

Full Length Research Paper

Evaluation of aqueous methanolic extract of *Sorghum bicolor* leaf base for antinociceptive and anti-inflammatory activities

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Sorghum bicolor (Family: Gramineae; Poaceae) is used traditionally for some ailments related to pain and inflammation. This study was therefore aimed at investigating possible antinociceptive and anti-inflammatory effects of this plant. The aqueous methanolic (70% methanol) extract of the leaf base, its aqueous and ethylacetate fractions were evaluated for antinociceptive activity using acetic acid-induced writhing test (test on chemical pain) and tail flick test (test on mechanical pain) in Swiss albino mice. The site for antinociception was determined using formalin test in Wistar rats. Anti-inflammatory activity was evaluated using egg albumin-induced hind paw oedema in Wistar rats. Acute toxicity studies of all the extracts were also carried out on rats and mice to determine their LD₅₀. The aqueous methanolic extract and its aqueous fraction exhibited a significant ($P < 0.05$) antinociceptive activity while the ethylacetate fraction did not show antinociceptive effect. The aqueous methanolic extract showed higher percent pain inhibition in the early phase of formalin test but did not inhibit inflammation. The calculated oral LD₅₀ value for the aqueous methanolic extract was ≥ 2000 mg/kg in both mice and rats. In rats, the calculated intraperitoneal LD₅₀ value 48 h post treatment observation was 1,414.2 mg/kg while in mice, the calculated intraperitoneal LD₅₀ value was 1341.6 mg/kg for 48 h post treatment time. The intraperitoneal LD₅₀ value for both aqueous and ethylacetate fractions were ≥ 2000 mg/kg 48 h post treatment. The study revealed that *S. bicolor* leaf base contains analgesic components which are probably more in the aqueous fraction of the crude extract. This effect appeared centrally mediated. The extract did not show anti-inflammatory property.

Key words: *Sorghum bicolor*, fractions, antinociception, anti-inflammation, acute toxicity.

INTRODUCTION

Sorghum is the common and scientific name for numerous cultivated annual grasses of the genus *Sorghum*. Different parts of *Sorghum bicolor* (Linn.) Moench syn. *S. vulgare* (Linn.) Pers. (Family: Gramineae; Poaceae) belonging to the above classification are widely used ethnomedicinally for different ailments. It has been reported that a decoction of 50 g seed in a litre of water is boiled down to half a litre as a folk medication for kidney and urinary complaints (Grieve, 1931). The use of the seed for breast disease, the stem for tubercular swellings and the use of sorghum plant as a folk remedy for cancer,

epilepsy and stomach ache have been reported (Watt and Breyer-Brandwijk, 1962; Duke and Wain, 1981). The seeds of the plant are decocted for bronchitis, cough and other chest ailments in Brazil while Arubans poultice hot oil packs of the seeds on the back of those suffering pulmonary congestion (Morton, 1981). *S. bicolor* is one out of the four herbal components (*Piper guineenses* seeds, *Pterocarpus osun* stem, *Eugenia caryophyllum* fruit and *S. bicolor* leaves) of sickle cell drug (NIPRI-SAN[®]) developed by National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria (Wambebe et al., 2001). *S. bicolor* is also one out of the three plant components of Jubi Formular[®] (*Parquetina nigrescens*, *S. bicolor* and *Harungana madagascariensis*), a commercial herbal preparation manufactured by Health Forever Products Ltd., Lagos, Nigeria and recom-

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mended for the support of treatment of sickle cell anaemia, cancer, arthritis among others (Erah et al., 2003). The major signs exhibited by these clinical conditions for which different parts of *S. bicolor* plant are used in folklore medicine include pain, inflammation or both. Presently, no scientific justification has been reported on the use of this plant in the relief of pain and undesirable inflammatory process. The objective of this study was to authenticate the traditional use of the plant, evaluate the potential value of the plant as analgesic and anti-inflammatory agent and for possible drug development. This is in line with the belief that medicinal plants are important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990).

MATERIALS AND METHODS

Plant preparation and extraction

The dry mature leaves of *S. bicolor* were collected from Maganawa town, Sokoto State, Nigeria between November and January, 2006. 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 at 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.

