Comparative effects of *Sorghum bicolor* leaf base extract on tissues isolated from some body systems of experimental animals

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The knowledge of the effect(s) of substances to be used as medicines on different body systems is very vital in drug development *vis-a-vis* drug safety. The effects of aqueous methanolic extract of *Sorghum bicolor* leaf base were tested on cardiovascular system-related *in vitro* models (such as isolated rat atria and rat portal vein), gastrointestinal system-related *in vitro* models (such as isolated rabbit jejunum, guinea pig ileum, rat stomach fundus strip) and reproductive system-related *in vitro* models (such as isolated rat uterus and rat vas deferens). The results showed that the extract did not alter the intrinsic myogenic contraction of both isolated rat atria and rat portal vein. The extract caused relaxation of rabbit jejunum and guinea pig ileum but contraction of rat stomach fundus strip. The extract did not alter the rhythmic contractions of stilboestrol pre-treated rat uteri. It, however, caused minimal contractions on the isolated rat vas deferens. The results of the studies therefore suggest that the extract may not have detrimental effect on the cardiovascular system if use as drug. The results also showed that the extract has the potential of being developed into antispasmodic and/or antimotility agent as a remedy for gastro-intestinal system related problems. Results also suggest that the extract may be non-uterogenic and may not be contraindicated in pregnancy. It may, however, have effect on the male reproductive secretary activity and ultimately, fertility.

**Key words:** *Sorghum bicolor*, atria, portal vein, jejunum, ileum, stomach fundus, uterus, vas deferens.

INTRODUCTION

*Sorghum bicolor* (Linn.) Pers. (Family: Gramineae; Poaceae) is an annual plant extensively used in traditional medicine. Ethnomedicinal reports show that its parts are used as demulcent and diuretic (Duke and Wain, 1981; Grieve, 1984), astringent and haemostatic (Chiej, 1984), anti-abortive, emollient (Duke and Wain, 1981). It is also used as a remedy for malaria, breast disease, diarrhoea, tubercular swellings, helminthiasis, eczema (Watt and Breyer-Brandwijk, 1962), cancer, epilepsy, flux, stomachache (Duke and Wain, 1981), bronchitis, cough, pulmonary congestion and other chest ailments (Morton, 1981), kidney and urinary complaints (Grieve, 1931), purification of blood and stimulation of blood production (Okokoh, 1999), improving body defence system and fertility (Personal Communications: Ibrahim Muazzam ,Plant Taxanomist and G. Ali, animal attendant, all with National Institute for Pharmaceutical Research and Development, Abuja, Nigeria). Several relevant and specific *in vivo* and *in vitro* studies have been carried out in our laboratory to authenticate some of these ethnomedicinal claims. This was in consideration of the fact that the rationale for the utilisation of medicinal plants usually rests largely on long-term clinical experience or ancestral experience with little or no scientific...
data on their efficacy and safety (Marin-Bettolo, 1980; Zhu et al., 2002).

However, a general pharmacological screening of the extract was also carried out. This was important for establishment of the possible effect(s) of the extract of this widely used plant on other body systems considering that a drug may be useful and indicated for a particular ailment, yet having other desirable and undesirable effects on other body systems such as cardiovascular system, gastrointestinal system, reproductive system, central nervous system, among others. The scientific revelation of the effect(s) of the extract on these body systems offers the opportunity of outlining the possible side effects of the extract when indicated for clinical use. It is also useful in stating the health conditions under which the extract (a potential drug) must or must not be used. Hence, the indications, contra-indications, side effects and precautions usually stated in drug information leaflets. In addition, many reports have shown that research into ethnomedicinal uses of plants could lead to entirely new pharmacological properties and to the isolation of useful compounds (Sim, 1971; Taylor and Farnsworth, 1973; Todd, 1978; Sofowora, 1982; Klayman, 1985; Bharati, 2006). The present study was therefore aimed at providing some of these pharmacological information in addition to the other pharmacological studies and reports on S. bicolor already done and published from our laboratory.

