EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 12
Year: 2024

LONG TERM ADMINISTRATION OF HERBAL ALCOHOL BITTERS (1960 ROOTZ) ALONE AND IN COMBINATION WITH KOLAVIRON MAY INCREASE INTESTINAL MOTILITY AND TRANSIT IN ALBINO WISTAR RATS

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Article Received on 01/10/2024 **Article Revised on 21/10/2024** **Article Accepted on 10/11/2024**

ABSTRACT
This study was aimed at investigating the effects of long term administration of herbal alcohol (1960 rootz) bitters alone and in combination with kolaviron on intestinal motility and transit in albino wistar rat model. Fifteen (15) female rats weighing 100-190g were used. The rats were randomly divided into three groups containing five (5) rats each. Group 1 was control group and was fed with normal rat chow and 0.2ml normal saline. Group 2, herbal alcohol only, received 0.3ml/100g body weight and fed normal rat chow and water. Group 3, herbal alcohol + kolaviron received 100mg/kg body weight of kolaviron and 0.3ml/100g rat body weight of herbal alcohol alongside with normal rat chow and water. The results obtained following 21 days administration of the drugs and experiment, showed that at low doses of ACh concerntration, (10^{-9}M), there was a significant ($P<0.05$) increase in maximum contraction of the ileal smooth muscle in the test groups (herbal alcohol only and herbal alcohol + kolaviron) as compared to control. At moderate doses of ACh concerntration (10^{-7}M), the result showed a significant ($P<0.05$) increase in the herbal alcohol group compared to control and herbal alcohol + kolaviron test group. Also, the result showed that at high doses of ACh concerntration (10^{-5}M), the test groups had a significant ($P<0.05$) increase in maximum contraction as compared to control. The test groups showed an increase in intestinal motility but no effect on transit. However, a combination of both drugs further increased intestinal motility. Therefore, both drugs (herbal alcohol + kolaviron) may be beneficial to people suffering from constipation. However, it should be taken with caution.

KEYWORDS: Herbal alcohol bitters, 1960 rootz, kolaviron, Garcina cola, intestinal motility, transit.

INTRODUCTION
It has been reported that about 80% of the World's population comprising of majorly people living in the developing world now depend almost completely on traditional medicine, using plants as local treatments.[1,2,3] The use of medicinal plants, or extracts from them, has been traditionally practiced worldwide in the prevention and treatment of several chronic diseases such as cardiovascular diseases, intestinal inflammatory disease, inflammatory bowel disease, arthritis, diabetes, allergies, multiple sclerosis, Parkinson's and Alzheimer's diseases, and others.[4] Reasons alluded to this trend are economic viability, accessibility, and ancestral experience.[5]

Although alcohol consumption is socially accepted across many cultures, heavy and prolonged alcohol intake can lead not only to physical dependence but also to devastating long-term health problems. Regardless of the type and dose of beverage involved, alcohol facilitates the development of gastroesophageal reflux disease by reducing the pressure of the lower esophageal sphincter and esophageal motility. Fermented and non-distilled alcoholic beverages increase gastrin levels and acid secretion. Low alcohol doses accelerate gastric emptying, whereas high doses delay emptying and slow bowel motility.[6] Studies have earlier on been reported on the effect of fasting on intestinal motility and transit.[7] The herbal substances considered in this study are kolaviron- the active component of bitter kola and those of herbal alcoholic bitters (1960 Rootz). Reports are indicative of the ability of the active components of this species of kola and of flavonoids from other plants such as those present in herbal alcoholic bitters (1960 Rootz) to arrest inflammation and other ailments when used in traditional medicine. The rate of consumption of these drugs in the Northern part of Cross River state, Nigeria is becoming alarming and there is still paucity of information as regards the effects of chewing or

including bitter kola and consuming herbal alcoholic bitters either separately or concomitantly on rate of small bowel motility and transit. Thus the need for this study.

2.0 MATERIALS AND METHODS

2.1. Chemicals

2.1.1 Kolaviron extraction

Kolaviron was extracted from fresh seeds of the kola (3.5kg). Powdered dried seeds of G. kola were extracted with light petroleum ether (b.p. 40-60^{o}C) in a Soxhlet extractor for 24 hours. The defatted, dried marc was repacked and then extracted with methanol in a Soxhlet extractor. The extract was concentrated and diluted to twice its volume in distilled water and partitioned with chloroform. The concentrated chloroform fraction gave a yellow-brown solid known as Kolaviron. The purity and identity of Kolaviron was determined by subjecting it to thin-layer chromatography (TLC).