The aqueous-methanolic extract was further partitioned into non-polar, medium polar and very polar components using the solvents; hexane, ethylacetate and water (aqueous). 10.15 g of the 70% methanolic extract was dissolved in distilled water and then gently mixed separately with each of the solvents in a separating funnel and allowed to stand for about 30 min to produce two immiscible layers that were then separated. The process was repeated until the upper partitioning solvent became clear. All the portions (hexane, ethylacetate and aqueous portions) were concentrated to small volumes in a rota vapour and finally concentrated on water bath for subsequent use. The hexane portion of the crude extract was greenish, fatty/oily and very small with a yield of 0.5% w/w (a probable indication of presence of only very small quantities of non-polar components in the crude extract). Ethylacetate portion appeared shiny, deep brownish-black in colour, clumped up but not sticky. It had a yield of 95.9% (w/w) (constituting the major component) while the aqueous component appeared deep brownish, clumped up but sticky. It gave a yield of 3.6% (w/w).

Animals

Wistar rats (155.6-269.0 g) and Swiss albino mice (15.0 - 29.1 g) of both sexes were used for the studies. They were obtained from the Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD, Abuja. The experimental animals were separated for two weeks in the experimental room for acclimatization. They were

housed in appropriately designed cages suitably bedded with wood shavings. The animals were maintained under normal environmental temperature (26-28°C) with approximately normal 12 h day and night illumination cycle. The animals were fed *ad libitum* with standard NIPRD formulated feed and had free access to water from Abuja Municipal water supply except where uniform hydration was needed in the course of the experiment. The experimental rooms were cleaned and disinfected regularly. Soiled wood shavings were replaced often. The feed and water containers as well as the animal cages were also washed regularly.

In the experimental grouping of the animals, their age/body weight and sex of the animals were taken into consideration to achieve approximately equal conditions among the groups. The animals were identified using picric acid solution to mark unique numbers on individual animals. Small cards indicating the study number, group number, animal number and dose level were stuck to different cages for identification.

The 'Principles of laboratory animal care' (NIH Publication # 85 - 23, 1985) were followed in the study.

Drugs and chemicals

The drugs and chemicals used in these studies included Aspirin (Sigma, USA), glacial acetic acid (Searle, Essex, England), methanol (Fluka Chemie, Switzerland), formaldehyde (M and B, England), hexane (BDH Chemicals Ltd, Poole, England), ethylacetate (BDH Chemicals Ltd, Poole, England), Triton x-100 (Lubley, England).

Acute toxicity study (LD₅₀)

The modified method of Lorke (1983) was adopted for the studies. The estimation of the median lethal dose (LD₅₀) values for the aqueous-methanolic extract, its ethylacetate and aqueous fractions was done using Swiss albino mice (15.0 - 29.1 g) and Wistar rats (172.6-269.0 g) of both sexes. The test routes were intraperitoneal (i.p.) and oral (p.o.) for the aqueous-methanolic extract and only intraperitoneal route for the fractions. The extract administration was done in biphasic manner using doses ranging from 100- 2000 mg/kg. The animals were observed for 72 h for behavioural effects such as nervousness, ataxia, excitement, alertness, dullness and death. The LD₅₀ was calculated as the geometric mean of the dose that caused 100% mortality and that which caused 0% mortality.

Acetic acid-induced writhing test in mice

The test was done as described by Koster et al. (1959). Adult Swiss albino mice of either sex weighing 15.1 - 29.0 g were used for the investigation. They were grouped into five (n = 5). Normal saline (20 ml/kg i.p.) was given to the first group to serve as the negative control. The second to fourth groups received graded doses of the extract (100, 200, 400 mg/kg i.p.). Acetyl salicylic acid (100 mg/kg i.p.) was administered to the fifth group to serve as the reference standard. At intervals of 30, 60, 90 and 120 min. post administration, 0.75% glacial acetic acid was administered intraperitoneally to each mouse at the dose of 10 ml/kg. 5 min. after acetic acid injection, the number of writhes (abdominal constrictions) made by each mouse within 10 min was counted using a counter. The percent writhes for the treated groups was calculated in relation to the control group. The activity was also expressed as percent inhibition of nociception (reduction in episodes of writhing between saline control and treated groups).

Tail flick test

The modified techniques of Takagi et al. (1966) and Huang et al.

