British Journal of Medicine & Medical Research
4(6): 1335-1344, 2014

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Effect of Masfon-Aloe vera Drink on Intestinal Motility and Transit in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OVO designed the study, coordinated the research, and wrote the first draft of the manuscript. Authors IDE and OOE managed the analysis and interpretation of data. Authors AAN and AAB wrote the protocol and managed the literature searches. Author AAB supervised and guided the entire experimental procedure. All authors read and approved the final manuscript.

Original Research Article

Received 27th August 2013
Accepted 9th October 2013
Published 25th November 2013

ABSTRACT

The effect of Masfon-Aloe vera drink on intestinal transit and motility was investigated in this study. Thirty (30) albino Wistar rats were divided into 2 batches of 15 rats each, batch 1 for intestinal motility, and batch 2 for intestinal transit experiments. Each batch was further divided into three groups of 5 rats each (control, low dose and high dose). All groups were fed with normal rat chow and water for 21 days. In addition, the control received 0.3 ml of normal saline (0.9% NaCl solution) while the low dose (LD) and high dose (HD) experimental groups received Masfon Aloe vera drink (1 ml and 3 ml/kg body weight orally, once daily respectively). The study was carried out at the Department of Physiology, University of Calabar, Nigeria. At the end of the study duration, intestinal motility and transit studies were conducted. Result for intestinal motility showed the extract (Masfon-Aloe vera drink), at either low or high dose did not significantly change the frequency and amplitude of the spontaneous contraction (basal contraction) of the rat ileum when compared with the control. Graded concentrations of Acetylcholine (10^{-8} to 10^{-4}) was observed to produce a dose dependent increase in contraction of the rat ileum which was significantly ($p<0.001$) greater in the LD and HD groups at lower concentrations of 10^{-8} and 10^{-7}M. The mean percentage intestinal transit which was not

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significantly different (p = 0.226, p=0.892) among the groups was 42.16 ± 2.63 in the low dose, 37.75 ± 5.15 in the high dose groups compared to 36.91 ± 3.01 in the control. Administration of atropine produced significantly higher (p<0.01) relaxations in the LD (5.00 ± 0.41 mm) and HD groups compared with control [relaxation in mm, mean ± SEM (2.25 ± 0.25) in control vs (5.00 ± 0.41) and (10.75 ± 0.25) in LD and HD respectively]. Masfon-Aloe vera drink administered at these concentrations "did" not significantly alter the basal motility and transit of the rat ileum, but was observed to potentiate Ach induced contraction of the rat ileum, and also "augmented" the relaxant effect of atropine.

Keywords: Masfon-Aloe vera drink; intestinal motility; intestinal transit; wistar rats.

1. INTRODUCTION

The spontaneous movements or contractions of the small intestine is termed intestinal motility. These movements of the small intestine mix and churn the intestinal content (chyme) and propel it towards the large intestine [1,2]. The movements of the small intestine occur in the absence of extrinsic innervations but require an intact myenteric plexus. In other words, myogenic electrical activity of the smooth muscle provides the basis for the intestinal movement [2,3].

Aloe barbadensis miller, the true aloe vera plant otherwise called the Curacao aloe is a subtropical plant with a rich and long history of use by humans in support of health [4]. Aloe vera, a member of the Liliacea family is a succulent perennial plant that grows in a clump and has long, spiky, grey-green leaves. Out of the 275 species, 42 of them belong to Madagascar region (Africa), 12 – 15 to Arabian peninsula and the rest are distributed over south Africa, with *Aloe barbadensis miller* being the most widely distributed [5]. Known by such common names including Chinese Aloe, Indian Aloe, True Aloe, Barbados Aloe, Burn Aloe, First Aid plant, Wand of heaven and Miracle plant [6,7], *Aloe barbadensis miller* from documented researches has been shown to affect diverse body systems such as the integumentary [8], endocrine [9,10], immune [11], cardiovascular [12] and gastrointestinal tract [13].

The experimental substance used for the research is termed Masfon-Aloe vera drink. Masfon – Aloe vera drink is a high multi-mineral extract of the aloe vera plant which was recently formulated (Ekabua, K, University of Calabar, 2009, Personal communication).