MATERIALS AND METHODS

Plant Preparation and Extraction

The dry mature leaves of S. bicolor were collected from Maganawa town, Sokoto State, Nigeria between the months of November and January. The plant was authenticated by a Plant Taxonomist, Mr. Ibrahim Muazzam of Herbarium Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The specimen was deposited in NIPRD’s Herbarium with voucher specimen number 3815. The dark red portions of the leaves attached to the suckers of the plants were cut out from the entire leaves (the portion of the leaves especially claimed to be used ethnomedicinally). They were then pulverized in a mortar. Two hundred grams (200 g) of the pulverized sample was cold macerated successively in 5 l of 70% v/v methanol over 96 h period on a shaker (GFL D 3006 mgH, Germany) to ensure maximum extraction. The extract was then filtered using clean cotton wool. The filtrate was placed on water bath to allow evaporation of the solvents and consequent concentration of the extract for subsequent studies. A yield of 23.6% w/w extract was obtained.

Animals

Adult Wistar rats (127.3 – 220.5 g), adult guinea pigs (345.0 – 418.9g) and adult rabbits (2.2 – 2.8 kg) of both sexes were used for the studies. They were obtained from the Animal Facility Centre in NIPRD. The experimental animals were separated for two weeks in the experimental room for acclimatization and were maintained under normal environmental temperature (26 – 28°C) with approxi- mately normal 12 h day and night illumination cycle. The animals were fed ad libitum with NIPRD formulated feed which was standard for each of the animal species except when starvation was needed in the study. They also had free access to water from Abuja Municipal water supply. The ‘Principles of laboratory animal care’ (NIH Publication # 85-23, 1985) were followed in the study.

Drugs and chemicals

The drugs and chemicals used for the studies included: Acetylcholine, histamine, 5-hydroxytryptamine, atropine (all from Sigma, USA), adrenalin hydrochloride injection (Sinochem Mingbo Ltd, China), stilboestrol injection (May and Baker Ltd, England), oxytocin injection (Rotex Medica, Germany), D (+) – glucose monohydrate (dextrose, LNL, Nigeria), sodium chloride, potassium chloride, sodium dihydrogen orthophosphate, sodium hydrogen bicarbonate, calcium chloride, magnesium chloride, magnesium sulphate, potassium dihydrogen orthophosphate (all from BDH, Poole, England), methanol (Fluka Chemie, Switzerland).

Studies on Isolated rat atri

Adult Wistar rats of both sexes were sacrificed following a blow on the head. The thoracic region of each was opened, and the heart quickly removed and put in Ringer Lorke’s solution constituted of NaCl 90 g; 10 % KCl 42 ml; D-glucose 10 g; NaHCO₃ 5 g; molar CaCl₂ 10.8 ml; dissolved in 10 l of distilled water. This was maintained at temperature of 30°C and aerated with 95% oxygen and 5% carbon dioxide mixture. The atria were then carefully removed and suspended in a 25 ml organ bath containing Ringer Lorke’s solution. At equilibration, the effects of adrenaline and graded concentrations of the leaf base extract were tested on the tissues and recorded on microdynamometer recorder set at sensitivity of 10.0 mV and speed of 95 mm/min.

Studies on isolated rat portal vein

Adult Wistar rats of either sex were used. The rats were sacrificed after a blow on the head. The abdomen of each was opened and the portal vein isolated. The isolated portal vein from each rat was suspended in a 25 ml organ bath containing Ringer Lorke’s solution (constituted as above), maintained at 37°C and aerated with a mixture of 95% oxygen and 5% carbon dioxide. The tissue was allowed to equilibrate. The spontaneous rhythmic myogenic contractions of the tissue as well as the effects of adrenaline and the leaf base extract on the intrinsic myogenic activity of the portal vein were recorded at a sensitivity of 6 mV and speed of 5 mm/min.