2.1.2 Other drugs used

Three standard drugs were used in the course of this experiment. They are: Acetycholine, Atropine, and Propranolol. Acetycholine was obtained from the department store, while Atropine and Propranolol were obtained from Betz Pharmacy, Calabar.

Composition of Tyrode Solution: NaCl-0.8g, KCl-0.01g, NaH_2P_4-0.05g, $CaCl_2$-0.02g, $NaHCO_3$-0.01g. Distilled water was added to make up 1000ml.

2.1.3 Animal management

Fifteen adult albino Wistar rats weighing between 100g-190g were used for this study. The animals were housed in the animal holding of the Department of Human physiology, Cross River University of Technology, Okuku. The animals were fed with standard rat pellet (Vital feed) and given water liberally. Animals were housed in clean plastic cages under natural light and dark cycle and at room temperature. All animals were handled in accordance with the guidelines for animal research as detailed in the Guidelines for the Care and Use of Laboratory Animals by the National Research Council of the National Academy of Sciences, 2011.

2.1.4 Experimental Design

The rats were kept under standard animal house conditions at room temperature. They were been acclimatized for one week, after which the animals were randomly divided into three (3) groups, thus.

Group I: normal control animals were given standard feed normal saline only (0.2ml).
Group II: the animals in this group were administered herbal alcohol only (0.3ml/100g body weight).
Group III: this group was administered with Kolaviron(100mg/kg body weight) and herbal alcohol (0.3ml/100g body weight).
The animals had free access to water and standard rat chow (Vital feeds).

2.1.5 Drug Administration

Kolaviron and Herbal Alcohol (1960 rootz) were administered to the rats during the course of study. Kolaviron was dissolved in tween 80 and 4.8ml of distilled water was added to the mixture. It was administrated at a dose of 100mg/kg body weight of the rat, while herbal alcohol (30%v/v) was administrated at a dose of 0.3ml/100g rat. Animals in the control group received equal volume of water and proper feed was given. Administration was done orally using an orogastric tube for 21 days.

2.1.6 Phytochemical Screening

Chemical tests were carried out on the alcoholic mixture of 1960 herbal drink using standard procedures to identify the constituents as described by Trease, *et al.,*[8] and Sofowara,[9] The phytochemicals tested for include flavonoids, glycosides, tannins, alkaloids, saponins, steroids and terpenoids).

2.1.7 Quantitative Determination of the Phytoconstituents in 1960 Herbal Drink

The alkaloid, tannins, saponins and flavonoid were determined by the method described by Obadoni & Ochuko.[10]

2.1.8 Determination of Small Intestinal Motility

The determination of intestinal motility and transit was done using the method described by Obembe *et al.* (2008).[11] Animals were starved of food but allowed access to normal saline for 24 hours prior to the experiment to ensure complete emptying of the small intestine. The animals were sacrificed by cervical dislocation. A cut was hurriedly made through the linea alba to expose the intestine. The proximal ileum was recognized and cut off, then placed into a beaker containing Tyrode solution. The tissue was aerated continuously with an Aerator to keep the tissue alive and the temperature in the beaker was maintained within 34-37^{o}C to preserve energy for contraction when mounted in the tissue bath.

The ileum was then cut into small segments of about 2-3cm in length and mounted at one end to a fixed support in an organ bath. The other end of the ileum was fixed to a writing horizontal balance (lever) at a tangent to a revolving kymograph drum set to rotate at a velocity of 0.01 revolutions per second. The tissue was allowed to equilibrate for 60 minutes. Within the equilibration period, the bathing solution was replaced with Tyrode solution at 15 minutes interval to avoid accumulation of metabolites.

After the equilibration period, the basal equilibration response was obtained. The tissue was then challenged with graded doses of acetycholine (10^{-4}-10^{-9} M) and later with atropine (0.1mg), at an interval of 1min/administration. Finally, propranolol (0.1mg) was introduced into the organ bath and its corresponding effects on the tissue was noted and recorded accordingly.

Log-concentration was plotted against the amplitude of contraction (expressed as tension in grams) from the results.

