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Saponins are involved in the analgesic and anti-inflammatory properties of *Ficus platyphylla* stem bark

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ABSTRACT

The analgesic and anti-inflammatory properties of saponins (FPS) from the methanol extract of *Ficus platyphylla* stem bark were studied in rodents. FPS significantly attenuated acetic acid-induced writhes in mice and inhibited responses in both phases I & II of formalin-induced nociception. FPS demonstrated significant antinociceptive activity in Analgesy-meter model of nociception and significantly attenuated albumin-induced oedema in rats. Morphine significantly ($p<0.05$) inhibited responses in both phases I & II of formalin-induced nociception and increased the threshold of mechanically induced nociception in rats. The effects of FPS on formalin-induced nociception and the threshold of mechanically-induced nociception were less compare to the effects of morphine. Acetylsalicylic acid (ASA), significantly ($p<0.05$) reduced acetic acid induced writhes in mice, attenuated responses in the late phase II of formalin- induced nociception and albumin-induced oedema in rats, but failed to attenuate responses in the early phase I of the formalin-induced nociception in rats. The effects of FPS on the late phase of formalin-induced nociception were greater than the effects of ASA. Effects of FPS on acetic acid- writhes in mice were less compare to the effects of ASA. Our results provided evidence that saponins are implicated in the analgesic and anti-inflammatory effects observed in our earlier studies on the crude methanol extract of *Ficus platyphylla* stem bark, thus supporting the isolation and development of the saponin components of this medicinal plant as analgesics and anti-inflammatory agents.

***Keyword**: Ficus platyphylla;* saponins; anti-nociception; anti-inflammation

INTRODUCTION

The search for chemical agents to ameliorate pain and inflammation has been an effort of man throughout recorded history. Medicinal plants are known to be important sources of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). Consequently, research on plants which are employed for the treatment of pain and inflammation is one of the potential and logical strategies in the search for new analgesic and anti-inflammatory drugs (Elisabetsky et al., 1995). Currently available analgesics and anti-

inflammatory drugs are often not suitable in all patients and are often accompanied by debilitating adverse effects including a high propensity for tolerance (opiates) effects, sedation and gastrointestinal effects (Vongtau et al., 2004; Burke et al., 2006; Gutstein and Akil, 2006). The development of new pharmacological agents that can overcome these barriers has become a major goal in drug research and development.

Preparations of the stem bark of *Ficus platyphylla Del.-Holl (Family: Moraceae)* have been used in Nigerian traditional medicine to treat psychoses, depression, epilepsy, pain and inflammation for many years and their efficacies are widely acclaimed among the Hausa communities of Northern Nigeria (Audu, 1989). The cold water extract, decoction or powder of the stem or root bark are usually taken orally while the powder is often mixed with food and eaten or placed in burning charcoal and inhaled (Audu, 1989). Our previous studies revealed that the crude methanol extract of *Ficus platyphylla* stem bark contains sedative principles with potential anticonvulsant, neuroleptic, analgesic and anti-inflammatory properties (Gamaniel et al., 2000; Amos et al., 2002; Chindo et al., 2003; 2009). Since saponins, which form the major components of the crude methanol extract are believed to have analgesic and anti-inflammatory properties (Gupta at al., 1969; Adzu et al., 2003., Taesotiku et al., 2003; Asongalem et al., 2004; Ojewole, 2008), we hypothesized that saponins may be involved in the analgesic and anti-inflammatory effects of the crude methanol extract earlier reported (Amos et al., 2002). Here, we evaluated the analgesic and anti-inflammatory properties of the saponin rich fraction as a step toward the isolation of biologically active components of extract of *Ficus platyphylla* and to complement earlier studies (Chindo et al., 2008; 2009) on this medicinal plant, which is already in common use.

MATERIALS AND METHODS

Animals

Swiss albino mice (18 - 25 g) and Wistar rats (180–250 g) of either sex maintained at the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja were used. All animals were housed under standard conditions of temperature (25 ± 2 ^{o}C), and light approximately (12/12 h light / dark cycle) and fed on standard diet (Ladokun Feeds, Plc, Ibadan, Nigeria) and water *ad libitum*. These animals were approved for use by the AFC committee after reviewing the protocol for good laboratory practice and animal handling, which is in compliance with the National Institute of Health's *Guide for the Care and use for Laboratory animals* (Publication No. 85-23, revised 1985).