Studies on Isolated Rabbit Jejunum

This is as was earlier reported by Nwinyi and Kwanashie (2009). Adult rabbits of both sexes used for the study were starved of feed for about 18 h. They were sacrificed after a blow on the head. Each abdomen was cut open and segment of the jejunum (about 2 – 3 cm long) removed and put in Ringer Lorke’s solution constituting (dextrose, LNL, Nigeria), adrenalin hydrochloride injection (Sinochem Mingbo Ltd, China), stilboestrol injection (May and Baker Ltd, England), oxytocin injection (Rotex Medica, Germany), D (+) – glucose monohydrate (dextrose, LNL, Nigeria), sodium chloride, potassium chloride, sodium dihydrogen orthophosphate, sodium hydrogen bicarbonate, calcium chloride, magnesium chloride, magnesium sulphate, potassium dihydrogen orthophosphate (all from BDH, Poole, England), methanol (Fluka Chemie, Switzerland).
Studies on isolated guinea pig ileum

Adult guinea pigs of both sexes used for the study were starved of feed for 18 h. They were sacrificed after a blow on the head and the abdomen of each guinea pig was cut open. About 2 cm strips of the guinea pig ileum was removed, the adhering mesentery dissected out. This was mounted in a 25 ml organ bath containing aerated tyrode solution of the above composition and maintained at 37°C. The set up was connected to microdynamometer recorder set at sensitivity of 3.0 mV and speed of 24 mm/min. Following equilibration, the effects of histamine, acetylcholine and graded concentrations of the leaf base extract were tested on the tissue (Nwinyi and Kwanashie, 2009).

Studies on isolated rat stomach fundus strip

Adult Wistar rats of both sexes were used. They were sacrificed after giving a blow on the head. Each abdomen was cut open and the stomach removed. The pyloric region was cut off from the fundus region of the stomach. A strip of the stomach fundus was then made and mounted in aerated Krebs solution constituted of NaCl 69 g; 10 % KCl 35 ml; 10 % MgSO₄ 2H₂O 29 ml; 10 % KH₂PO₄ 16 ml; D-glucose 20 g; NaHCO₃ 21 g; molar CaCl₂ 25.2 ml; dissolved in 10 l of distilled water. The set up was connected to microdynamometer recorder set at sensitivity of 3.0 mV and speed of 24 mm/min. At equilibration, the effects of acetylcholine, histamine, 5-hydroxytryptamine (5-HT) and the leaf base extract were tested on the tissues (Nwinyi and Kwanashie, 2009).

Studies on isolated rat uterus

Adult female Wistar rats were pre-treated with stilboestrol (1 mg/kg s.c.) 48 h before the experiment to induce oestrous. Each rat was then sacrificed following a blow on the head and the abdomen cut open to reveal the fallopian tubes (uterine horns). The horns were dissected free of the adhering tissues. About 2 cm strip was cut out and mounted in a 25 ml organ bath containing De Jalon’s solution made of NaCl 90 g; 10% KCl 42 ml; D-Glucose 15 g; NaHCO₃ 5 g; molar CaCl₂ 2.7 ml; dissolved in 10 l of distilled water. The solution was maintained at 37°C and aerated with 95% oxygen/5% carbon dioxide mixture. At equilibration, the effects of oxytocin, acetylcholine and graded concentrations of the leaf base extract were recorded on microdynamometer set at sensitivity of 2.0 mV and speed of 24 mm/min.

Studies on isolated rat vas deferens

Adult male rats were stunned and bled. The lower abdomen of each rat was cut open along the midline. The intestine was moved to one side to reveal the vas deferens by the prostate and urethra. The vas deferens on each side was cut at one end and the urethra at the other end. It was dissected off the surrounding tissue and suspended in a 25 ml organ bath containing Kreb’s solution (constituted as above) maintained at 37°C and aerated with 95% oxygen and 5% carbon dioxide mixture. At equilibration, the effects of acetylcholine, adrenaline, leaf base extract, histamine and atropine were recorded on microdynamometer recorder set at sensitivity of 6.0 mV and speed of 24 mm/min.