2.1.9 Determination of Small Intestinal Transit

Small intestinal transit was determined using the method by Udo *et al.*, (2013).[12] The rats in the various experimental groups were starved for 24 hours prior to the experiment but had unhindered access to drinking water. A cut was hurriedly made through the linea alba to curtail bleeding. The duodenum was then identified, at the extension of the pyloric sphincter, while the ileocecal sphincter was also prominent at the cecal end. The duodenum was cut away from the pyloric sphincter and the ileum was also cut at the ileocecal sphincter. The small intestine was immediately straightened and the location of the indicator was identified along the small intestine. A thread was used to tie the intestine at the point where the indicator stopped. Using a measuring tape, the total length of the small intestine was measured and recorded. The length travelled by the indicator was also measured and recorded. The small intestinal transit was calculated as.

- $\frac{\text{Length travelled by marker substance}}{\text{Total length of small intestine}} \times 100\%$
- Values were recorded and statistically analyzed

2.2 Statistical Analysis

Results were expressed as Mean ± SEM and analyzed using one-way analysis of variance (ANOVA), Bonferonis multiple comparison test was adopted using GraphPad prism version 5.0 statistical packages, $p<0.05$ was considered significantly different and $p<0.001$ was considered extreme significant difference.

4.0 RESULTS

4.1 Comparison of basal height of contractions of the rat ileum

The mean values of the basal height of the rat ileum for the control and test groups when compared show that the group administered with herbal alcohol only (1.13±0.13) was significantly reduced ($p<0.05$) when compared to control (1.50±0.29) and significantly increased ($p<0.05$) in group administered with herbal alcohol and kolaviron (2.13±0.13) when compared to control and herbal alcohol only groups (fig. 5).

4.2 Comparison of percentage maximum contraction of the ileal smooth muscle to graded concentrations of Ach in the different experimental groups

At 10^{-9}M concentration, the percentage maximum contraction was 32.14±6.26, 48.44±1.56, and 46.67±0.95% for control, herbal alcohol and herbal alcohol + Kolaviron respectively, showing a significant ($p<0.05$) increase in the herbal alcohol only and herbal alcohol + Kolaviron groups compared to control, but there was no significant difference between the test groups.

At 10^{-8}M concentration, the percentage maximum contraction was 47.62±9.72, 60.94±1.66, and 51.19±1.19% for control, herbal alcohol only and herbal alcohol + Kolaviron respectively, showing a significant ($p<0.05$) increase in the herbal alcohol + Kolaviron group compared to herbal alcohol only and control. The same result occurred in the groups with 10^{-7}M Ach.

At 10^{-7}M concentration, the percentage maximum contraction was 62.50±12.16, 68.23±2.99, and 62.14±0.24% for control, herbal alcohol only and herbal alcohol + Kolaviron respectively.

At 10^{-6}M concentration, the percentage maximum contraction was 71.43±13.88, 73.96±3.45, and 71.19±0.24% for control, herbal alcohol only and herbal alcohol + Kolaviron respectively, showing a significant ($p<0.05$) increase in herbal alcohol group only, but there was no significant difference between the herbal alcohol + Kolaviron and control groups.

At 10^{-5}M concentration, the percentage maximum contraction was 77.98±12.95, 81.25±3.71, and 81.00±1.00% for control, herbal alcohol only and herbal alcohol + Kolaviron respectively, showing a significant ($p<0.05$) increase in the test groups compared to control.

All the groups attained a maximum (100%) contraction at 10^{-4}M concentration of ACh (fig. 6 and 7).

4.3 Comparison of effect of atropine sulphate on intestinal motility in control and test groups

The mean values of group administered with herbal alcohol only (-300.00±0.00) shows no significance difference at ($p>0.05$) when compared to control (-287.50±12.50), but there was extreme significant decrease ($p<0.001$) when group administered with herbal alcohol and kolaviron (-396.43±3.57) was compared to control (-2.87.50±12.50) shown in fig. 8 below.

4.4 Comparison of effect of propranonol on intestinal motility in control and test groups

The mean value of group administered with herbal alcohol and kolaviron (-415.00±5.00) signified a slight decrease at ($p<0.05$) when compared to control (-350.00±28.87), but the group administered with herbal alcohol only (-483.33±16.67) shows an extreme significant decrease ($p<0.001$) when compared to control (-350.00±28.87) shown in fig. 9 below.

4.5 Comparison of intestinal transit (cm) in control, herbal alcohol only and herbal alcohol + Kolaviron treated groups

The mean intestinal transit in herbal alcohol only (77.00±0.20) and herbal alcohol + Kolaviron (68.00±6.68) were lower when compared to control group (80.00±0.41). There was no significant difference among the three groups (fig. 11).