(1996) were used. The study involved the use of Ugo Basile Analgesymeter (Cat No. 7200) for the evaluation of force-induced pain stimulus. This instrument exerts a force that increases at a constant rate (a certain number of grams per second). The force is continuously monitored by a pointer moving along a linear scale. Force was applied to tail of each mouse placed on a small plinth under a cone-shaped pusher with a rounded tip. The scale was read at points where each mouse suddenly withdrew its tail. These marked the points at which these mice felt the force-induced pain. The nociceptive response of every mouse was taken prior to treatment to establish the baseline values. Five groups of Swiss albino mice ($n = 5$) of either sex weighing between 15.7-21.7 g were used for the study. The first group received normal saline (20 ml/kg i.p.) to serve as the negative control. Mice in groups two, three and four received the aqueous-methanolic extract (100, 200, 400 mg/kg i.p.) respectively. Acetyl salicylic acid (100 mg/kg i.p.) was given to mice in group five to serve as a reference standard. The test was repeated as described above and the nociceptive responses measured every 30 min. over a 120 min. observation period.

This study was also carried out on the aqueous (100 - 400 mg/kg i.p.) and ethylacetate (100-400 mg/kg i.p.) fractions of the aqueous-methanolic extract of *S. bicolor*. Normal saline (20 ml/kg i.p.)-treated group and acetyl salicylic acid (100 mg/kg i.p.) treated group served as the negative control and reference standards respectively.

Formalin test

The procedure followed was that of Dubuisson and Dennis (1977). Adult Wistar rats of either sex weighing 155.6 - 244.0 g were used. They were grouped into five (of five rats each). Normal saline (20 ml/kg i.p.) was given to rats in group one to serve as negative control. The aqueous-methanolic extract (100, 200 and 400 mg/kg i.p.) was given to rats in groups two, three and four, respectively. Acetylsalicylic acid (100 mg/kg i.p.) was administered to group five rats. 30 min post treatment, 50 μ l (0.05 ml) of 2.5% formalin was injected under the planter surface of the left hind paw. The rats were then placed in transparent boxes for observation. The severity of pain was recorded as scores: (0), rat walked or stood firmly on the injected paw; (1) rat partially elevated or favoured the paw; (2), rat elevated the paw from the floor; or (3), rat licked, bit or shook the paw. The cut off points for the observations were every 2 min for the first 10 min (early phase) and at every 5 min for the period between the 10th and 60th min (late phase).

Anti-inflammatory studies

The study was done according to the method of Winter et al. (1962) as was modified by Akah and Nwambie (1994). The Wistar rats used for the investigation were deprived of water during the experiment to ensure uniform hydration and minimize variability in oedematous response (Winter et al., 1963). They were divided into five groups ($n = 5$) of both sex and body weight between 172.3 - 216.5 g. The first group received normal saline (20 ml/kg i.p.) and served as negative control. Three doses of the aqueous-methanolic extract (100, 200 and 400 mg/kg) were administered intraperitoneally to the second, third and fourth groups respectively while acetyl salicylic acid (ASA, 100 mg/kg i.p.) was given to the fifth group as a reference standard. Inflammation was then induced 30 min later by injecting 0.1 ml of fresh egg albumin into the sub-planta surface of the right hind paw of each of the rats. The principle and technique of volume displacement were adopted for the measurement of paw volume (cm^3) using LETICA Digital Plethysmometer (LE 7500) earlier calibrated with 0.15% Triton x-100. Zero readings were taken twice before injection of egg albumin (0 min) and at 20 min intervals after the injection of egg albumin over a 2 h (120 min) period. The

oedema at every interval was calculated in relation to the mean paw volume before the injection of the egg albumin. Activity for the treated groups was expressed as percent inhibition of inflammation in relation to the control group.

Statistical analysis

The results of the studies were expressed as mean \pm SEM. The differences between the control and treated means were analysed using two-way analysis of variance (ANOVA) as appropriate. Student t-test and Least Significant Difference (LSD) were applied where ANOVA showed significant difference. P-values < 0.05 were taken to be statistically significant. Results were presented as tables and diverse charts (histograms, line graphs, etc.) as appropriate.

Compliance with good laboratory practice (GLP)

The studies were carried out according to Good Laboratory Practice (GLP) Regulations of Organization for Economic Cooperation and Development-OECD (UNDP/World Bank/WHO, 2001).

