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Comparative antioxidant potential of *Momordica charantia* leaf extract and metronidazole on castor oil-induced dysentery in Wistar rats

Tugbobo Oladimeji Samuel *, Oyewole Tosin Ayodele and Owabumoye John Babatope

Department of Science Laboratory Technology, Federal Polytechnic, Ado-Ekiti, Nigeria.

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Abstract

Dysentery has been rampaging with high mortality most especially among children in developing countries. The synthetic drugs available are quite unaffordable, scarce and not reliable as they often present side effects which are detrimental to health. In vitro antioxidant potential of a native plant in Yoruba black race locally called 'Ijirin' (*Momordica charantia*) leaf extract against castor oil-induced dysentery and subtle comparison with efficacy and health effect of metronidazole in wistar rats were investigated in this study. The concentration of bioactive components in the extract were also examined and investigated while the inhibitory antioxidant potential against oxidative stress between the extract and drug along stomach villi while interfering with the electrolytes were also compared. The extract demonstrated high inhibitory and antioxidant potential where frequency of stooling and intestinal content movement were reduced significantly ($P<0.05$) in rats treated with the extract compared with animals treated with test drug. However, the pharmacological strength of both drug and extract are concentration dependent while it could be inferred from this study that dysentery could be managed or prevented with zero side effect by dietary intake of *Momordica charantia* leaves.

Keywords: Dysentery; *Momordica charantia*; Wistar-rats; Metronidazole; Antioxidant

1. Introduction

Orthodox drugs available for treating various diseases including dysentery have not been effective in their pharmacological activity. Nevertheless, they are expensive, scarce and often present side effects which are grossly inimical to human health most especially in rural areas. Hence, need for alternative medicine for effective treatment of these diseases causing high mortality. Larger population in sub-Saharan Africa mostly rely on medicinal plants to treat their ailments (Hostettman *et al.*, 2000). Some of these medicinal plants have been investigated for their antioxidative properties which is good for the treatment of such diseases. Many of the metabolites from these plants exhibit potent antioxidant activity *in vitro* (Sofidiya *et al.*, 2006). Most of the free radical scavenging potential in herbs and spices is due to the redox properties of phenolic compounds which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. The importance of free radicals and reactive oxygen species (ROS) has attracted increasing attention. The free radical mediated reaction are involved in degenerative or pathological process such as cancer, rheumatoid, diarrhea, coronary heart disease as well as Alzheimer's disease. Dysentery is an irregular bowel movement that is characterized by increase in rate of watery stool (Caramia *et a.l,* 2015). This has led to an increase in the mortality, resulting in about ten million death especially in infants, mostly in the developing countries (Mohd *et al.*, 2004). Biologically, viruses do also cause dysentery in adults (Okolo *et al.,* 2013). Besides, parasite like *Giardia* can also cause dysentery (Shemsu *et al.*, 2013). The activities of these organisms lead to an increase in the intestinal movement due to influx of water and ions. This leads to watery stool which further leads to excessive loss of water leading to dehydration of the body and imbalance of electrolytes which invariably could cause fatality (Shah *et al.*, 2007).

* Corresponding author: Tugbobo Oladimeji Samuel

2. Material and methods

2.1. Collection of Plants

'Ijirin' (*Momordica charantia*) was harvested within the Federal Polytechnic, Ado-Ekiti campus and was authenticated at the Plant Physiology Unit, Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti, Nigeria. Castor oil and metronidazole were purchased from Bell, Sons & co. Ltd. and Parker Pharmaco. Ltd., United Kingdom while all other reagents are of analytical grade.

2.2. Extract Preparation

Leaves of *Momordica charantia* were cleaned, rinsed and air dried at room temperature until constant weight was obtained. The dried leaves were then milled into powder form which was later dissolved 2kg powder leaves for 24hrs in ratio (1:1) with 5litres methanol. After 24hrs, the extract was filtered and the filtrate was evaporated with rotary evaporator to reduce its moisture content after being freeze-dried.

2.3. Experimental Design

70 male wistar rats average weight between 165-170g were purchased from Physiology Department, University of Ibadan, Nigeria. These rats were kept in metabolic cages at the animal house in the Department of Science Laboratory Technology for 2weeks to acclimatize prior to commencement of the experiment. The rats were divided into equal halves where 35 rats was used to investigate the frequency of the stooling and were divided into seven (7) groups containing five rats each. Group A rats are control and was administered 1ml of distilled water orally while group B was administered 1ml castor oil to induce dysentery. Group C, D, E and F were orally administered 1ml of the extract containing 100, 200, 400, 600mg/kg body weight respectively. Group G was orally administered 1ml of standard drug (metronidazole) containing 2mg/kg body weight.