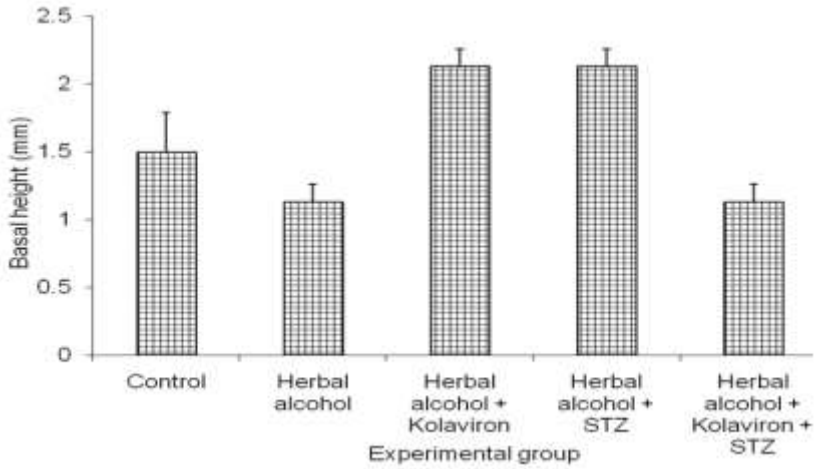

Figure 5: Comparison of basal heights of ileal smooth muscle contraction in the different experimental groups. Values are expressed as Mean ± SEM, n = 4.

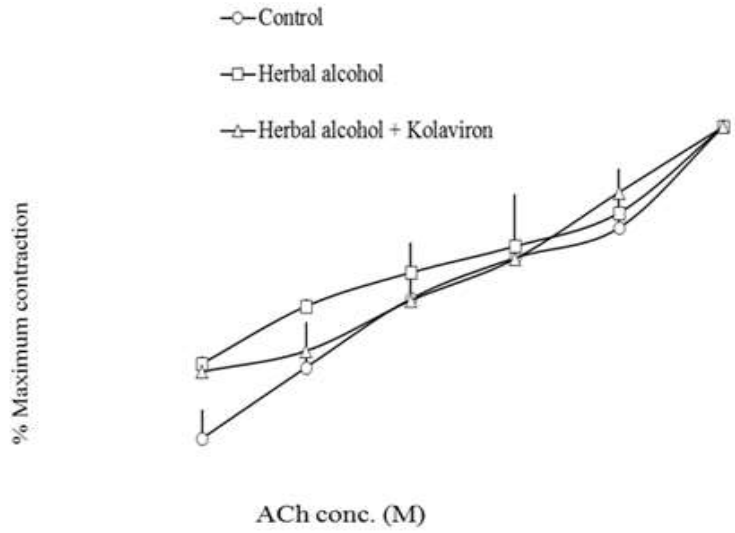

Figure 6: Percentage maximum contraction of the ileal smooth muscle to graded concentrations of ACh in the different experimental groups in rats.

Values are expressed as mean ± SEM, n = 4.

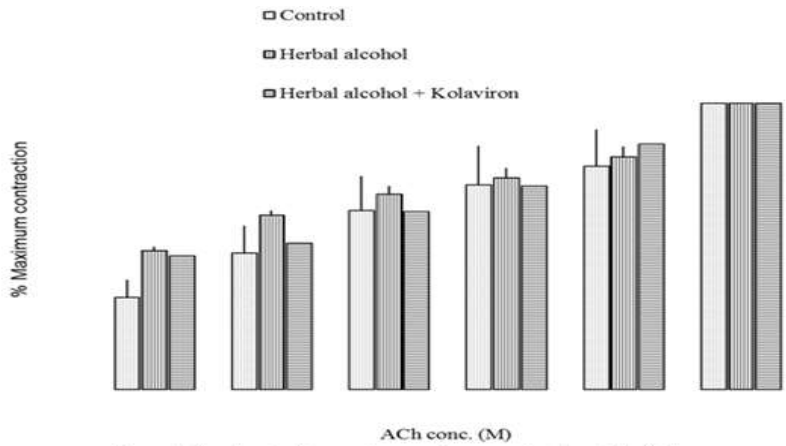

Figure 7: Bar charts of percentage maximum contraction of the ileal smooth muscle to graded concentrations of ACh in the different experimental groups in rats.

Values are expressed as mean ± SEM, n = 4.