The present investigation was undertaken to assess the effects of this plant extract on GI motility but specifically to i) assess the effect on the basal rate of contraction, ii) Ach induced contraction, iii) intestinal transit and iv) atropine response. There is paucity in scientific documentation of Masfon – Aloe vera drinks effect on intestinal motility and transit despite the folkloric claims by individuals that it helps in bowel activity. In one previous study, crude Aloe vera gel was observed to significantly reduce percentage intestinal transit in normal Wistar rats [20]. This study was thus designed to ascertain the effect of this novel Aloe vera formulation on Intestinal motility and transit and also to weigh this effect against the outcome of similar studies conducted with other Aloe vera extracts if any.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty wistar albino rats were purchased from the Animal house of the Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria. The animals were allowed to acclimatize in the research laboratory for two weeks. They were housed under standard environmental conditions and allowed free access to normal rat feed and drinking water throughout the duration of the experiment. Ethical conditions regarding animal use and handling was strictly followed.

2.2 Plant Extract

The plant extract that was used for the study is termed Masfon – Aloe vera drink. This extract which was recently formulated is enclosed in a two liter plastic bottle and is marketed by Abishua ventures, Calabar (Ekabua, K, University of Calabar, 2009, Personal communication).

2.3 Preparation of Tyrode's Solution

The Tyrode's solution has the following composition: NaCl 0.8 gm%, KCl 0.02 gm%, $CaCl_2$ 0.02 gm%, NaH_2PO_4 0.005 gm%, $NaHCO_3$ 0.01 gm% and glucose 0.1 gm%. They were weighed into a beaker, then diluted with small amount of distilled water and then made up to 1 L with a measuring cylinder.

2.4 Experimental Design

Thirty (30) wistar albino rats weighing between 120 and 300 g were used for the study. They were divided into two weight matched batches of 15 rats each. Batch 1 was utilized for intestinal motility experiment while batch 2 was used for carrying out intestinal transit study. Each batch was in turn divided into three groups of 5 rats each (control, low dose and high dose). All the animals were acclimatized for 14 days and exposed to normal temperature and a 12/12 hours light/dark cycle. In addition, the control received 0.3 ml of normal saline (0.9% NaCl solution) while the low and high dose experimental groups received 1 ml/kg and 3 ml/kg dosage of the plant extract orally once daily for 21 days. The dosage of Masfon – Aloe vera drink used was derived from a dose response curve of an earlier study conducted on Aloe vera gel, where an ED_{50} of 0.1 ml/100g was documented [13].

2.5 Measurement of Intestinal Motility

This was done on isolated rat ileum [14]. The rats that were used for this experimental study were starved for 24 hours prior to the time of the experiment to ensure that there were no food particles in the stomach and in the small intestine. The rats were sacrificed by stunning and an incision was made along the linea alba to expose the small intestine. The proximal ileum was located, isolated and then placed in a container of Tyrode solution and aerated. Using a meter rule, the ileum was then cut into segments that were 2 cm long. One end of a segmented ileum was attached to a fixed support (hook) in an organ bath while the other end of the ileum was attached to a horizontal writing lever tangential to the kymograph drum by using a thread. The Tyrode solution in the tissue bath was flushed 3 times, after which the tissue was allowed to equilibrate. After the recording of a consistent basal tracing, the tissue

was then challenged with graded doses of acetylcholine (Ach) and atropine. These drugs were injected into the tissue bath to study their effect on the ileal tissue.

2.6 Measurement of Intestinal Transit

The intestinal transit experiment was done following a modified method of Marthar-Perez [15]. The rats were starved 24 hours before the start of the experiment. Using a 2 ml syringe fitted with an oral cannula, a dose of 2 ml of 3 percent solution of gum arabic and charcoal was administered orally to each of the rats. The animals were allowed for an hour to allow for movement of the dye, after which the rats were sacrificed and the small intestine was cut out and spread. The distance travelled by the dye in each rat and the entire length of the small intestine was measured using a measuring tape.

The intestinal transit expressed in percentage was calculated thus:

$$\frac{\text{Length travelled by the dye}}{\text{Total length of the small intestine}} \times 100$$

2.7 Statistical Analysis

Results were expressed as mean ± SEM. Three or more variables were compared using the analysis of variance (ANOVA), followed by post hoc multiple comparisons test. $P<0.01$, 0.001, 0.05 was considered as statistically significant. Computer software SPSS version 17.0 and Excel Analyzer was used for the analysis.

3. RESULTS

3.1 Comparison of Both Basal Rate of Contraction and Percentage Maximum Contraction of the Rat Ileum to Graded Concentrations (10^{-8} to 10^{-4}M) of Acetylcholine (Ach) in the Different Experimental Groups

Basal rate of contraction in the HD (1.88 ± 0.13) and LD (1.75 ± 0.14) did not differ significantly from that of the control (1.63 ± 0.13) (Fig. 1).