RESULTS

Acute toxicity studies (LD₅₀)

No overt toxicity sign or death was observed in rats and mice 72 h post oral treatment with 100-2,000 mg/kg doses of *S. bicolor* leaf base extract. The oral median lethal dose (LD₅₀) of the extract in rats and mice was therefore ≥ 2000 mg/kg p.o. The rats treated intraperitoneally (i.p.) with the leaf base extract (100-2,000 mg/kg) showed no overt toxicity sign or death 24 h post treatment. However, all the rats treated with 2,000 mg/kg i.p. dose became recumbent and died within 48 h of the intraperitoneal treatment while those treated with 100 - 1,000 mg/kg i.p. doses neither showed toxicity signs nor death 72 h post i.p. treatment. For the estimation of the intraperitoneal median lethal dose (LD₅₀ i.p.) in rats, assessment based on 24 h post treatment showed a median lethal dose (LD₅₀) $\geq 2,000$ mg/kg i.p. since no overt toxicity sign or death was observed in i.p.-treated rats after 24 h. However, an assessment based on 48 h post i.p. treatment observation gave a calculated median lethal dose of 1,414.2 mg/kg i.p. in rats. The mice treated with doses of the extract $\leq 1,200$ mg/kg i.p. showed neither toxicity signs nor death 24 h post treatment. At the dose of 1,500 mg/kg i.p., the mice were calm, dull, with increased respiratory rate. At this dose, mortality of 66.7% and 100.0% occurred within 24 h and 48 h of i.p. treatment respectively.

The mice treated i.p. with 2,000 mg/kg dose was calm, dull and recumbent with increased respiratory rate. A mortality of 100.0% occurred at this dose within 24 h. The calculated intraperitoneal medial lethal dose in mice was 1,248.0 mg/kg i.p. and 1,341.6 mg/kg i.p. for 24 h and 48 h post treatment observations, respectively.

For the ethylacetate fraction of *S. bicolor* leaf base

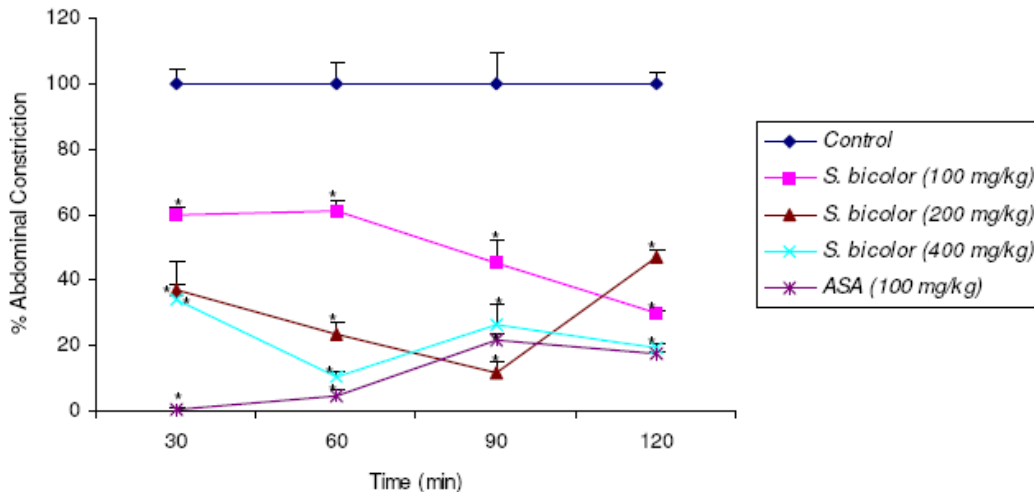


Figure 1. Effect of aqueous methanolic extract of *S. bicolor* leaf base. (100 - 400 mg/kg i.p.) on glacial acetic acid-induced abdominal constriction in mice. * $P < 0.05$; statistical difference from control (ANOVA; Least Significant Difference – LSD, $n = 5$).

extract, 33.3% and 66.7% of 1000 and 2000 mg/kg i.p.-treated mice were dull, immobilized with increased respiration within 12 min post administration. All the mice later recovered and no further toxicity signs or death was observed 24, 48 and 72 h post intraperitoneal administration. The intraperitoneal LD_{50} of ethylacetate fraction of *S. bicolor* leaf base extract in mice is therefore ≥ 2000 mg/kg.

For the aqueous fraction of *S. bicolor* leaf base extract, only 33.3% of mice treated intraperitoneally with the dose of 2,000 mg/kg were dull, immobilized with increased respiration within 10 min of administration. The mice also recovered and no further toxicity sign or death was observed 24, 48 and 72 h post i.p. administration. The intraperitoneal LD_{50} of aqueous fraction of *S. bicolor* leaf base extract in mice is therefore ≥ 2000 mg/kg.