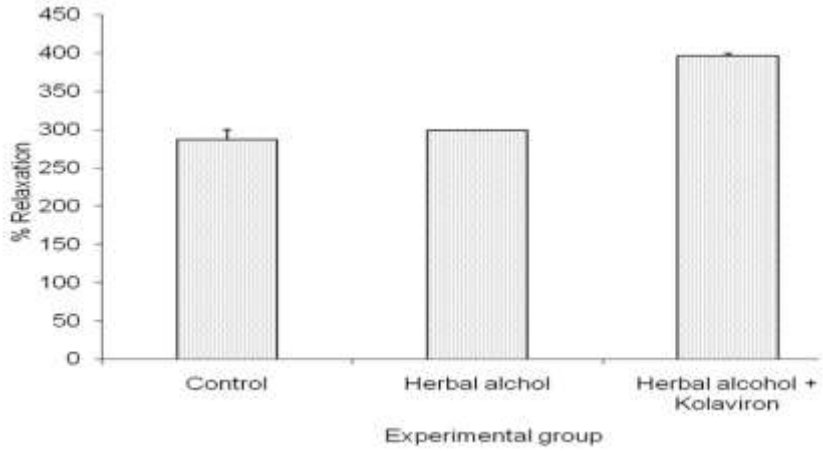

Figure 8: Comparison of effect of atropine sulphate on intestinal motility in control and test groups.Values are expressed as Mean ± SEM, n = 4.

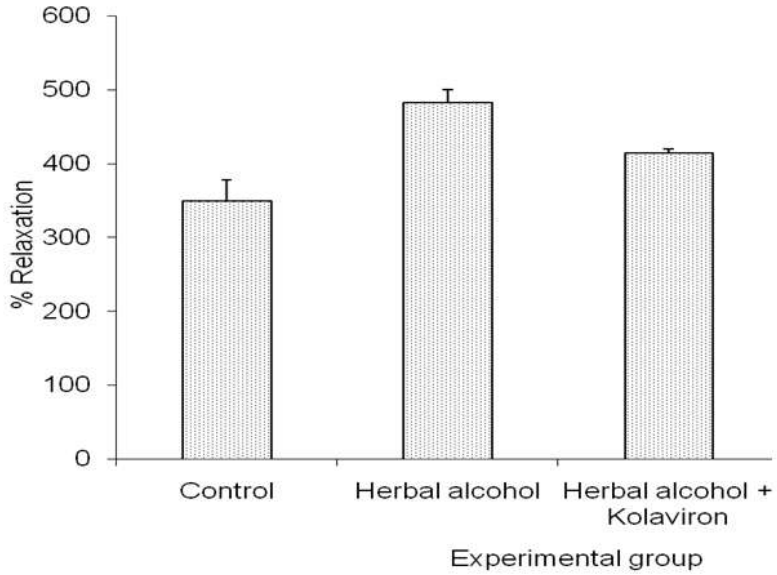

Figure 9: Comparison of effect of propranolol on intestinal motility in control and test groups. Values are expressed as Mean ± SEM, n = 4.

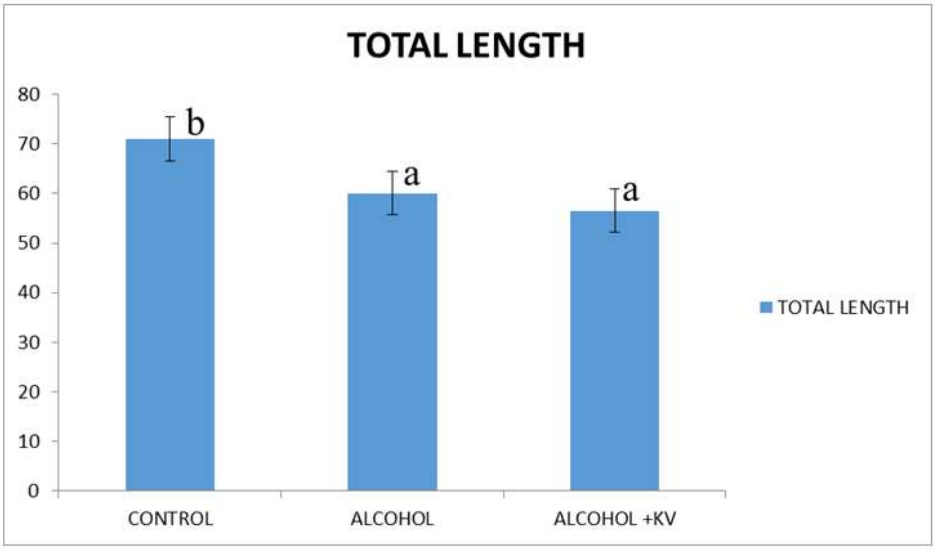

Fig. 10: Showing comparison of total length as compared with control and other test groups.