Graded concentrations of Acetylcholine (Ach) was observed to produce a dose dependent increase in contraction of the rat ileum in all the experimental groups. At lower concentrations of Ach (i.e. 10^{-8}M and 10^{-7}M); the increase was greater in low dose (LD) and high dose (HD) groups compared with control (Fig. 2).

At 10^{-8}M concentration, a percentage maximum contraction of 11.61 ± 0.89% was produced in the control, while LD and HD produced 39.06 ± 1.56% and 50.63 ± 0.63% respectively, showing a significant ($p<0.001$) increase in LD and HD compared with control.

At 10^{-7}M concentration, the percentage maximum contraction was 31.25 ± 0.89%, 45.31 ± 1.56% and 63.13 ± 0.63% for control, LD and HD groups; this also showed a significant contraction in LD and HD ($p<0.001$) than control.

At higher concentrations of ACh (10^{-6}M and 10^{-5}M), the increase in contraction was greater in control than in the extract treated rats. All the groups attained a maximum (100%) contraction at 10^{-4} M concentration of ACh (Fig. 2).

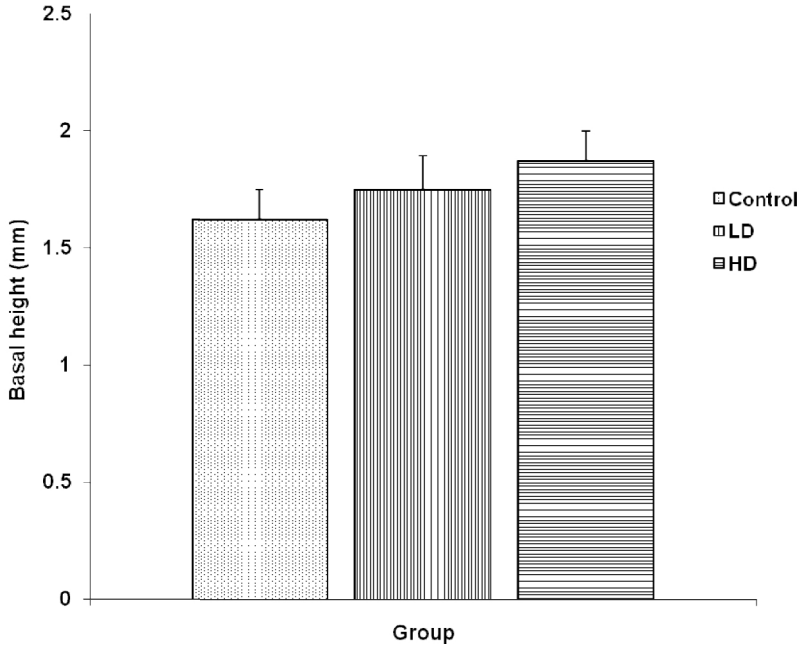

Fig. 1. Basal height of contraction of the rat ileum in control and test groups.
Values are mean + SEM, n = 5.

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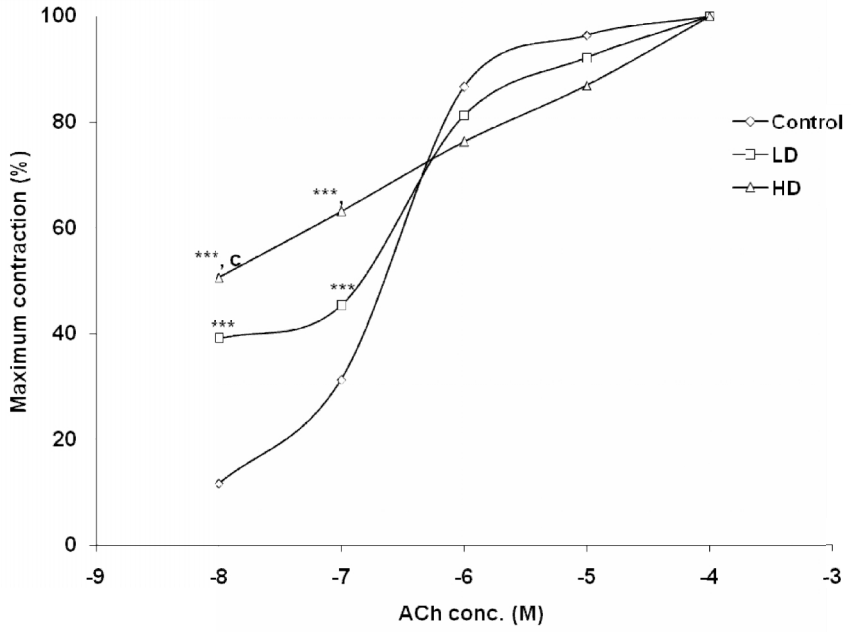