Acetic acid-induced writhing test

The aqueous-methanolic leaf base extract of *S. bicolor* significantly ($p < 0.05$) reduced the number of acetic acid-induced abdominal constrictions (writhes) in mice at all the tested doses (100, 200 and 400 mg/kg i.p.). The antinociceptive effect was dose-dependent having a dose-pain reduction effects calculated to be 47.6, 70.7 and 76.4% for 100, 200 and 400 mg/kg doses respectively. The effect occurred throughout the observation period of 120 min. The maximal antinociceptive effect for 100 mg/kg dose was at 120 min having writhing of 29.9% (equivalence of pain inhibition of 70.1%). The maximal antinociceptive effect for 200 mg/kg dose was at 90 min with percent writhes of 11.8% (equivalence pain inhibition of 88.2%) while that of 400 mg/kg dose was at 60 min with writhes of 10.5% (pain inhibition equivalence of 89.5%). The result was comparable to that of acetyl salicylic acid

(100 mg/kg i.p.; Figure 1).

Tail flick test

The aqueous-methanolic leaf base extract of *S. bicolor* (100-400 mg/kg i.p.) generally increased the ability of mice to withstand force-induced pain (mechanical pain). The antinociceptive effect occurred throughout the observation time of 120 min. The antinociceptive effect was however significant ($p < 0.05$) at different intervals for the different doses. The results compared favourably with acetylsalicylic acid (100 mg/kg i.p.; Figure 2).

The aqueous fraction (100 - 400 mg/kg i.p.) of *S. bicolor* leaf base extract also increased the ability of mice to withstand mechanical pain. The aqueous fraction at the tested doses of 100, 200 and 400 mg/kg i.p. produced significant ($p < 0.05$) antinociceptive effect in comparison with the zero minute (0 min) reading of the mice. The result is comparable to that of acetylsalicylic acid (100 mg/kg i.p.). Ethylacetate fraction on the other hand did not show antinociceptive effect at the tested doses (100-400 mg/kg i.p.; Figure 3).

Formalin test

The aqueous-methanolic extract dose-dependently reduced formalin-induced pain in both early and late phases of the experiment. However, the percent pain inhibition between 0 - 10 min were 10.2, 38.8 and 55.1% for 100, 200 and 400 mg/kg i.p. doses of the extract, respectively, while the percent pain inhibition between 15 - 60 min were 5, 32.9 and 37.9%, respectively, for 100, 200 and 400 mg/kg i.p. doses of the extract. This was unlike acetylsalicylic acid (100 mg/kg i.p.) that showed

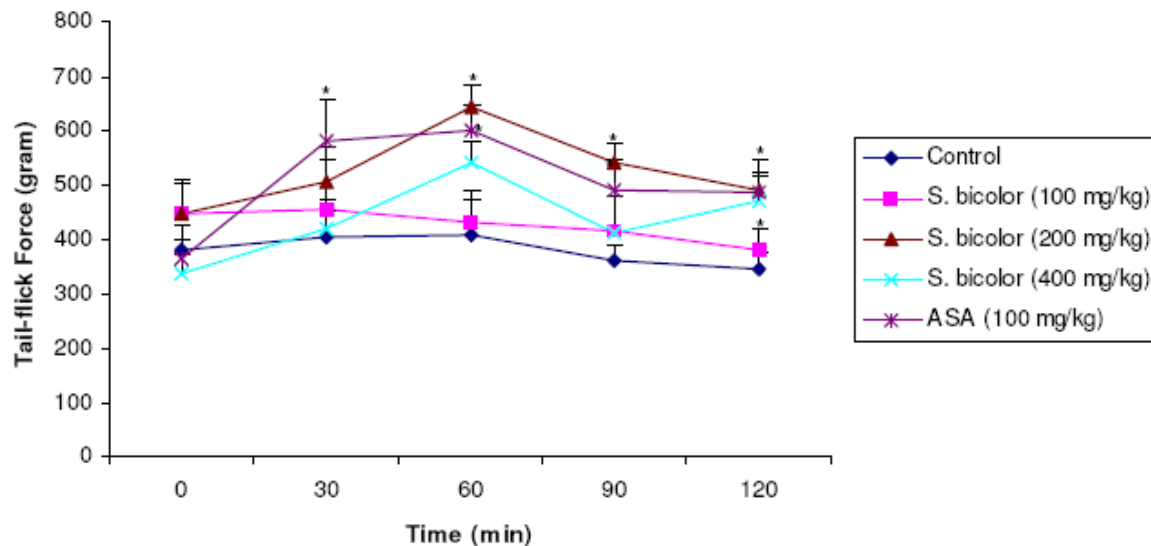


Figure 2. Effect of aqueous methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on force-induced pain in mice. * = $P < 0.05$; statistical difference from control (ANOVA, Student t-test).

