumatism (Anyensu, et al., 1978). In northern Nigeria, a decoction of the leaves with natron is given for dysentery in humans and for similar conditions in horses (Burkill, 1985). The aim of present work is to investigate the effect of *M. balsamina* and *S. indica* on castor oil induced diarrhea, compare the efficacy of both plants and attempt to provide a scientific basis for their traditional use in the treatment of diarrhea.

MATERIALS AND METHODS

***Collection and preparation of plant**: M. balsamina* Linn was collected from Babale, Plateau State in June 2005 and authenticated at the School of Forestry, Jos by Mr. Abdulkareem. While *S. indica* was collected from the University of Jos, premises in September 2005, by uprooting the whole plant and shaking off the soil and other debris from the roots. They were authenticated by Prof. S.W.H Husseini of the Department of Botany, University of Jos. The air-dried plants were milled to a coarse powder and macerated in water for 24 hours. The extracts were concentrated to dried powder (Sofowora, 1993) and the resultant powder stored in the desicator till use.

***Collection and maintenance of animals**:* New Zealand rabbits (*Oryctolagus cuniculus*) weighing 1.0 Kg and Wister Albino rats (150-220g) of both sexes were obtained from animal house of Pharmacology

Dept., Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. The animals were fed with standard laboratory feeds (Vital Feeds, Nigeria) and tap water (*ad libitum*)

This research was carried out in University of Jos, according to the rules in Nigeria governing the use of laboratory animals as acceptable internationally.

Effects on isolated rabbit jejunum: Rabbits were sacrificed by a blow on the head, dislocating the neck, and exsanguination. Segments of the jejunum, about 2.0 cm long, were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution (NaCl, 136.8; KCl, 2.7; CaCl, 1.3; NaHCO3, 12.0; MgCl, 0.5; NaPO4, 0.14; glucose, 5.5. millimole). The tissue was mounted in a 50 ml organ bath containing Tyrode's solution maintained at 37^0C and aerated with air. A load of 0.5 g was applied. Equilibration period of 60 minute was allowed during which the physiological solution was changed at every 15 min. At the end of the equilibration period, the effects of Acetylcholine ($1x10^{-5}$ g/ml), Histamine ($1x10^{-5}$ g/ml), and Adrenaline ($1x10^{-5}$ g/ml) were determined. The effects of graded doses of the extracts of Leaves and stem of *M balsamina* (0.4, 0.8 and 1.6 mg/ml) and whole plant of *S. indica* (0.2, 0.4 and 0.8 mg/ml) were recorded by using Ugo Basile Unirecorder 7050. Also the effects of the various doses of the two extracts in the presence of antagonists: Atropine ($1x10^{-5}$ g/ml), and Nifedipine ($1x10^{-7}$ g/ml), which were incubated for 3 minutes prior to the introduction of the extract, was determined by using Ugo Basile Unirecorder 7050, instrument. The contact time for each concentration was 1 min, which was followed by washing three times. The tissue was allowed a resting period of 15 min before the next addition. Responses were recorded iso-metrically using Ugo Basile Unirecorder 7050 (Amos, et al., 1998).

Effects on castor oil-induced diarrhea in rats: The method described by Galvez, et al., (1993) was employed though modified to suit experimental needs. Wister albino rats (weighing 150-220g) were randomly placed into six groups each of six animals and housed in separate cages. One cage housed one animal during the experiment. Animals of first group received 1ml distilled water orally, while those in groups 2, 3 and 4 were pre-treated with *M. balsamina* Linn extract (0.1 g/kg, 0.25 g/kg, and 1.0 g/kg respectively) intra-peritoneally. The standard drug Loperamide (50 mg/kg, intraperitoneally) was administered to the fifth group and the last group was pre treated with distilled water, IP. Animals of groups 2 to 6 received castor oil orally using the orogastric canula 30 minutes after pre treatment with the extracts or standard drugs or distilled water.