Fig. 2. Percentage maximum contraction of the rat ileum to graded concentrations
(10^{-8} and 10^{-4}) of ACh. Values are mean ± SEM, n = 5.
****p<0.001 vs control; c = p<0.001 vs LD.*

3.2 Percentage Transit of the Small Intestine in the Different Experimental Groups

The mean percentage intestinal transit in the different experimental groups was also recorded. There was a slight increase in percentage intestinal transit in the test groups when compared to the control, this increase was not significant as shown in Fig. 3. The mean percentage intestinal transit was 36.91 ± 3.01 % in the control group, 42.16 ± 2.63 % in the low dose group and 37.75 ± 5.15 % in the high dose group (Fig. 3).

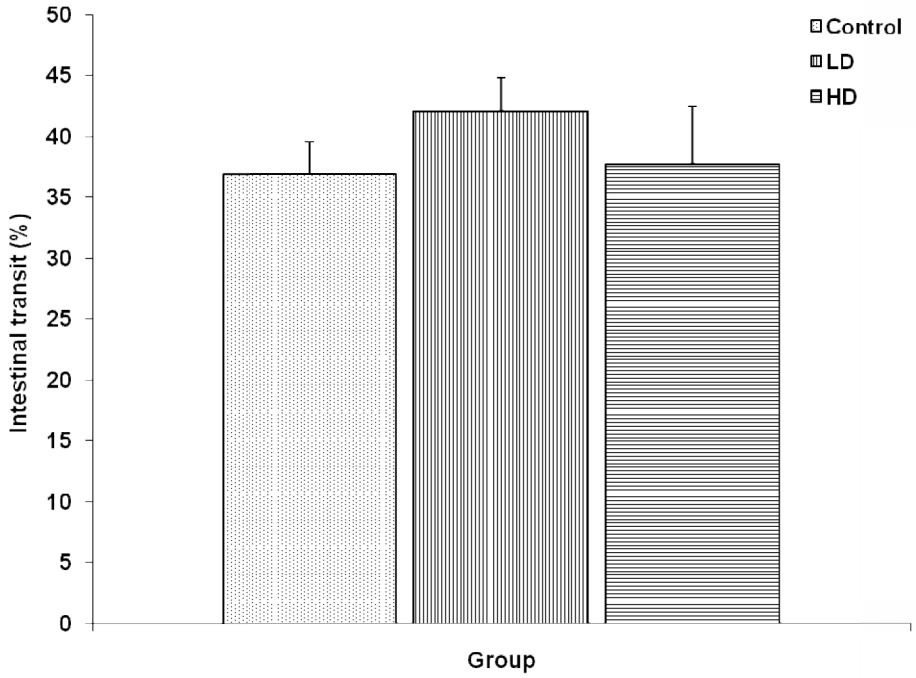

Fig. 3. Comparison of percentage intestinal transit in the different experimental groups

Values are mean ± SEM, n = 5.

3.3 Effect of Atropine on Intestinal Motility in the Control and Test Groups

Administration of atropine in the control produced maximum relaxation of 2.25 ± 0.25 mm, while in the low and high doses it was 5.00 ± 0.41 and 10.75 ± 0.25 mm respectively. This showed a significantly ($p<0.01$) increased relaxation in the tests groups than in control. In addition, relaxation was in turn significantly ($p<0.001$) higher in high dose compared with low dose group (Fig. 4).

