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International Journal of Current Research
Vol. 7, Issue, 04, pp.14496-14500, April, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ACUTE TOXICITY STUDY AND PARASYMPATHOLYTIC EFFECT OF BURANTASHI ETHANOL EXTRACT ON AN ISOLATED SMOOTH MUSCLE PREPARATION

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ARTICLE INFO

Article History:
Received 20th January, 2015
Received in revised form
07th February, 2015
Accepted 22nd March, 2015
Published online 28th April, 2015

Key words:

Acetylcholine,
Burantashi,
Gastrointestinal tract,
Parasympatholytic,
Pausinystalia yohimbe,
Receptors,
Smooth muscles.

ABSTRACT

The acute toxicity and parasympatholytic effects of a powder made from the stem bark of *Pausinystalia yohimbe*, popularly called Burantashi was evaluated using carefully designed *in vivo* charcoal meal transit in rats and *in vitro* isolated tissue models. Results obtained revealed an acute toxicity value of about 1000mg/kg body weight. All doses of Burantashi ethanol extract (BEE) also elicited a significant ($p< 0.05$) relaxation effect on the smooth muscles of the rabbit jejunum. In the *in vivo* work, 100, 200 and 400mg/kg of BEE inhibited the movement of charcoal meal in the rat's gastrointestinal tract by 8.61±0.89, 12.30±1.06 and 25.06±1.30% respectively and compared favorably with the effect of atropine (1mg/kg) which yielded a mean % inhibition of 34.27±4.15%. On isolated rabbit jejunum, BEE significantly inhibited rhythmic contractions at all final bath concentrations (FBC) administered. BEE also significantly blocked the contractile effect of acetylcholine but did not significantly affect that of propranolol (a non selective beta receptor blocker). The results obtained suggest that excessive and habitual consumption of burantashi could be deleterious to health, but mild to moderate use/consumption could be of medicinal value. The extract may contain substances with potent parasympatholytic properties which may be of value in the management of diseases associated with excess activity of the parasympathetic arm of the autonomic nervous system, and may yet serve as template for the development of more synthetic parasympatholytics of clinical significance.

INTRODUCTION

The use of burantashi, a powder derived from the bark of an African tree, *Pausinystalia yohimbe,* as food additive to barbecued meat (suya) has become a common practice in Nigeria where its consumption now spreads like a wild fire from the northern to the southern parts of the Country. Paucity of research information in this regard is evidence that no scientific investigation has so far been made to ascertain the direct effect of this popular suya additive on the gastrointestinal tract where it (burantashi) makes first contact after the mouth. It is therefore imperative to scientifically study this popularly consumed herbal preparation with a view to ascertain its health implications, whether deleterious or of medicinal value. *Pausinystalia yohimbe* is an evergreen tree belonging to family Rubiaceae. The plant is native to South, West and Central Africa where it is commonly found in the forest and jungles of Cameroun, Congo, Gabon, Nigeria and Equatorial Guinea (Duke, 1985; en.wikipedia.org/wiki/ Pausinystalia). *Pausinystalia yohimbe* usually grows up to 30 meters high and possesses a heavily fissured grey-brown coloured bark usually spotted with lichen. The erect stems branch extensively, with ovate or elliptical leaves. The tree is popularly known to contain yohimbine, an alkaloid which has been extensively used for sexual erectile dysfunctions (en.wikipedia.org/wiki/Pausinystalia). The bark extract has also been used traditionally as tonic for the management of exhaustion, chest pain, skin disorders and inflammations (en.wikipedia.org/wiki/Pausinsystalia). Parasympatholytic agents are substances that block the action of neurotransmitter acetylcholine in the parasympathetic outflow and thereby inhibit cholinergic nerve impulses by selectively occupying the muscarinic receptors to which acetylcholine molecules should bind to. This interaction between parasympatholytics and muscarinic receptors is the basis for the use of parasympatholytic agents to manage disorders caused by over activity of the parasympathetic innervation including diarrhea, incontinence, gastrointestinal cramps, gastritis, peptic ulcers, motion sickness with vomiting etc (Guyton and Hall, 1996).