Following treatment with castor oil, the animals were then placed in separate cages over clean white paper and were inspected up to six hours (by an observer unaware of the particular treatment) for the presence of the characteristic diarrheal droppings; their absence was recorded as a protection from diarrhea (Diurno, et al., 1996) and the percentage protection calculated. The same procedure was used for *S. indica* extract (1.5 g/kg and 3.0 g/kg). *S. indica* extract was administered orally. Intravenous administration of *S. indica* has been reported to be very toxic in mice with an LD_{50} less than 0.15 g/kg. (Otimenyin, et al., 2006).

Statistical analysis: The result on castor oil-induced diarrhea was analyzed by Two Way ANOVA and Student't' test. They were regarded as significant when $P<0.05$.

RESULTS

Effects on isolated rabbit jejunum: The effects of the plant extracts on jejunum revealed that *M. balsamina* produced an initial transient contraction followed by relaxation (Figure-1) while *S. indica* produced sustained contraction which was reduced by atropine but not nifedipine (Figure-2, 3). The initial contraction observed with *M. balsamina* was antagonized by atropine and to a greater extent by nifedipine.

Effects on castor oil-induced diarrhea: The extracts and loperamide (50 mg/kg) significantly ($P< 0.05$) protected rats against castor oil-induced diarrhea when compared with the control. *M balsamina and S. indica* gave 87.5% (Table-1) and 64.5% (Table-2) protection respectively, while loperamide gave 100% protection (Table-1, 2).

DISCUSSION

In developing countries, diarrhea is a major cause of infant mortality and morbidity (Shoba and Thomas, 2001). Despite the availability of vast spectrum of approaches for diarrheal management, a vast majority of the people in these developing countries rely on herbal drugs for the management of diarrhea. WHO has encouraged studies for treatment and prevention of diarrheal diseases using traditional medical practices (Atta and Mouneir, 2004). *M. balsamina and S. indica* are examples of medicinal plants used locally for the management of diarrhea. In present study, the extracts of both plants exhibited antidiarrheal activity. Diarrhea induced by castor oil, occurs when there is hydrolysis of the oil by intestinal lipases resulting in the release of ricinoleic acid. The ricinoleic acid released produces an irritating reaction on the wall of the intestine thus enhancing the peristaltic activity of the small intestine. Also ricinoleic acids like other anionic surfactants reduce the net absorption of water and electrolytes (Almieda, et al., 1995) causing diarrhea. This effect coupled with the former is responsible for the diarrhea observed when castor oil is administered. Loperamide, a drug widely used in the management of diarrhea disorders was reported to be effective in the prevention of diarrhea induced by castor oil, prostaglandins, and cholera toxin (Farack, et al., 1981). The pharmacological effect of loperamide is due to its anti-motility and anti-secretory properties (Karim and Adeikan, 1977). From our investigation, it is likely that the plant extracts mediate their effects through similar mechanisms. Prostaglandins are implicated in the patho-physiology of diarrhea, (Haruna, et al., 1997). The phyto-chemical analysis of *M. balsamina* (Otimenyin, et al., 2005) *and S. indica* (Otimenyin, et al., 2006) revealed that they contain flavonoids. Flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) (Moroney, et al., 1988) there by inhibiting prostaglandin production. The activation of LOX is induced by fatty meals while COX1and COX-2 is by diarrhea-genic agents. Though several constituents are present in the extracts, it is most likely that flavonoids, singly or in combination with tannins, and possibly other constituents, are responsible for the observed anti-diarrhea effects of *M. balsamina* and *S. indica*.