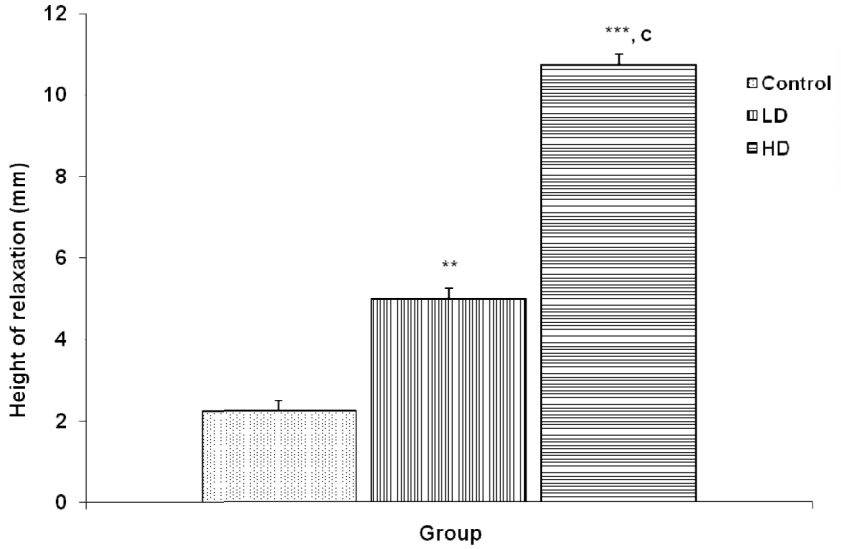

Fig. 4. Height of relaxation of the rat ileum to Atropine in the different experimental groups

*Values are mean ± SEM, n = 5. **p<0.01, ***p<0.001 vs control; c = p<0.001 vs LD.*

4. DISCUSSION

The extract (Masfon-Aloe vera drink) did not significantly change the frequency and amplitude of the spontaneous contraction (basal contraction) of the rat ileum when compared with the control. Intracellular calcium from internal reservoirs have been documented to play a role in the development of the basal tone of intestinal smooth muscles [16,17]. It is possible that the interaction of phytochemicals of this plant extract might have reduced the mobilization of calcium from internal stores, hence the non significant change in basal tone as observed in the test group.

Effects of small and large doses of acetylcholine (10^{-8} to 10^{-4}) on amplitude of contraction in the experimental groups showed a dose dependent increase in contraction. This result is in line with those documented by several previous studies involving other plant extracts [16,17]. Administration of low doses (10^{-8} and 10^{-7}) increased contraction in the test groups compared to the control while larger doses of Ach (10^{-6} to 10^{-5}) caused an opposite effect (contraction was increased in the control group compared to the test group). Spasmogenic activities in plants including Aloe vera might be due to the presence of plant constituents like terpenoids, sterols, flavonoids, tannins, phenolic compounds and alkaloids [18]. It is possible that at lower doses of Ach, agents in the extract, namely the above phytochemicals some of which may be cholinomimetic in action act synergistically with Ach to bring about a greater contraction in the test doses. Tachyphylaxis was observed to set in faster at higher concentrations in the extract administered groups compared with the control, hence the observed reduced contraction in these groups compared to controls. One of the documented mechanism of action of tachyphylaxis is to reduce the number of receptors through which a particular drug exerts its effects [19]. Hence, it is possible that the extract has tachyphylactic constituents that might have reduced the expression of M3 cholinergic receptors, which explains the reduced contraction in the extract treated groups when administered with high doses of Ach.

Percentage transit increase as observed in the test groups was not significant when compared with control. This result is in contrast to a previous study where crude aloe vera gel caused a significant decrease in intestinal transit in normal wistar rats [20]. Intestinal transit is controlled by both neural and myogenic mechanisms [21]. This result which relates with that for basal rate of contraction might have also been attributed to the interaction of phytochemicals of the plants extract which did not significantly alter the muscles myogenic contraction.

The involvement of cholinergics in the activity of Masfon – Aloe vera is also evidenced with the results of atropine, the extract was observed to cause further reduction in ileal motility following administration of atropine i.e. upon administration of atropine the reduction in intestinal motility was significantly higher in the extract treated groups compared with the control.

It can be probably inferred from the results obtained that the plant extract would not significantly alter GI motility in normal healthy states, but in disease conditions like diarrhea or constipation, the plants extract may act in a synergistic manner with foreign agents like drugs that act as antagonist or agonist of the M3 cholinergic receptor.

It is not known which component of the plants extract had the effect from this study design. Additionally, a graded concentration of the plants extract was not used to effectively ascertain the extracts effect on the basal rate of contraction in normal rats. The effect of Aloe vera on rats with reduced GI motility was not studied and possibly this would be required in order for it to ascertain fully the extracts effect on GI transit.

5. CONCLUSION

Masfon - Aloe vera drink administered at these concentrations “did” not significantly alter the basal motility and transit of the rat ileum but was observed to potentiate Ach induced contraction of the rat ileum, and also augments the relaxant effect of atropine.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENTS

We acknowledge the contribution of Dr. (Mrs). Kufre Ekabua for providing the extract that was used for this research and Mr Umoh Ededet for assisting in the laboratory. These colleagues – Nna Victor and Victoria Okon assisted in proof reading the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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