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Due to the widespread consumption of burantashi along with barbecued meat (suya), the current study was designed to evaluate the acute toxicity status and gastrointestinal effect of Burantashi ethanol extract (BEE) via carefully controlled *in vivo* and *in vitro* experimental approaches.

MATERIALS AND METHODS

Collection of plant leaves and preparation of plant extract

Pausinystalia yohimbe ground stem bark (burantashi) was obtained from local herbal practitioners in Lafia, Nasarawa State, Nigeria. Fifty (50) grams of the powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using methanol as solvent. Extraction temperature was maintained at 70^0C for 48 hours. At the end of the period, the ethanol was evaporated at low temperature in an electric oven to obtain a crude extract which weighed 8.75g and represented a yield of 17.50%.

Animals

Thirty mice (20-25g), 25 rats (120-160g) and 5 rabbits (1.8-2.5kg) obtained from the Animal production unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. They were housed under specific pathogen free (SPF) conditions with 13 H/11 H light/dark schedule and were provided standard feed and water *ad libitum*, but starved for 12hours prior to commencement of experiment. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985), as expressed by Akah *et al.* (2009). The study was carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Acute toxicity (LD_{50}) test of Burantashi Ethanol Extract (BEE)

Result obtained from an initial pilot study using five mice was used for this acute toxicity dose design. Twenty five mice of both sexes weighing 25-35g were divided into 5 groups of 5 mice each and were assigned graded oral doses of the extract in the order 250, 500, 1000, 2000 and 3000mg/kg body weight. The mice were kept in aluminum cages after administration and allowed free access to feed and water. Observation was made for toxicity signs and number of deaths in each group within 24 hours and LD_{50} value for the extract was determined using Locke's method as reported by Enegide *et al.* (2013).

In vivo effect of BEE on charcoal transit in rats

Twenty five rats of both sexes were divided into 5 groups of 5 rats each. Group 1 was administered 0.2ml normal saline orally. Group 2 received 1mg/kg Atropine (i.p). Groups 3, 4 and 5 received oral administrations of BEE at doses 100, 200 and 400mg/kg respectively. Thirty minutes later, 0.2ml of activated charcoal meal was given orally to all the rats. The animals were all sacrificed in a further 30 minutes by suffocation in a chloroform chamber. Each animal was opened and the full length of the small intestine was measured. The distance travelled by the charcoal meal was also measured and expressed as a percentage of the length of the intestine using the formula:

$$\text{Percentage distance moved by charcoal meal} = \frac{\text{Distance moved by charcoal}}{\text{Full length of intestine}} \times 100$$

Percentage Inhibition for the in vivo study was evaluated using the expression

$$\text{Percentage Inhibition} = \frac{A-B}{B} \times 100$$

Where A = % distance moved by charcoal in control
B = % distance moved by charcoal in test

Preparation of intestinal smooth tissue for in vitro isometric contraction effect of BEE

The method of Uchendu, (1999) was adopted. In this method the rabbits were killed by stunning and decapitation. The abdomen was cut open and the jejunum was carefully isolated and transferred into tyrode solution that was continuously bubbled with air and maintained at 37^0C (pH 7.4) The tyrode solution had the following composition: NaCl (8g), KCl (0.2g), $CaCl_2$ (0.2g), $NaHCO_3$ (1g), NaH_2PO_4 (1g), $MgCl_2$ (0.1g) and Glucose (2g). The jejunum, about 2-3cm in length was cut out and suspended vertically in a 35ml organ bath by means of ligatures attached at one end to a tissue holder and at the other end to an isometric force displacement transducer connected to a digital physiological recorder (Medicaid Physiopac) and computer screen for displaying isometric contractions. Resting tension in the muscle strip was readjusted, just sufficient to remove the slack. The preparation was allowed to equilibrate within 30 minutes of mounting. After regular rhythmic contractions were recorded, dose-response relationships were established for acetylcholine, noradrenaline and BEE. EC_{50} values of the drugs were also administered in the presence their respective antagonists, atropine for acetylcholine and propranolol for noradrenaline. EC_{50} doses of acetylcholine and noradrenaline were also administered in the presence of BEE. For all administrations, a minimum time of 1 minute was allowed for individual tissue responses before being washed 3 times with Tyrode solution. Concentration of test substances given in the text are all final bath concentrations (FBC), except otherwise indicated.