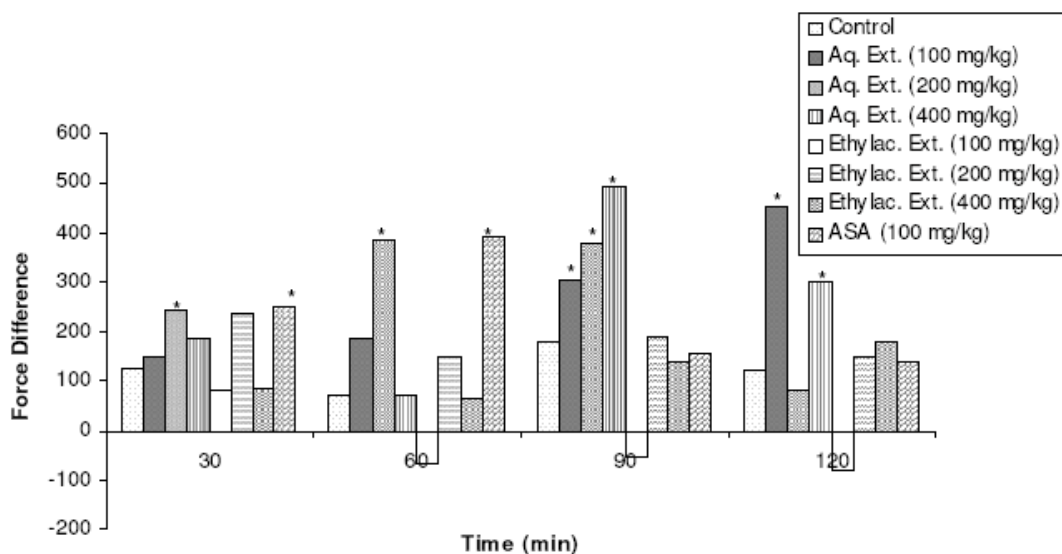


Figure 3. Effect of the aqueous and ethylacetate fractions (100 - 400 mg/kg i.p.) of *S. bicolor* leaf base extract on force induced pain in mice. * = $P < 0.05$; statistical difference from control (ANOVA, Student t-test). Ext. = Extract; Aq. = aqueous; Ethylac. = ethylacetate. Note: The plotted force values were the differences between the force exerted at zero minute and all the time intervals.

higher percent pain inhibition of 81.4 % in the late phase (15-60 min) and only 24.5% in the early phase (0-10 min; Table 1).

Anti-inflammatory studies

The results revealed that intraperitoneal administration of aqueous-methanolic leaf base extract of *S. bicolor* (100, 200, 400 mg/kg i.p.) did not inhibit fresh egg albumin-

induced inflammation (measured as oedema in cm^3) in rats. Significant ($p < 0.05$) increase in oedema was rather recorded at the dose of 400 mg/kg i.p. of the extract. The statistical comparison was done with the normal saline treated group (Figure 4).