2.4. Biochemical Assays

- **Na^+-K^+-ATPase Activity:** The method of (Bewaji *et al*., 1985) was used for this test. 400µl of 200mM NaCl/40 mMKCl/60 mMTris (pH 7.4). This procedure was repeated using the remaining 35 wistar rats for both the enteropooling and gastro-intestinal motility experiments.
- **Induction of Dysentery:** Castol oil-induced dysentery was conducted using method described by (Bamisaye *et al*., 2013). The test animals were fasted for 18hours prior to induction but were allowed access to free water. After 1hour of treatment with the drug and extract, each rat was orally administered 1ml of castor oil and the time interval between oil administration and first stooling was recorded.

2.4.1. Determination of Gastro-intestinal motility

The method described by (Gerald *et al.*, 2007) was used. After 1hour of dysentery induction, all animal groups were administered 1ml of 10% barium sulphate solution. 30minutes after all the rats were sacrificed. The distance traveled by barium sulphate solution was measured and expressed as percentage of total length of small intestine (Gerald *et al.*, 2007).

$$\% \text{ Inhibition in intestinal } = \frac{\text{Positive control group} - \text{Treated group} \times 100\%}{\text{Positive control group}}$$

2.5. High Performance Liquid Chromatography Assay

This was carried out using method described by (Mingtao *et al.*, 2015).

2.6. Lipid Peroxidation (TBARS) Assay

Normal healthy rats were sacrificed under mild diethyl ether anesthesia and intraluminal fluid was collected from the alimentary canal. The lipid peroxidation assay was carried out using the modified method of (Ohkawa *et al.*, 2009) to determine levels of oxidative stress in the endothelial layer of the stomach villi.

2.7. Determination of Anti-Enteropooling

The accumulation of intraluminal fluids was determined with the method of (Havagiray *et al.*, 2004). The test animals were fasted for 18 hours prior to the experiment and were allowed free access to water. Immediately after dysentery has been induced, the extract and metronidazole were administered accordingly and after 1hour, the animals were

sacrificed accordingly. The small intestine was excised and content was aspirated into a measuring cylinder. The volume and weight of the contents were measured and weighed respectively. The percentage volume and weight of the intestinal fluids were then determined using the formula below:

$$\% \text{ Reduction in Volume of intestinal fluid} = \frac{\text{Average volume of Positive group} - \text{Average volume of Treated group x } 100\%}{\text{Average Volume of Positive control group}}$$

$$\% \text{ Reduction in Weight of intestinal fluid} = \frac{\text{Average weight of Positive control group} - \text{Average weight of Treated group x } 100\%}{\text{Average weight of Positive control group}}$$

2.8. Statistical Analysis

Data pool expressed as ± standard error of mean (SEM) of five determinations and analysis of variance (ANOVA) using SPSS software and significant differences were defined as $P<0.05$

3. Results

Table 1 HPLC Characterization of aqueous-methanol extract of *Momordica charantia* leaf

Phytochemicals	Area	Concentration	% Composition
Isoquercitrin	209.39	0.020	**2.01**
Quercitrin	203.90	0.020	**1.96**
Kaempferol	412.62	0.400	**39.72**
Rutin	334.30	0.033	**3.17**
Limonene	201.38	0.020	**1.94**
1,8-cineole	297.38	0.028	**2.83**
Beta-bisabolene	291.05	0.028	**2.77**
Quercetin	927.86	0.096	**9.59**
Gallic acid	86.45	0.008	**0.81**
Saponin	88.43	0.007	**0.81**
Catechin	202.32	**0.018**	**0.90**
Avicularin	203.80	0.021	**0.86**
Flavonoids	621.31	0.024	**6.73**

Table 2 Inhibitory effect of aqueous-methanol extract of *Momordica charantia* leaf on frequency of stooling in castol oil-induced dysentery Wistar rats

Group	Total No of Faeces	No of wet faeces	% Inhibition of defaecation
1	3.92±0.01	0.02±0.01	98.79^{a}
2	8.35±0.06	5.23±0.13	32.60^{e}
3	8.31±0.03	5.21±0.08	32.20^{e}
4	6.82±0.01	4.92±0.10	29.69^{f}
5	9.89±0.01	2.35±0.04	69.13^{d}
6	8.35±0.03	2.63±0.07	69.37^{c}
7	6.24±0.02	1.79±0.05	70.65^{b}

Table 3 Inhibitory effect of the extract on mean distance traveled by barium sulphate in castor oil-induced dysentery Wistar rats

Group	Mean distance (mm)	% Inhibition
1	29.01±4.14	30.92[a]
2	62.80±2.90	0.00[f]
3	62.00±3.81	0.97[e]
4	60.40±2.16	2.90[d]
5	57.50±3.36	6.40[c]
6	57.40±3.93	6.52[c]
7	54.80±5.42	9.66[b]