Statistical analysis

Results were expressed as Means$\pm$ standard error of mean (SEM) and analysed using one way Analysis of variance. P-values less than 0.05 at 95% level of significance were considered as being significant.

RESULTS

Acute toxicity

Deaths were recorded in most groups within 24 hours of acute toxicity study. All animals that died had serious signs of

toxicity with aggression, convulsions, lifting of fore limbs followed by fall backwards before eventual death. Deaths were recorded in all groups that were administered up to 1000 mg/kg body weight. LD_{50} value using Locke's method is as shown below:

$LD_{50} = (a \times b)^{½}$

Where

a = highest dose that produced no death and
b =minimum dose that produced 100% death
$= (500 \times 2000)^{½}$
= 1000mg/kg

In vivo effect of BEE on charcoal transit in rats

When compared to the control group, all doses of BEE significantly ($P<0.05$) reduced the distance moved by charcoal meal in the rat's gastrointestinal tract. 100, 200 and 400mg/kg of BEE inhibited charcoal transit by 8.61 ±0.89, 12.30±1.06 and 25.06±1.30% respectively and compared favorably with the effect of atropine (1mg/kg) which yielded a mean percentage inhibition of 34.27±4.15% (Table 1).

Table 1. *In vivo* effect of BEE on charcoal meal transit in rats

Group	Treatment (mg/kg)	Mean % distance moved by charcoal	Mean % inhibition of charcoal movement
1	0.2 normal saline	92.77 ± 6.68	-
2	Atropine (1)	60.98 ± 7.32*	34.27 ± 4.15
3	BEE (100)	84.78 ± 1.18*	8.61 ± 0.89
4	BEE (200)	81.36 ± 2.17*	12.30 ± 1.06
5	BEE (400)	69.52 ± 3.21*	25.06 ± 1.30

*= p< 0.05 for test versus control

In vitro effect of BEE on an isolated rabbit jejunum

Acetylcholine produced a dose dependent increase in the amplitude of the rhythmic contractions of the isolated jejunum (Table 2), while noradrenaline produced relaxation (Table 3).

Table 2. *In vitro* effect of Acetylcholine on an isolated rabbit jejunum

FBC (µg/ml)	Mean basal amplitude (mm)	Amplitude in response to Ach (mm)	% rise in amplitude
0.014	7. 00 ± 0.00	10.33 ± 0.52*	47.57
0.029	7.00 ± 0.00	13.24 ± 0.48*	89.14
0.057	7.00 ± 0.00	17.67 ± 0.17*	152.43
0.114	7.00 ± 0.00	22.00 ± 0.52*	214.29
0.229	7.00 ± 0.00	24.15 ± 0.34*	245.00

*= p< 0.05 for test versus basal values

Table 3. *In vitro* effect of Noradrenaline on an isolated rabbit jejunum

FBC (µg/ml)	Basal amplitude (mm)	Amplitude in response to NA (mm)	% inhibition
0.014	11.15 ± 0.13	6.20 ± 0.49*	44.39
0.029	13.20 ± 0.42	7.00 ± 0.81*	46.07
0.057	14.10 ± 0.23	7.12 ± 0.25*	49.50
0.114	11.00 ± 0.19	6.18 ± 0.11*	43.82
0.229	12.25 ± 0.09	4.25 ± 0.20*	65.31