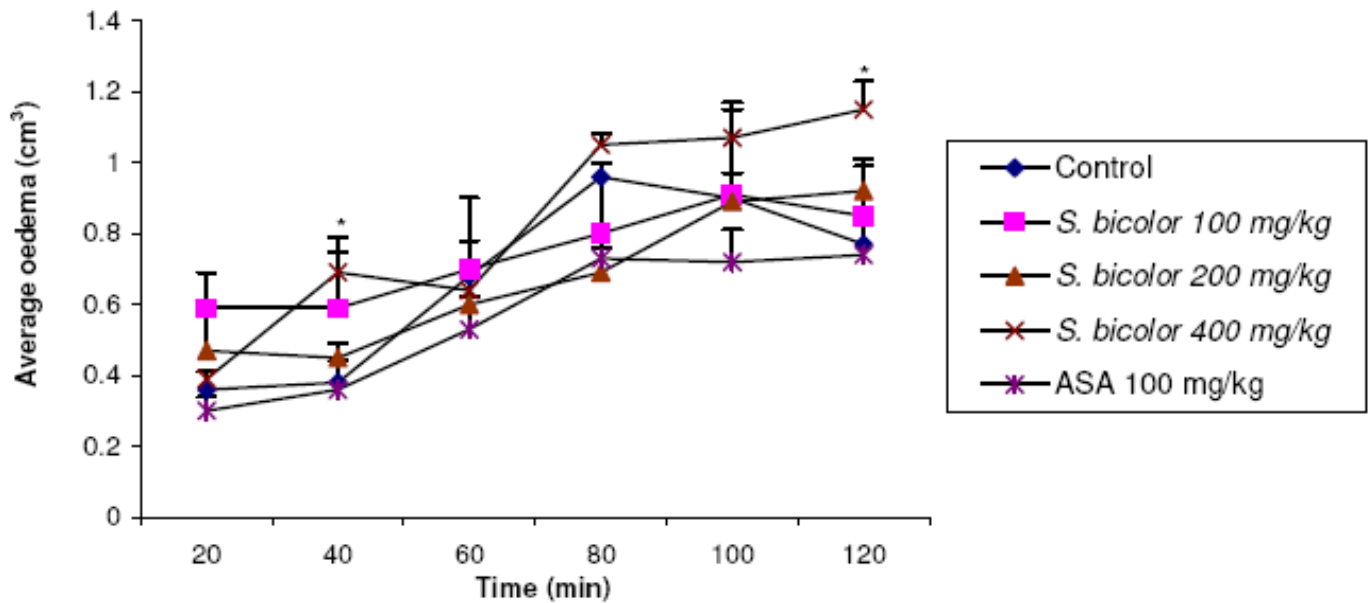
DISCUSSION

The use of abdominal constriction (writhing test) model

Table 1. Effect of methanolic extract of *S. bicolor* leaf base (100, 200, 400 mg/kg i.p.) on early and late phases of formalin-induced pain in rats.

Treatment	Early phase		Late phase	
	Score of pain	% pain inhibition	Score of pain	% pain inhibition
Control	1.96 ± 0.6	-	2.80 ± 0.3	-
<i>S. bicolor</i> 100 mg/kg i.p.	1.76 ± 0.6	10.2	2.66 ± 1.2	5.0
200 mg/kg i.p.	1.20 ± 0.6	38.8	1.88 ± 0.5	32.9
400 mg/kg i.p.	0.88 ± 0.5	55.1	1.74 ± 0.6	37.9
ASA (150 mg/kg i.p.)	1.48 ± 0.4	24.5	0.52 ± 0.4*	81.4

*p < 0.05; statistical difference from control (Student t-test).

**Figure 4.** Effect of aqueous-methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on fresh egg albumin-induced paw oedema in rats. * = P < 0.05; statistically different between treated and control group (two-way ANOVA; Student t-test, n = 5).

was adopted for the evaluation of the aqueous-methanolic leaf base extract for antinociceptive effect on chemical pain. The writhing response is thought to partly involve local peritoneal receptors (Bentley et al., 1983; Mat et al., 1997; Atta and Alkofahi, 1998). The result of the study showed that the extract significantly ($p < 0.05$) and dose-dependently reduced the number of acetic acid-induced abdominal constrictions (writhes) in mice. This probably suggests an antinociceptive property. This effect progressed over the 120 min (2 h) observation period suggesting a possible prolongation of antinociception. Although the use of abdominal constriction (writhing) model for detection of antinociceptive activity has been reported to be more sensitive when compared with other models such as tail flick model (Collier et al., 1968; Bentley et al., 1981), the present study revealed that antinociceptive evaluation of the aqueous-methanolic extract carried out on mechanical pain using tail flick test

gave similar results as that of writhing test model. The extract was able to increase the ability of mice to withstand force-induced pain (mechanical pain). The antinociceptive effect also seen throughout the observation period of 120 min (2 h) with the tail flick test confirmed the prolonged antinociceptive property. This is an indication that the leaf base extract has the potential of being developed into analgesic. The tail flick test on the aqueous and ethylacetate fractions also revealed that the antinociceptive property was retained in the aqueous fraction while the effect was not seen in the ethylacetate fraction. This suggests that the component responsible for the antinociception is polar in nature. These results corroborate the ethnomedicinal use of the plant parts for stomachache, breast disease, tubercular swelling, kidney and urinary complaints. These conditions are usually associated with pain. The antinociceptive property of the plant leaf may have therefore been taken advantage of

traditionally for pain relief. This may also be a reason for the inclusion of *S. bicolor* leaves as one out of the four herbal components (*P. guineenses* seeds, *P. osun* stem, *E. caryophyllum* fruit and *S. bicolor* leaves) of the sickle cell drug (NIPRISAN[®]) developed by National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, considering that sickle cell is a condition usually associated with pain.

