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The effects of aqueous extracts of *Hibiscus sabdariffa* Linn. calyces of *var. ruber and var. intermedius* on intestinal transit in rats

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The effects of aqueous extracts of *Hibiscus sabdariffa l.* calyces of *var. ruber* and *var. intermedius* on intestinal transit were determined in experimental rats. The dried calyces of *H. sabdariffa l.* were pulverized and 10% extracts of both *var. ruber* and *var. intermedius* were made and administered orally at varying doses. Test rats were given the 10% extracts of both the species of *H. sabdariffa* at 0.5 ml/100 g,1 ml/100 g and 2 ml/100 g body weight, while control rats received normal saline. After 30 min, each animal was then given 1.5 ml of the dye solution orally. About 1 h after administering the dye, each rat was sacrificed and the intestine carefully dissected out. The length of the intestine and the transit point of the orally administered dye were then measured. The transit point was calculated as a percentage of the total length of the intestine. The extracts of both *var. ruber* and *var. intermedius* of *H. sabdariffa l.* caused a reduction in transit points of the dye. The extract of *var. intermedius* was more effective than *var. ruber*. The reduction in transit point and the increase in transit time by both extracts indicates that the plant possess astringent effect and could be used at appropriate dose as constipating agent or for stabilizing diarrhea. The acute toxicity study of *H. sabdariffa l.* in rats was found to be above 5,000 mg kg^{-1}.

Key words: Astringent, aqueous extract, *Hibiscus sabdariffa*, intestinal transit, *var. ruber, var. intermedius.*

INTRODUCTION

Hibiscus sabdariffa has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (Olaleye, 2007). *H. sabdariffa Linn* is a herb belonging to the malvaceae family, growing about 0.5 to 3.0 m high, with a strong tap-root. It is cultivated for leaf, fleshy calyx, seed or fibre according to the respective properties of the two major varieties *var. ruber* (red) and *var. intermedius* (green) (Dalziel 1973). *H. sabdariffa l.* is grown in Central and West Africa, South East Asia, and elsewhere in parts of West Indies, Jamaica and Central America. It is commonly known as roselle or red sorrel (English), karkade (Arabic), yakuwa (Hausa), amukan (Yoruba) and okworo ozo (Ibo). The thick, red and fleshy, cup-shaped calyces of the flower are consumed worldwide as a cold beverage and as a

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hot drink (sour tea). These extracts are also used in folk medicine against many complaints that include high blood pressure, liver diseases and fever (Dalziel, 1973; Wang et al., 2000; Ross, 2003; Bako et al., 2009). The red anthocyanin pigments in the calyces are used as food colouring agents (Esselen and Sammy, 1975). In light of this, the study is designed to evaluate and compare the astringent effect of *H. sabdariffa l.* species extracts of *var. ruber* and *var. intermedius.*

MATERIALS AND METHODS

Animals

Thirty five albino rats of both sexes weighing between 130 to 150 g were used. These rats were obtained from the Department of Human physiology, Ahmadu Bello University, Zaria. They were randomly divided into seven groups of five rats per group (n = 5). Each group was kept in separate standard cage with 12 h light/dark cycle condition in the animal room of the Department of Human Physiology, Ahmadu Bello University Zaria. They were fed on commercial feeds with tap water *ad libitum*. The cages were cleaned every day, with food and water changed daily. The animals were starved 24 h before the experiment but water was allowed.

Plants

The two *H. sabdariffa l*. species (*var. ruber and var. intermedius*) were bought in Samaru-Zaria market Kaduna state, Nigeria. The plants was identified in the Department of Biological Sciences, Ahmadu Bello University Zaria, authenticated with a voucher number 1056 and deposited in the Herbarium section. Extraction was conducted using maceration method in the Department of Pharmacognosy and drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The respective calyces of both plants were sun dried separately, pulverized and then sieved. Twenty five grams (25 g) of both the powdered calyces were weighed out and dissolved in 250 ml of distilled water to make 10% extract. The resultant extracts were filtered and kept in refrigerator before use.

Dye

The dye was prepared by a modified method of Uwagboe and Orilikwe (1995). 95 ml of 10% aqueous suspension of charcoal (BDH, England) was mixed with 5 ml of Giemsa stain (BDH, England).