*= p< 0.05 for test versus basal values

BEE at all concentrations administered produced strong and uniform relaxation effect on the smooth muscles of the isolated jejunum. The effect of BEE compared favourably with that of noradrenaline (Table 4). The effect of acetylcholine (EC_{50} = 0.035µg/ml) was effectively blocked by atropine (0.029µg/ml) (Fig.4), while that of noradrenaline (EC_{50} = 0.012µg/ml) was also blocked by propranolol. BEE (28.57µg/ml) like atropine significantly blocked acetylcholine induced smooth muscle contractions but had no effect on the activity of propranolol.

Table 4. *In vitro* effect of BEE on an isolated rabbit jejunum

FBC (µg/ml)	Basal amplitude (mm)	Amplitude in response to PYBE (mm)	% inhibition
14.28	09.00 ± 0.25	2.14 ± 0.07*	76.22
28.57	8.00 ± 0.85	2.11 ± 0.17*	73.62
57.14	8.00 ± 0.34	1.98 ± 0.35*	75.25
114.30	8.00 ±0.25	1.13 ± 0.19*	85.86
228.60	8.00 ± 0.32	1.10 ± 0.16*	86.25

*= p< 0.05 for test versus basal values

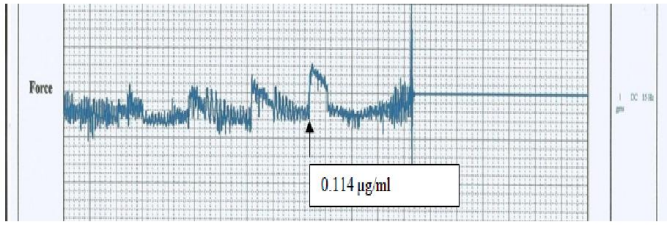

Fig. 1. Effects of graded doses of Acetylcholine on an isolated rabbit jejunum

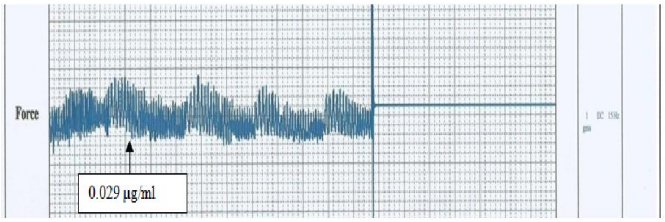

Fig. 2. Effects of graded doses of Noradrenaline on an isolated rabbit jejunum

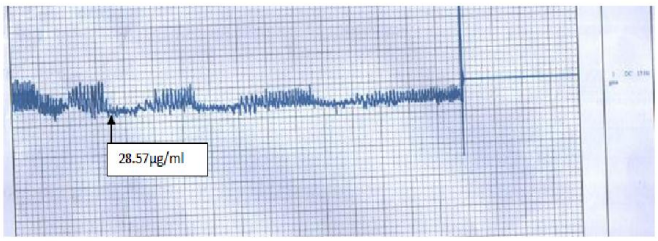

Fig. 3. Effects of graded doses of BEE on an isolated rabbit jejunum

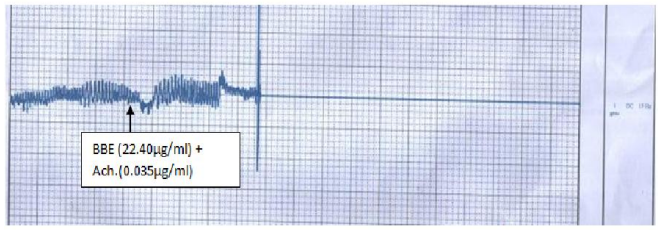

Fig. 4. Effect of BEE on Acetylcholine induced contractions on the rabbit jejunum