Analgesics are classified into two which include the opioid analgesics (such as morphine and related compounds like codeine) and the antipyretic analgesics (also called non-opioid analgesics such as aspirin, acetanilide and phenacetin). The opioid analgesics are more consistently effective than antipyretic analgesics in pain associated with trauma or with deep structures such as viscera. The antipyretic analgesics however, are more useful in the treatment of pain associated with headache, connective tissue, arthralgia and pains arising from integumental structures rather than viscera.

Drugs such as narcotics (which opiates are) act mainly centrally while drugs such as aspirin, hydrocortisone and dexamethasone (which are non-opioids) are primarily peripherally acting (Ahmadiani et al., 1995). In the present investigation, formalin test was adopted to elucidate the possible site (central or peripheral) of antinociceptive activity observed in the aqueous-methanolic extract. The result revealed that the extract dose-dependently reduced formalin-induced pain in both early (0-10 min) and late (15-60 min) phases of the experiment. However, the percent pain inhibition in the early phase was more than the inhibition in the late phase of the experiment. This was unlike aspirin that showed higher percent pain inhibition in the late phase. Dubuisson and Dennis (1997) and Tjolsen et al. (1992) reported that in formalin test, nociception occurs in two phases. The first phase starts immediately after formalin injection and continues for 5 min, after which nociception appears to diminish. The second phase is marked by a return to high levels of nociception beginning 15 - 20 min after formalin injection and continuing for 60 min. The first phase is probably a direct result of stimulation of nociceptors in the paw, while the second phase may reflect the inflammation process, and at least to some degree, the sensitization of central nociceptive neurons (Coderre et al., 1990; Coderre and Melzack, 1992). This method is very useful for elucidating the mechanism of pain and analgesia (Tjolsen et al., 1992). Drugs such as narcotics which act mainly centrally inhibit both phases of formalin-induced Pain while drugs, such as aspirin, hydrocortisone and dexamethasone which are primarily peripherally acting only inhibit the late phase (Chen et al., 1995; Elisabetsky et al., 1995; Santos et al., 1995).

Therefore, the inhibitory action of the extract on both early and late phases suggests that the central mechanism may be involved. Also to note is that the second phase of formalin test is related to a peripheral inflammatory process. However, the present investigation

showed that the leaf base extract could not inhibit inflammation (oedema) induced by fresh egg albumin in rats. It can therefore be deduced that the peripheral mechanism may not be involved in the antinociceptive effect of *S. bicolor* leaf base extract. This therefore suggests that the extract of *S. bicolor* leaf base (by the classification of analgesics into opioids and antipyretics) may be of the opioid analgesic type rather than the antipyretic analgesic. This is also in line with the report of Carlsson and Juma (1987) that tail flick test is very sensitive to centrally acting drugs. Thus, analgesic effect of the extract in the tail flick model provides additional evidence for central antinociceptive action of the extract. Subsequent study will involve the interaction of the antinociceptive action of the extract with opioid antagonists such as naloxone, to see if the effect could be reversed as is typical of opioid analgesics.

Further classification of analgesics showed that some drugs are known to be clinically effective analgesics and antipyretics but lack significant anti-inflammatory properties. Such drugs include phenacetin, acetaminophen while other drugs are potent anti-inflammatory agents but lack or have only weak analgesic properties. Such drugs include phenylbutazone. Others have analgesic and anti-inflammatory properties example, aspirin. The present investigation has shown that *S. bicolor* leaf base extract did not inhibit egg albumin-induced inflammation, while acetic acid-induced writhing test on chemical pain and tail flick test on mechanical pain showed it to have a significant ($p < 0.05$) analgesic property. The extract therefore behaved like phenacetin and acetaminophen which have analgesic but no anti-inflammatory property.

In conclusion, these results showed that *S. bicolor* leaf posses significant ($p < 0.05$) anti-nociceptive activity which suggests the presence of active principles with the potential of being developed into analgesics.

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