Diarrhea can also be caused by the ingestion of poorly absorbable materials, or inflammatory and dysmotility problems of the gastro-intestinal tract (Ahlquist, et al., 2001). Drugs that have effect on the motility or propulsion rate of the GIT may have antidiarrhea effect. The extract of *M. balsamina* relaxed spontaneously contracting rabbit jejunum after transient contraction. The transient contraction observed was reduced by atropine and Nifedipine (a Ca channel blocker). Nifedipine potentiated the relaxation effect of *M. balsamina,* this suggest that *M. balsamina* contain substances with Ca channel blocking properties. According to Gilani, et al., (2005) the spasmolytic constituents of various plants are mediated through blockage of calcium channels. The total relaxation produced by the extract at higher concentration (Fig-3) and the potenciation of the smooth muscle relaxing effect of *M. balsamina*, supports that assertion. The total relaxation by the combined effect of Nifedipine and *M. balsamina* may be due to a synergistic blockage of calcium channels hence producing a relaxation that could not be produced by the extract alone. The blockage of the calcium channels would result in reduced influx of calcium ions into the sarcoplasmic reticulum, thus causing a reduction in cytosolic calcium ion concentration which in turn causes a reduced binding of calcium to the protein calmodulin. The calcium-calmodulin complex should activate myosin light chain kinase with the resultant phosphorylation of the

light chains. If such phosphorylation occurs, interaction between actin and myosin is promoted resulting in smooth muscle contraction (Gilani, et al., 2005). Hence inhibition of calcium would result in a break in the cascade producing relaxation. Potiencation of the relaxation effect of *M. balsamina* by Nifedipine suggests that smooth relaxing effect of *M. balsamina* may be mediate through calcium channel blockade.

On the rabbit jejunum, *S. indica* at concentrations used produced sustained maximum contractions (Fig-4). However, in atropinized preparation, the spasmogenic activity observed was reduced and in the presence of Nifedipine, no blockade was observed though there was a reduction in the duration of contraction. It can be inferred from this that the contractions observed were mediated through muscarinic cholinoceptors present in the gastrointestinal tract. The effect of *S. indica* on the rabbit jejunum contradicts it use in the management of diarrhea as supported by the inhibitory effect of the extract on castor oil induced diarrhea. Almeida, et al., (1995) reported that *S. cayenesis* (a species under the same genus with *S. indica)* extract exhibited antidiarrhea activity through its positive action on intestinal transport of water, and gastrointestinal propulsion. Their results supported the results obtained from *invivo* study of the effect of *S. indica* on castor oil induced diarrhea, but not the *invitro* results. They stated that *S. cayenesis* reduced gastrointestinal propulsion rate, but from this result the extract of *S. indica* caused sustained contraction of isolated rabbit jejunum. Anyensu (1978) stated that *S. indica* is used by traditional healers in the management of constipation as well as diarrhea. He noted that it is often mixed with natron (NaCl) for the management of diarrhea. NaCl used (alone or in combination with the plant effect) may be responsible for the antidiarrhea effect of the plant. NaCl is one of the components of oral rehydration therapy. It is known to assist in the absorption of glucose (from the gastrointestinal tract) which is normally impaired during diarrhea. The principle behind this therapy is the replacement of lost body fluids. The arguments for the use of this therapy is based on believe that death resulting from diarrhea is mainly due to body fluid depletion. This therapy is particularly useful in children, because severe alteration in body fluid volume can be fatal.

From *in vitro* experiments results, the effect produced by *S. indica* at the doses employed would be more useful in the management of constipation rather than diarrhea.

Some antidiarrhea agents like morphine increase the tone and rhythmic contractions of the intestine but diminish propulsive activity, the overall effect being constipation. This activity is mediated through μ and δ receptors through both peripheral and central sites of action (Rang, et al., 2001). It is possible that the contraction produced by *S. indica* may be the same as the one produced by morphine, in which case this result will support the findings of Almeida, et al., (1995) that the extract of *S. cayenesis* reduced gastrointestinal propulsion rate. If this is the case, the results obtained support the use of *S. indica* for the management of diarrhea. It is possible that the contraction observed was same as that of morphine and it may diminish the propulsive activity of the GIT. The net effect will be an antidiarrhea effect.

The observed relaxation also explains why *M balsamina* extract could protect the rats against diarrhea induced by castor oil.

Some plants show antidiarrhea properties by their antimicrobial activities (Karim, and Adeikan, 1977) or by blocking the eicosanoids (prostaglandins and cogeners) (Christopher, et al., 1996). The mechanism of anti diarrhea properties of these plants is yet to be ascertained, it is hoped that further studies into the mechanism of action will give a better explanation to their antidiarrhea properties.

