ABSTRACT

The effects of the aqueous leaf extract of Combretum micranthum were studied on gastrointestinal smooth muscle of rodents. The extract was screened using isolated rabbit jejunum, guinea pig ileum and rat uterus. The extract produced relaxation of isolated rabbit jejunum and guinea pig ileum. The relaxation of guinea pig ileum was inhibited by phentolamine. The effect of the extract on rabbit jejunum and guinea pig ileum may involve adrenergic receptors. The extract had no effect on pregnant and non-pregnant isolated rat uterus. The result of preliminary phytochemical screening of the extract showed that, the aqueous leaf extract contains alkaloids, flavonoids, glycosides, saponins, tannins and phlobatannins. The properties of the extract may be due to the presence of these active constituents of pharmacological importance that bear relevance to its therapeutic claims in traditional medicine.

Keywords: Combretum micranthum, Rabbit jejunum, Rat uterus and Guinea pig ileum.

INTRODUCTION

Plants have always been a common source of medications, either in the form of traditional preparations or as a pure active principle. Most drugs of plant origin used by medical practitioners are in form of extract of the part or whole plant for example digoxin from Digitalis lanata (Trease and Evans, 1989) and the discovery of tubocurarine from Chondodendron tomentosum (Hans, 1996).

Combretum micranthum is locally known as farargeza (Hausa), Okan (Yoruba) and Nzaotego (Igbo) and belongs to the family of Combretaceae. It is widely distributed in savannah regions and in some places near the coast as a shrub or a tree and found in Northern part of Nigeria, where it is used for the treatment of diarrhoea, skin disease and to stop bleeding (Burkill, 1985). Combretum micranthum has a pleasant taste and appreciated as a digestive aid (Burkill, 1985). Combretum micranthum, is of great medicinal importance, used in the treatment of diarrhoea and various skin diseases. Personal communication with herbalist showed that, leaf of C. micranthum is used in the management of diarrhoea, blood clotting, pain and tumor. Forty years ago, a plague in the Gambia locally named "Alibain", claimed many lives but a lot of people were cured using hot tea of C. micranthum leaves (Laxen, 2001). The leaves of C. micranthum are used traditionally as cough syrup, treatment of malaria, fever and all ailments of the liver and gall bladder (Trease and Evans, 1996). The leaves of C. micranthum and C. racemosum are used as herbal remedy for the treatment of diarrhoea and various skin diseases (Kolaand Benjamin, 2002). An aqueous extract of the fresh leaves of C. micranthum was reported to contain antibacterial agent (Udum et al., 2012). Some species of Combretaceae family have been investigated and have been revealed to possess a large number of bioactive compounds, which exert strong antioxidant and antimicrobial activities (Karou, et al., 2005). Combretaceae family is rich in tannins, saponins, sterols, carbohydrates, glycoside and trace of alkaloid (Trease and Evans, 1997). The objective of this study was to investigate the phytochemical constituents and the effect of aqueous leaf extract of Combretum micranthum on isolated rabbit jejunum, guinea pig ileum and rat uterus.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaves of Combretum micranthum were collected from Malumfashi L.G.A, Katsina State. Plant was identified and authenticated in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, by comparing with voucher specimen number 900257. Leaves were air dried under the shade at room temperature (30°C) for 28 days and then ground into a fine powder using pestle and mortar. About 700 g of powdered material was soaked in water for two weeks and maceration method was used in the extraction. The extract was concentrated on water bath at temperature of 60°C.

Animals

Rats (weighing 150 – 180g), a rabbit (weighing 2.0 - 2.5 kg) and guinea pigs (weighing 250 – 300g) of either sex were used for the experiments. Animals were obtained from animal House of Faculty of Pharmaceutical sciences, Ahmadu Bello University Zaria. Animals were kept in a well-ventilated room, fed with a pelleted grower mash (vital) and water provided ad libitum. Rabbit and Guinea pig were starved for 24 hours before the experiment.
Preliminary Phytochemical screening

Preliminary screening of the aqueous leaf extract of *Combretum micranthum* was conducted using standard methods of Harbone (1989) and Trease and Evans (1997).

Isolated Tissue Studies

Method of Amos *et al.* (2000) was used. An overnight starved rabbit (weighing 2.0 – 2.5kg) and an adult guinea pig (weighing 300 – 400g) which had free access to water were sacrificed by a knock on the head, exsanguinated and the abdomen opened. Segment of the rabbit jejunum and guinea pig ileum of about 2 to 3cm long each were removed and mounted in a 25 ml organ bath containing Tyrode’s solution at 37°C and aerated with air. Thirty minutes equilibration period was allowed and the physiological solution was changed every 15 minutes. At the end of the equilibration period, the effects of extract and acetylcholine on the rabbit jejunum were evaluated. The effect of the extract on the guinea pig ileum was investigated, while phentolamine and propranolol were used as antagonists on guinea pig ileum. The doses used for the extract (mg/ml), acetylcholine (×10^{-8}mg/ml), phentolamine (×10^{-7}mg/ml) and propranolol (×10^{-7}mg/ml) are 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4. The contact time for each concentration was 1 minute while time cycles were 3 minutes. The responses were recorded on microdynamometer 7050 with speed of 24mm/min and sensitivity of 2.