Compliance with good laboratory practice (GLP)

The studies were carried out according to GLP regulations of Organization for Economic Cooperation and Development – OECD (UNDP/World Bank/WHO, 2001).

RESULTS

Effect on isolated rat atria

The aqueous methanolic extract of S. bicolor leaf base (0.4 – 3.2 mg/ml) produced no effect on rat atria. However, adrenaline (0.04 µg/ml) enhanced the contractile amplitude of these tissues (Figure 1).

Effect on isolated rat portal vein

The aqueous methanolic extract of S. bicolor leaf base (0.04 – 5.12 mg/ml) did not alter the intrinsic myogenic contraction of rat portal vein. Adrenaline (0.04 – 0.16 µg/ml) on the other hand increased the contractile amplitude of these tissues (Figure 2).

Effect on isolated rabbit jejunum

The aqueous methanolic extract of S. bicolor leaf base (0.04 – 2.56 mg/ml) produced a concentration-dependent relaxation of rabbit jejunum. This effect was in contrast to those of acetylcholine (0.004 – 0.016 µg/ml) and histamine (0.4 – 0.8 µg/ml), which caused contraction of the same tissues (Figure 3).

Effect on isolated guinea pig ileum

The aqueous methanolic extract of S. bicolor leaf base (0.04 – 5.12 mg/ml) did not produce any effect on smooth muscles of guinea pig ileum (Figure 4) except in one out of four preparations studied in which a slight relaxation was observed at extract concentrations of 1.28 – 5.12 mg/ml (Figure 5). Histamine (0.04 – 0.16 µg/ml) and acetylcholine (0.02 – 0.16 µg/ml) produced contraction of all the studied tissues (Figures 4 and 5).

Effect on isolated rat stomach fundus strip

The aqueous methanolic extract of S. bicolor leaf base (0.04 – 5.12 mg/ml) contracted the smooth muscles of rat stomach fundus strip. The effect was not concentration-dependent. Acetylcholine (0.04 – 0.16 µg/ml), histamine (0.08 – 0.16 µg/ml) and 5-hydroxytryptamine (5-HT; 0.004 – 0.016 µg/ml) also contracted these tissues but in a concentration dependent manner (Figure 6).

Effect on isolated rat uterus

The aqueous methanolic extract of S. bicolor leaf base (0.04 – 5.12 mg/ml) did not produce any effect on stilboestrol pre-treated uterus. Oxytocin (0.004 – 0.016 µg/ml) and acetylcholine (0.02 – 0.08 µg/ml) contracted...
Figure 1. Effect of aqueous methanolic extract of *S. bicolor* leaf base (0.4 – 3.2 mg/ml) on isolated rat atria.
these tissues in a concentration-dependent manner (Figure 7). The contractile effects of oxytocin (0.008 – 0.016 μg/ml) were not blocked by the extract (0.64 – 2.56 mg/ml; Figure 8).

**Effect on isolated rat vas deferens**

The aqueous methanolic extract of *S. bicolor* leaf base (0.4 – 25.6 mg/ml) caused minimal contractions on rat
Figure 3. Concentration-dependent relaxation effect of aqueous methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on isolated rabbit jejunum.

vas deferens. These contractions occurred at all the tested concentrations but they were not concentration-dependent. Acetylcholine (0.04 – 0.16 µg/ml) produced a concentration-dependent contraction of the tissues. The contractile amplitude of acetylcholine at these concentrations was not high. They were, however, higher than those of the extract at all the tested concentrations (Figure 9). Atropine (0.4 µg/ml) blocked the contractile effect of acetylcholine (0.16 µg/ml) on the tissue but did not block the contractile effect of the aqueous methanolic extract of *S. bicolor* leaf base (3.2 mg/ml) on same tissue (Figure 10).