The effects of these extracts on smooth muscle and castor oil-induced diarrhea justify their uses in the treatment of diarrhea in Plateau State, Nigeria.

CONCLUSION

From the tests carried out, *in vivo* and *in vitro*, *M. balsamina* at the doses employed has antidiarrhea properties evident by its ability to inhibit castor oil induced diarrhea and to cause an overall relaxation of rabbit jejunum smooth muscle. The relaxation is thought to be mediated through the blockade of calcium channels.

S. indica produced a significant inhibition of castor oil induced diarrhea at the doses employed but in *in vitro* test it did not produce relaxation that would have supported the earlier observation. Rather it produced increased contractions, which is mediated through activation of muscarinic cholinoceptors present in the gastrointestinal tract. The results obtained *in vitro* did not support the results from the *in vivo* tests.

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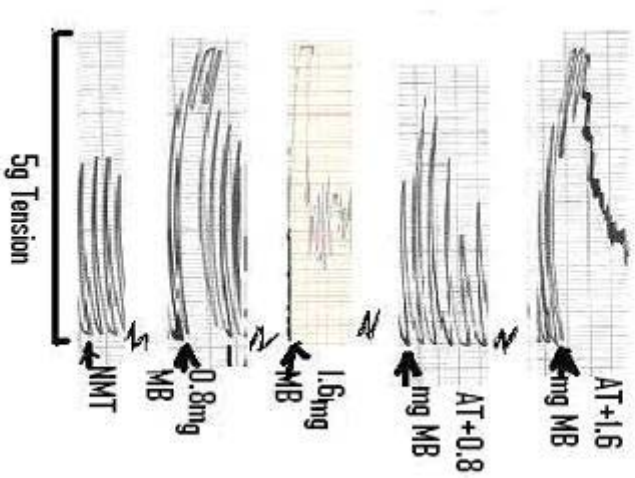

Figure-1: Effect of *Momordica balsamina* on rabbit Jejunum in the absence and presence of Atropine.
(NMT= Normal Tracing, AT= Atropine, MB= *Momordica balsamina*)
(Instrument used for measurement = Ugo Basile Unirecorder 7050)

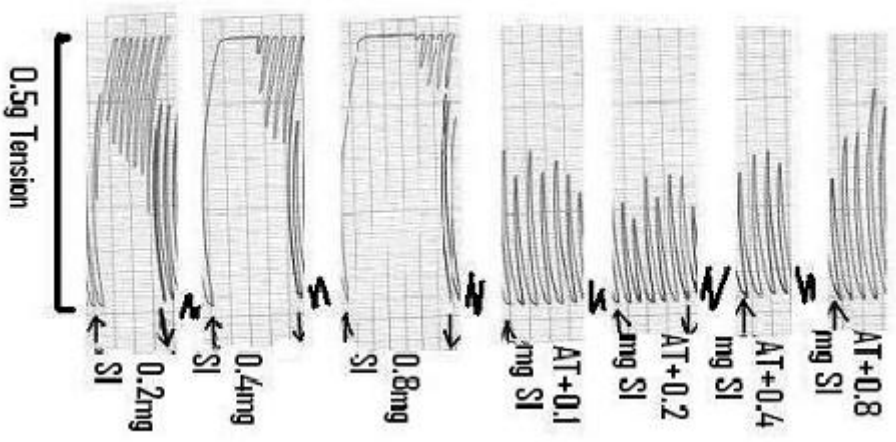

Figure-2: Effect of *Sarchytapharta indica* on rabbit Jejunum in the absence and presence of Atropine.
(AT= Atropine, SI= *Stachytarphata indica*)
(Instrument used for measurement = Ugo Basile Unirecorder 7050)