Intestinal transit study

The thirty five albino rats were grouped into control (Group I), *var. ruber*-treated (Group II to IV) and *var. intermedius*-treated (Group V to VII) as shown in Table 1. About 24 h before the experiment, food was withdrawn from the animals but water was allowed. During the experiment, each group received the appropriate dose of the respective extracts using oral cannula. Control group (normal saline), Group II to IV (*var. ruber*-extract) and Group V to VII (*var. intermedius*-extract) received the extracts, respectively.

Thirty minutes after administration of the extract, 1.5 ml of the dye was administered orally to each rats using oral cannula. The rats were then kept without food and water for one hour before the determination of transit point of the dye. The rats were sacrificed by overdose of chloroform at the end of the time. The peritoneum of the rats was opened and the entire length of the small intestines were carefully stretched and cut open. The lengths of the intestines from the pyloric junction as well as the distance transversed by the dye from the pyloric junction were measured.

Statistical analysis

The data collected are expressed as mean ± standard error of mean (SEM). The data obtained were analyzed using one way analysis of variance (ANOVA) and Turkey-Kramer *post hoc* test for multiple comparisons. The results were considered statistically significant if the p values were 0.05 or less (Betty and Jonathan, 2003).

RESULTS

The distance transversed by the dye from the pyloric junction was calculated as percentages of the entire length of the intestines. This was regarded as the percentages of the transit point of the dye at each dose of the extract. The mean and standard error of the mean (mean ± SEM) of these percentages were calculated for each group. Data from of the control group were regarded as zero administration of the extract. The level of significance between the transits point of each dose were determined using Turkey-Kramer post hoc test.

DISCUSSION

The results of the present study showed that both extracts of *var. ruber and var. intermedius* produced a significant reduction ($P < 0.01$) of the percentage transit point when compared with that of the control as shown in Table 2 and Figure 2. *Var. intermedius* seems to be more effective because it has activity at the lowest dose ($P < 0.05$) but the response is not dose-dependent. *Var. ruber* responded in a dose-dependent manner but the response was observed at the second dose as shown in Table 2 and Figure 1. The extracts of *H. sabdariffa* exhibited a relaxing activity by reducing the transit point. Reduction in the percentage transit point indicates a reduction in intestinal motility and an increase transit time (Oluwade et al., 2004).

H. sabdariffa is reported to inhibit the tone of various isolated muscle preparations that included rabbit aortic strip (Obiefuna et al., 1994) and rat ileal strip (Salah et al., 2002). The extract also rhythmically contracted rat uterus, guinea-pig tracheal chain and rat diaphragm. However, as the extracts contain organic acids and minerals, the effect of the extract on different smooth muscle preparations would be expected to be variable. The mechanism of action of *H. sabdariffa* aqueous extract on smooth muscles cannot be ascertained from this study. Although studies carried out by coworkers suggested that the overall effect is a direct relaxation of the smooth muscles, which is blocked by atropine through

Table 1. Animal groupings and treatments.

Extracts-treatment group	Animal groups	n	Dose of 0% aqueous extract of *var. ruber* (ml/100 g)	Dose of 10% aqueous extract of *var. intermedius* (ml/100 g)	Dose of 0.9% Nacl (ml/100 g)
Control group	I	5	-	-	1.0
Var. ruber-treated groups	II	5	0.5	-	-
	III	5	1.0	-	-
	IV	5	2.0	-	-
Var. intermedius-treated groups	V	5	-	0.5	-
	VI	5	-	1.0	-
	VII	5	-	2.0	-

Table 2. Mean percentage transit points of dye in the intestines of rats administered extracts.

Parameter / Group	Control normal saline 1.0 ml (5)	*Hibiscus sabdariffa l.*					
		Dose of 10% aqueous extract of *var. ruber* (ml/100 g)			Dose of 10% aqueous extract of *var. intermedius* (ml/100 g)		
		0.5 ml (5)	1.0 ml (5)	2.0 ml (5)	0.5 ml (5)	1.0 ml (5)	2.0 ml (5)
Mean (%) transit point	73.0±4.1	67.0±1.3	55.4±7.6	52.6±6.0	61.2±1.5	53.2±4.7	55.8±4.2
Level of significance		NS	S**	S**	S*	S**	S**

NS = Not significant; S* = significant (P < 0.05); S**=significant (P < 0.01).