**DISCUSSION**

The atrium houses the sino atrial node (SAN) from where impulse and rhythm are generated to the remaining parts of the heart. The heart is influenced by autonomic nervous system to modulate spontaneous cardiac rhythmic activity (Broadly, 1982; Willielt, 1986). The catecholamines released from the sympathetic nervous system act on post-synaptic adrenoceptors to increase heart rate and contractile force. These adrenoceptors are predominantly of the β-type (Carlson et al., 1977; Broddef et al., 1982). On the other hand, β-adrenergic antagonists slow the heart rate and decrease myocardial contractility (Mimran and Ducailar, 1988) and these antagonists have significant effect on cardiac rhythm and automacity. The present study showed that the tested concentrations of *S. bicolor* leaf base extract did not alter the intrinsic myogenic contraction of isolated rat atria while the contractile amplitude of these tissues were increased by adrenaline. In other words, the aqueous methanolic extract of *S. bicolor* leaf base neither produced effect similar to those of catecholamines nor those of β-adrenergic antagonists. This suggests that the extract may not have interfered with the mechanism(s) involved in modulation of cardiac rhythmic activity.

*S. bicolor* extract did not also alter the intrinsic myogenic contraction of rat portal vein. The spontaneous rhythmic myogenic contractions of the portal vein is also modulated by the autonomic nervous system. It also depends on the influx of extracellular calcium (Omogbai and Smith, 1990). Potassium chloride (KCl) evoked a sustained contraction of the rat portal vein which was blocked by chlorpropamid, which is thought to act by binding to and blocking an ATP-sensitive K+ channel,
which has been cloned (Philipson and Steiner, 1995). The production of no alterations in the contractility of the isolated rat portal vein by the tested concentrations of the aqueous methanolic extract possibly implies that the extract did not interfere with any of the contractile mechanisms. The absence of contractile alterations observed on both the rat atria and the portal vein possibly suggests that *S. bicolor* leaf base extract may not have detrimental effect on the cardiovascular system if in use as drug.

*S. bicolor* leaf base extract produced a concentration-dependent relaxation of rabbit jejunum in contrast to concentration-dependent contractility caused by acetylcholine and histamine on the same tissues. The extract did not produce any effect on smooth muscles of guinea pig ileum except in one out of four tissue preparations studied in which a slight relaxation was observed at extract concentrations of 1.28 – 5.12 mg/ml. These same tissues were contracted by histamine and acetylcholine. Ethnomedicinal reports have shown that parts of *S. bicolor* are used as remedy for diarrhoea (Watt and Breyer-Brandwijk, 1962). Studies carried out in our laboratory on aqueous methanolic extract of *S. bicolor* leaf base showed that the extract significantly \( p < 0.05 \)
and dose-dependently reduced the propulsive movement of charcoal meal through the gastrointestinal tract. This effect is indicative of reduction in peristaltic activity and ultimately, reduction in gastrointestinal motility. Further studies on the extract also showed a significant (P < 0.05) inhibition of castor oil-induced diarrhoea (Nwinyi and Kwanashie, 2009). Although, the mechanism for the relaxation observed on the rabbit jejunum and guinea pig ileum has not been elucidated, the relaxation could be the cause of the antimotility effect (spasmolytic effect) and subsequent anti-diarrhoeal activity observed. The significant (P < 0.05) inhibitory effect of *S. bicolor* leaf base extract on some of the gastrointestinal system-related activities therefore suggests that it also has the potential of being developed into antispasmodic and/or anti-motility agent. This also corroborated the use of *S. bicolor* plant in folklore medicine as a remedy for diarrhoea.

Conversely, the extract contracted the smooth muscles of rat stomach fundus strip in a manner that was not concentration-dependent as did acetylcholine, histamine and 5-hydroxytryptamine. The mechanism for this contractile effect on stomach fundus strip will further be elucidated to explain the reason for having contractile effect in the stomach and relaxation effect in the intestine (as was demonstrated on rabbit jejunum and guinea pig ileum).