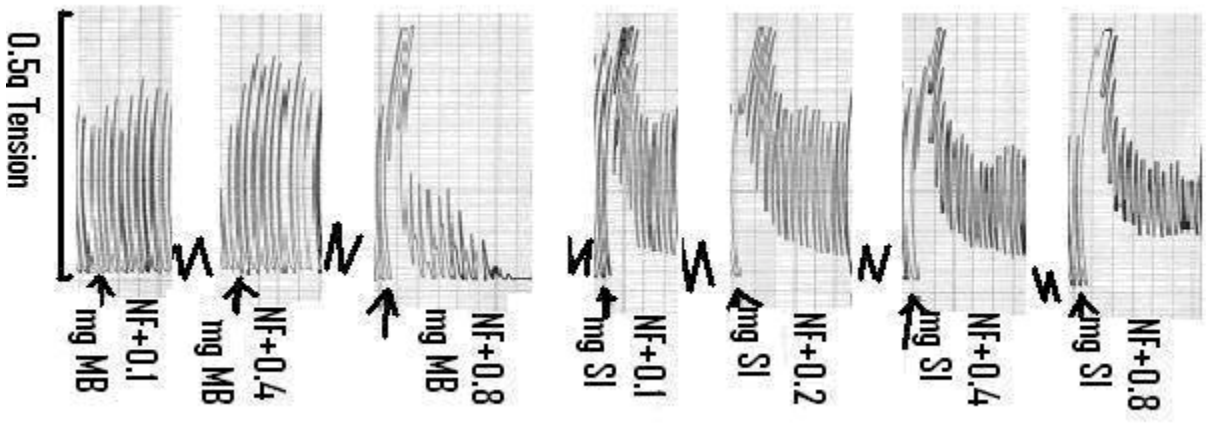

Figure-3: Effect of Calcium channel blocker (Nifedipine x10^{-5}) on transient contraction and accompanying relaxation by *Momordica balsamina* and on contraction by *Stachytarphata indica* on rabit jejunum.

(NF= Nifedipine, SI= *Stachytarphata indica*)

(Instrument used for measurement = Ugo Basile Unirecorder 7050)

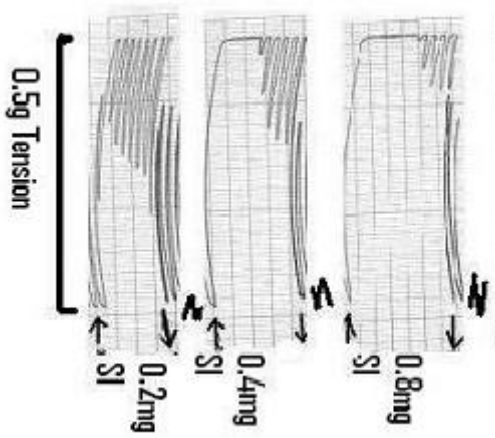

Figure-4: Effect of *Sarchytapharta indica* on rabbit Jejunum.

(SI= *Stachytarphata indica*)

(Instrument used for measurement = Ugo Basile Unirecorder 7050)

Table -1: Effect of *M. balsamina* Linn on castor oil induced diarrhea in albino rats.

Animal group	Frequency of diarrheal stool	% Inhibition of diarrhea
Control	4.0 ± 1.51	Nil
M. balsamina		
100 mg/kg (IP)	1.2 ± 0.50*	87.5
250 mg/kg (IP)	1.2 ± 0.48*	87.5
1000 mg/kg (PO	0 ± 0	0
Loperamide		
50mg/kg	0 ± 0	100
Water for injection	0 ± 0	0

*Indicates the Mean±SEM was statistically significant when compared with castor oil control and diphenoxylate (50mg/kg), at the significance level of $P<0.05$.

Table-2: Effect of *S. indica* on castor oil induced diarrhea in albino rats.

Animal group	Frequency of diarrheal stool	% Inhibition of diarrhea
Control	7.75 ± 0.92	Nil
S. indica		
1500 mg/kg (PO)	2.55 ± 0.29*	64.52
3000 mg/kg (PO)	3.15 ± 0.73*	60
Loperamide		
50mg/kg	0 ± 0	100
Water for injection	0 ± 0	0

*Indicates that the mean ± SEM was statistically significant when compared with the castor oil control, loperamide (50mg/kg) and water for injection at the significance level of $P<0.05$.