DISCUSSION

Intraperitoneal administration of burantashi ethanol extract (BEE) at doses up to 1000mg/kg was observed to cause toxicity in the animals used. The animals manifested toxicity signs in the form of tremor, restlessness, convulsions and eventual deaths. This suggests that BEE may contain toxic phytochemical agents which may be present beyond tolerable limits. Burantashi has been reported to contain the alkaloid yohimbine, which at high doses produces serious toxicity signs and death due to its ability to excessively raise the mean arterial blood pressure of treated animals (Ajayi *et al*., 2003). The results therefore show that excessive and constant consumption of burantashi can be toxic to the consumers and may be contributing to the increasing number of persons with high blood pressure in Nigeria. Several reports have shown that the rate of development of high blood pressure in Nigeria is on the increase (Ijioma and Emelike, 2014). Of much concern is the fact that a higher percentage of users are men above 40 years of age, who usually take burantashi in their desperate bid to enhance libido and sustain erection during sexual intercourse, but have no knowledge of the effect of such habit on their health, particularly their blood pressure.

It is established that the smooth muscles of the gastrointestinal tract is host to numerous muscarinic receptors of both M_2 and M_3 subtypes which play major role in intestinal contractility and peristaltic activity. While the M_3 receptors does so by triggering phosphoinositide hydrolysis, Ca^{2+} mobilization and direct contractile response, M_2 subtype does same by inhibiting adenylcyclase and Ca^{2+} activated K^+ channels and potentiating Ca^{2+} dependent, non selective conductance (Eglen, 2001; Ehlert *et al*., 1999; Ehlert, 2003). Thus, the administered acetylcholine in the *in vitro* experiment generated inositol, 1,4,5-triphosphate (IP_3) which evoked Ca^{2+} release from intracellular storage sites in the rabbit GIT smooth muscle cells and elicited contractions in the isolated tissue (Uchendu, 1999). This was the underlying physiological principle behind the movement of the charcoal meal along the intestines of the rats in the *in vivo* experiment, since the contractions are responsible for moving intestinal contents forward (Guyton and Hall, 1996; Sembulingam and Prema, 2010; Osim, 2002). Atropine (1mg/kg), a standard parasympatholytic agent inhibited the contractions induced by acetylcholine in the experiments conducted by competitively binding to muscarinic receptors (Rang *et al.,* 2007).

Noradrenaline in contrast caused relaxation of the isolated jejunum by binding to adrenergic receptors also found in the smooth muscle while propranolol, a non selective beta blocker provided sufficient block to this effect of noradrenaline. Burantashi ethanol extract (BEE) exerted a strong inhibitory effect on the rhythmic contractions of the isolated rabbit jejunum and also significantly ($p< 0.05$) blocked acetylcholine induced contractions with no effect on the activity of propranolol. The results suggest that BEE may contain parasympatholytic agents and may have achieved its effect by antagonizing the activity of acetylcholine via binding to available muscarinic receptors and as a result inhibiting intestinal peristaltic contractions as was observed. This observed parasympatholytic effect of burantashi extract seems to agree with the use of the extract in ethno medicine for the treatment of erectile dysfunctions. By blocking cholinergic pathway, the extract may display a typical adrenergic property of increasing blood flow to the arteries and arterioles of the penis, thus causing and sustaining erection. It is indeed established that agents which block cholinergic pathway usually raise blood pressure by increasing the force and rate of contraction of heart muscles which usually favors the erection process (Rang *et al.,* 2007). The ability of burantashi to increase blood pressure has also been reported. Conclusively, the excessive and habitual consumption of burantashi could be deleterious to health, but mild to moderate use/consumption could be of medicinal value. The inhibitory effect of the extract on the rhythmic contractions of the rabbit jejunum coupled with its significant blockade of acetylcholine induced contractions in the *in vivo* and *in vitro* experiments suggest that the extract may contain substances with potent parasympatholytic properties and may be of value in the management of diseases associated with excess activity of the parasympathetic arm of the autonomic nervous system, and may yet serve as template for the development of more synthetic clinically significant parasympatholytics.

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