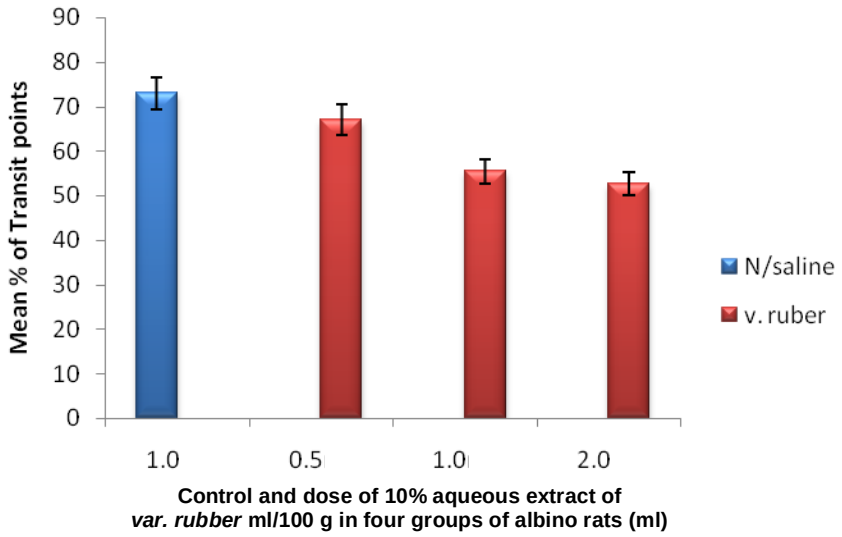

Figure 1. Mean percentage transit points of dye in the intestines of rats administered with *v. ruber*.

cholinoceptors as cholinergic antagonist. These responses may explain antibacterial activity by Olaleye (2007), the hypotensive action of the extract (Ali et al., 1991; Adegunloye et al., 1996) and the astringent effect of gastrointestinal smooth muscles.

Pectin, one of the phytochemical constituents (Odebiyi and Sofowora, 1978) of *H. sabdariffa* used as anti-diarrhea (Swinyard, 1975), could be responsible for the astringent effect. The acute toxicity study of *H. sabdariffa* in rats was found to be above 5,000 mg kg^{-1} according to Lorke's method (1983) which characterizes it to be safe for consumption. The astringent effect of the plant may

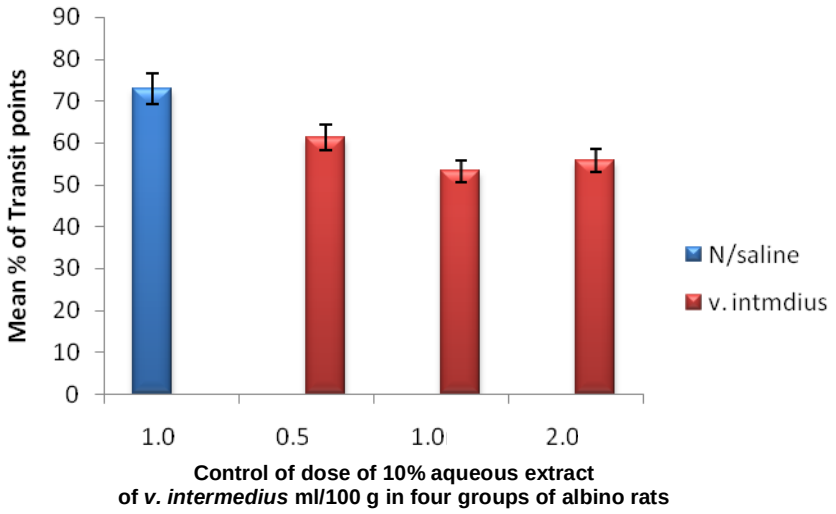

Figure 2. Mean percentage transit points of dye in the intestines of rats administered with *v. intermedius.*

be mediated through cholinoceptors as cholinergic antagonist, because atropine blocked the effect of the extract. *H. sabdariffa l.* species (*var. ruber* and *var. intermedius*) reduces the intestinal motility, though *var. intermedius* seems to be more effective because it responded at the lowest dose.

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