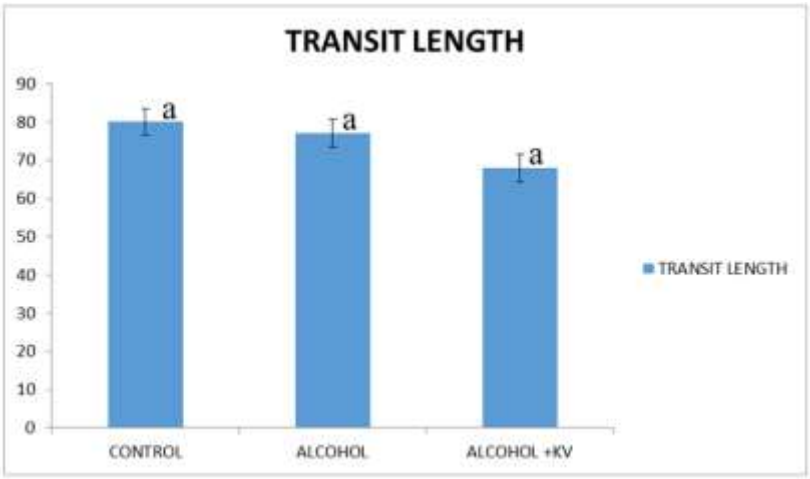

Fig. 11: Showing comparison of transit length as compared to control and other test groups.

5.1 DISCUSSION

This study was aimed at evaluating the effect of long term administration of kolaviron and herbal alcohol on intestinal motility and transit using albino wistar rat as model. After twenty one (21) days of administration of the drugs, the experiment on intestinal motility and transit were carried out.

The result of the study shows that the basal height of contraction in the rat ileum in the herbal alcohol + kolaviron test group was significantly ($p<0.05$) increased when compared to control and herbal alcohol alone (fig. 5). At low concentration of Ach (10^{-9}M), there was a significant ($p<0.05$) increase in maximum contraction of the ileum smooth muscle in the test groups (herbal alcohol + kolaviron group alone), compared to control. At moderate doses of ACH (10^{-7}M) there was a significant ($p<0.05$) increase in the herbal alcohol test group compared to control and herbal alcohol + kolaviron test group. However, at high doses of ACH (10^{-5}M), the test groups showed a significant increase ($p<0.05$) in max control compared to control (fig. 6 and 7).

This means that combination of herbal alcohol and kolaviron may increase intestinal motility compared to when herbal alcohol is administered alone but both groups increased intestinal motility. Kolaviron had earlier on been reported to exhibit potent anti-motility effect on destabilized gut homeostasis and could be the major compound in Garcinia kola responsible for the anti-diarrhoeal effect.[13] This report contradicts with this study and this may be due to the combination of kolaviron with herbal alcohol. Note that administration of herbal alcohol alone increased intestinal motility. The increase in contraction was blocked by administration of atropine in the test groups. With atropine administration, there was a significant decrease ($p<0.05$) in ileal contraction in the herbal alcohol +kolaviron test group compared to control. With propranolol administration, there was no significant difference among the groups eventhough the test groups showed an increase in ileal contraction.

There was no significant decrease in intestinal transit in the two test groups compared to control. Kolaviron has earlier been reported to decrease intestinal transit at dose of 100mg/kg body weight[13] this report does not agree with this study as this study showed that herbal alcohol + kolaviron group may not have any effect on intestinal transit. The herbal alcohol bitters (1960 Roots) is made up of other constituents which might have been responsible for the result as observed in this study. Therefore, administration of herbal alcohol + kolaviron and herbal alcohol may increase intestinal motility but may have no effect on intestinal transit.

5.2 CONCLUSION

These findings suggest that herbal alcohol when combined with kolaviron may increase intestinal motility compared to when herbal alcohol is consumed alone. However, herbal alcohol and kolaviron consumption may not have effect on intestinal transit. Therefore, it may not be suitable for patients suffering from diarrhea and therefore, their consumption should be with caution.

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