Table 4 Inhibitory effect of extract against oxidative stress in villi of castor oil-induced dysentery in wistar rats

Extract conc. (mg/ml)	Average Absorbance ±SD	% Inhibition	IC_{50}
Basal	0.09± 0.04[b]	-	-
Control	0.49± 0.19[a]	-	-
10	0.29±0.04[c]	40.51	1.62
20	0.29±0.04[c]	41.32	1.70
40	0.19±0.02[b]	61.82	3.70
80	0.15±0.06[b]	70.10	4.51
160	0.13±0.28[b]	72.82	4.77

Table 5 Inhibitory effect of Metronidazole against oxidative stress in castor oil-induced dysentery wistar rats

Extract conc. (mg/ml)	Average Absorbance ±SD	% Inhibition	IC_{50}
Basal	0.07±0.03[b]	-	-
Control	0.38±0.10[a]	-	-
10	0.28±0.04[c]	25.00	0.71
20	0.23±0.02[b]	38.91	1.95
40	0.18±0.08[b]	52.65	3.20
80	0.14± 0.05[b]	63.92	4.21
160	0.13±0.06[b]	67.07	4.47

4. Discussion

The study established the fact of an imbalance between the secretory and absorptive processes in the gastro-intestinal tube which normally leads to continuous stooling. This causes hypermotility causing an individual faeces to contain excessive fluid (Wibowo *et al*., 2021). Also, castor oil contains ricinoleic acid and thus, freely induces dysentery in animals and humans. The upper part of small intestine contains lipases which can act on castor oil to liberate the ricinoleic acid (Tunaru *et al.*, 2012). This acid then binds with prostaglandin receptor located on smooth muscle where its binding facilitates the intestine to accumulate fluids through inhibition of absorption. This process also enhances secretion of fluids and intestinal electrolytes. Hence, the *Momordica charantia* leaf extract could be responsible for the

observed significant (P<0.05) reduction of watery stools due to dysentery induced by the castor oil in this study. The activity of the extract may be attributed to its phytochemical constituents such as saponin, flavonoids and Avicularin as reported from earlier research (Ashok and Kumud, 2012). Most of anti-dysentery agents act by reducing gastrointestinal motility (Ezekwesili *et al.*, 2010). Hence, the observed inhibition of intestinal motility as the extract doses increased may be due to appreciable amount of bioactive phytochemicals found in the extract. This further corroborates the fact that whenever there is reduction in the intestinal muscle motility, there is corresponding increase of time a substance stays in the intestine, thereby creating a longer time for absorption (Silva *et al*., 2012). The observed reduction in intestinal propulsive movement of barium sulphate could be attributed to its inhibitory action on the intestinal motility of the extract. Besides, ricinoleic acid is an active metabolite in castor oil which causes intestinal mucosal to be irritated and inflamed. This leads to onward release of prostaglandins which eventually prevents the reabsorption of sodium chloride and water (Tunaru *et al*., 2012). The observed inhibition in hyper secretion of gastrointestinal contents and intestinal accumulation of fluids (enteropooling) as well as increase in reabsorption of water and electrolytes from the extract-treated rats may be due to the appreciable amount of flavonoids and saponins present in the extract. Flavonoids have been implicated to have inhibited prostaglandins release (Hamalain *et al.*, 2012). In addition, the extract antioxidant protective potential against oxidative stress in the villi in castor oil-induced dysentery rats was significantly (P<0.05) higher than that obtained from the test drug. From table 4, the *Momordica charantia* leaf extract demonstrated higher antioxidant inhibitory potential against oxidative stress in endothelial layer of the rat villi where highest extract inhibitory potential (72.82%) was observed at highest extract concentration (160mg/ml) as compared to (67.07%) highest metronidazole inhibitory potential which was equally obtained at highest test-drug concentration (160mg/ml). The inhibitory antioxidant potential of the extract could be attributed to its bioactive inherent phytochemicals.

5. Conclusion

The results obtained in this study actually justify the fact that *Momordica charantia* leaf extract demonstrates high inhibitory potential against frequency of dysentery, reduces gastrointestinal motility and fluid accumulation in the small intestine of the castor oil-induced dysentery wistar rats which could be attributed to the extract bioactive inherent phytochemicals. This further justify its wide use mostly by African herbal practitioners

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declare no conflict of interest in this research work.

Statement of ethical approval

This research work was granted approval by the authority of Ethical Research Management of Centre for Research Innovation and Development [CRID], Federal Polytechnic, Ado-Ekiti, Nigeria.

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