**RESULTS**

**Preliminary phytochemical study**

The preliminary phytochemical screening of the extract revealed the presence of valuable constituents as showed in Table 1. Alkaloids, flavonoids, glycosides, saponins, tannins and phlabotannins were present while sterols and terpenes were absent.

**Effects of aqueous leaf extract of *C. micranthum* and acetylcholine on isolated rabbit jejunum**

The relaxation effects of aqueous leaf extract of *C. micranthum* and contraction activities of acetylcholine on isolated rabbit jejunum are presented in Figure 1. Acetylcholine produced a dose-dependent contraction (Fig. 1a) while the extract produced a dose-dependent relaxation of rabbit jejunum (Fig. 1b). The relaxation effect of the extract produced no effect on acetylcholine (Ach) induced contraction of rabbit jejunum (Fig. 1c).

**Effects of aqueous leaf extract of *C. micranthum*, phentolamine and propranolol on isolated guinea pig ileum**

The activities of extract, phentolamine and propranolol on isolated guinea pig ileum were presented in Figure 2. The aqueous leaf extract of *C. micranthum* produced a dose-dependent relaxation effect on isolated guinea pig ileum (Fig. 2a). The relaxation effect produced by the extract on guinea pig ileum was inhibited by phentolamine but unabohlished by propranolol (Figures 2b and 2c).

**Effects of aqueous leaf extract of *C. micranthum*, propranolol and oxytocinon isolated rat uterus**

The effect of oxytocin and aqueous leaf extract of *C. micranthum* on pregnant and non-pregnant isolated rat uterus are showed in Figure 3. The extract showed no response on both pregnant and non-pregnant rat uterus but oxytocin produced contraction on both uterus (Figures 3a and 3b).

Table 1: Preliminary phytochemical screening of aqueous leaf extract of *C. micranthum*

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Inference</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
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<tr>
<td>Saponins</td>
<td>+</td>
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<tr>
<td>Sterols</td>
<td>-</td>
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<td>Tannins</td>
<td>+</td>
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<tr>
<td>Terpenes</td>
<td>-</td>
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<tr>
<td>Phlabotannins</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + Presence, Absence
Fig. 1: The relaxation and contraction effects of aqueous leaf extract of *C. micranthum* (mg/ml), and acetylcholine (×10^7 mg/ml) on rabbit jejunum, ALE= Aqueous LeafExtract and Ach= Acetylcholine.
DISCUSSION
The present study on the aqueous leaf extract of *C. micranthum* revealed the presence of chemical constituents which can induce relaxation of rabbit jejunum and guinea pig ileum. The preliminary phytochemical screening conducted on the aqueous leaf extract of *C. micranthum* showed that the leaf contained valuable constituents which are of great therapeutic importance. Some chemical constituents such as alkaloids, flavonoids, saponins and tannins found present in the extract are known to have various pharmacological activities on man and animals. The same authors had also reported that the aqueous leaf extract is less-toxic via *i.p* administration in mice and rats and can be used for folkloric medicine (Abdullahi *et al.*, 2014). The extract produced relaxation of isolated rabbit jejunum and guinea pig ileum, but the relaxation effect on rabbit jejunum did not antagonize contractions induced by acetylcholine.
Acetylcholine induced contraction of smooth muscle results from activation of muscarinic receptors and differences in muscarinic receptor are now known to exist (Bonner, 1989). The relaxant effect of the extract on guinea pig ileum was inhibited by phentolamine, a non-selective α-receptor antagonist. Phentolamine antagonist postsynaptic α1 receptors and presynaptic α2− receptors of smooth muscle and one of the adverse reactions of phentolamine is gastrointestinal disturbance as a result of hypersecretion (David, 2001). The relaxation effect of the extract on gastrointestinal smooth muscle may reduce gastrointestinal tract motility. The extract may produce its effect through α2-adrenoceptor because the jejunum and ileum have α1, β− adrenoceptors while uterus has α1, β2− adrenoceptors (David, 2001). The relaxation effect on rabbit jejunum and guinea pig ileum were not affected by acetylcholine and propranolol respectively. Oxytocin produced contractile response on pregnant and non-pregnant rat uterus, but the extract showed no response on pregnant and non-pregnant rat uterus. Hence, the result of this study suggests that aqueous leaf extract of C. micranthum could be considered safe to use in traditional medicine by pregnant and non-pregnant women. The adrenergic activity of the extract may be due to the presence of tannins and flavonoids and found to be responsible for the plant activity as claim by local herbalist. Several investigators have indeed attributed the antidiarrhoeal and anti-inflammatory properties of a number of plants to their flavonoids constituent (Oyewole, 2003). Flavonoids and tannins isolated from Scleroarya birrea bark and quercitrin were studied in rats and mice respectively and they were found to be involved in the anti-diarrhoea activity (Galvez et al., 1991; Galvez et al., 1993).

CONCLUSION
The aqueous leaf extract of C. micranthum contains pharmacological active principle(s), which may account for the beneficial effect of the plant in the management of diarrhoea as claim by traditional medicine.

REFERENCES