The rhythmic contractions of stilboestrol pre-treated uteri were not altered by the leaf base extract. This was validated by the contractile effects of oxytocin and acetylcholine on the same tissues. The extract therefore seems to be non-uterogenic. In addition to this, the contractile effect of oxytocin on the tissues was not blocked by the extract. Oxytocin is known to stimulate both the frequency and force of uterine contractions. These effects are highly dependent on oestrogen (Parker and Schimmer, 2001). This justified the pre-treatment of the uteri with stilboestrol, an active synthetic oestrogen (Livingstone, 1987). In addition to this, the contractile amplitude of oxytocin on the tissue was concentration-dependently reduced by the extract.

Oxytocin acts via specific G protein-coupled membrane receptors most closely related to the V1a and V2 vasopressin receptors (Parker and Schimmer, 2001). The ability of the extract to concentration-dependently reduce the contractile effect of oxytocin on rat uteri suggests that the oxytocin receptors were partially blocked by the extract at the tested concentrations.

Higher concentrations of the extract may likely block oxytocin completely. The ethnomedicinal report that *S. bicolor* plant is used as antiabortive agent (Duke and Wain, 1981) is corroborated by this result and the mechanism for its antiabortive activity possibly involves the blockade of oxytocin receptors. There is also a possibility of its usefulness in threatened abortion. *S. bicolor* leaf base extract on the other hand caused minimal contraction of rat vas deferens. Acetylcholine also contracted these tissues.

However, the contractile effect of acetylcholine on the tissue was blocked by atropine confirming involvement of cholinergic mechanism while the contractions produced by the extract on the tissues were not blocked by atropine. This suggests that the extract may have contracted vas deferens via mechanism(s) other than
Figure 7. Effect of aqueous methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on isolated rat uterus.

Figure 8. Effect of aqueous methanolic extract of *S. bicolor* leaf base (1.28 – 2.56 mg/ml) on oxytocin-induced contraction of isolated rat uterus.
Figure 9. Effect of aqueous methanolic extract of *S. bicolor* leaf base (0.4 – 25.6 mg/ml) on isolated rat vas deferens.

Figure 10. Interaction between atropine (0.4 μg/ml), acetylcholine (0.16 μg/ml) and aqueous methanolic extract of *S. bicolor* leaf base (3.2 mg/ml) on rat vas deferens.
cholinergic mechanism. Further studies will be carried out to elucidate the mechanism of action of *S. bicolor* leaf base extract on rat vas deferens.

Summarily, the models adopted in the present studies showed the differential effects of aqueous methanolic extract of *S. bicolor* leaf base on tissues isolated from three body systems (the cardiovascular, gastrointestinal and reproductive systems). The lack of alteration in the intrinsic myogenic contraction of isolated rat atria and rat portal vein possibly suggests that the extract may not have detrimental effect on the cardiovascular system if developed and used as drug. The relaxation of isolated rabbit jejunum and guinea pig ileum shown in the present report as well as some of our laboratory findings on effect of *S. bicolor* leaf base extract on some gastrointestinal models showed significant (p < 0.05) inhibitory effects on some of the gastro-intestinal system-related activities. This suggests that drug developmental advantage can be taken of these inhibitory effects to develop anti-spasmodic and/or anti-motility agents for subsequent therapeutic uses for gastro-intestinal system related problems. The study has also revealed non alteration of the rhythmic contractions of rat uteri by *S. bicolor* leaf base extract suggesting that the extract could be non-uterogenic and may therefore be a contributory reason why it may not be contra-indicated in pregnancy if developed as drug. Infact, it could be useful in threatened abortion due to its antagonistic effect on oxytocin. The extract on the other hand caused minimal contractions on the isolated rat vas deferens. This could have effect on the secretary activity of the male gender and possibly justifies its folkloric use for fertility. Drug developmental advantage can therefore be taken of the authenticated properties of *S. bicolor* leaf base.

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