Plant material

The plant material was collected from Zaria in Kaduna State, Nigeria. The plant was identified and authenticated by Mallam I. Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (no. 4035) was deposited at NIPRD Herbarium for future reference.

Extraction of saponin components of the plant

The method described by Ebata et al. (1996) was employed for the extraction of saponin rich fractions of *Ficus platyphylla* stem bark with some modifications. Briefly, 100 g of the coarse powder was sequentially extracted with solvents of increasing polarity including hexane, ethylacetate, dichloromethane and methanol, each for 6 h using a soxhlet apparatus. The resulting extracts were concentrated to dryness *in vacuo* at 40 ^{o}C using a rotary evaporator. The methanol extract was partitioned between water and butanol (1:3) to give butanol and aqueous fractions. The butanol fraction was then dissolved in methanol and added dropwise to diethylether. A precipitate was formed, which was separated from the solution by filtration to give the saponin rich fraction designated FPS. The FPS was used for analgesic and anti-inflammatory studies.

Formalin test

The method used for this test was similar to that described by Dubuisson and Dennis (1977) and modified by Vongtau et al. (2004). Adult wistar rats were divided into six groups of six rats each and pre-treated p.o. as follows: group I received normal saline as control; groups II, III and IV received FPS at doses of 25, 50 and 100 mg/kg, p.o. respectively, while groups V and VI received acetylsalicylic acid (150 mg/kg, p.o.) and morphine (4 mg/kg, *s.c.*) respectively. Thirty minute after these treatments the rats were administered 50 µl of a 2.5% solution of formalin subcutaneously under the plantar surface of the left hind-paw. They were then placed in an observation chamber in such a way as to ensure an un-obstructed view of the injected paw and monitored for 1 h. Severity of pain responses were recorded based on the following scale: (0) rats walked or stood firmly on injected paw; (1) the injected paw was favored or partially elevated; (2) the injected paw was clearly lifted off the floor; (3) the rat licked, chewed or shook the injected paw. Anti-nociceptive effect was determined in two phases, the early phase (I) being recorded during the first 5 min while the late phase (II) was recorded during the last 45 min with a 10 min lag period in between the two phases.

Acetic acid test

The method described by Koster et al. (1959) was used with some modifications. The mice were randomly-divided into five test groups of six mice each. The first three groups were pre-treated with 25, 50 and 100 mg/kg, p.o. of FPS, respectively. Two other groups received normal saline 3 ml/kg and acetyl salicylic acid 150 mg/kg orally (p.o.), respectively, to serve as controls. Thirty minutes after pre-treatment, each mouse was injected with 10 ml/kg of an aqueous solution of 0.7% acetic acid *i.p.* and placed in a transparent Perspex observation box. After a five minute lag period, the number of writhes (a syndrome characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) for each mouse was counted for five minutes at 30 and 60th min respectively.

Pain threshold test

The study involved the use of Analgesymeter (Model 7200, Ugo Basile, Italy) as described by Vongtau et al. (2004). The force is monitored by a pointer moving along a linear scale and the rat's response is taken to be the point at which it struggles or its paw slips off the plinth of the instrument. Rats were divided into five groups of six rats each and pre-treated as follows: Groups I, II and III received FPS at doses of 25, 50 and 100 mg/kg p.o., respectively, while groups IV and V received normal saline and morphine (4 mg/kg, *s.c.*) respectively. Thirty minutes after treatment, readings were taken at thirty minutes intervals for 120 min.

Anti-inflammatory test

This test was carried out using a modification of Winter et al. (1963) as described by Akah and Nwambie (1994). The rats were divided into five groups of six rats of either sex and pre-treated as follows: Group I (control) received normal saline (3 ml/kg p.o.), groups II, III and IV received 25, 50 and 100 mg/kg of FPS p.o., respectively, while group V received acetyl salicylic acid (150 mg/kg, p.o.). After 30 min of post-drug administration, rats in each group were injected with 50 µl/kg raw egg albumin in the sub-plantar surface of the left hind-paw thereby inducing pedal oedema. A digital plethysmometer (Letica, Spain LE7500) was used to measure the volume of paw oedema (volume displacement) for a period of 120 minutes, with readings taken at 20 min intervals, i.e. 20, 40, 60, 80, 100 and 120 min after albumin administration.

Statistical analysis

Data were expressed as mean $\pm$ S.E.M with *n* indicating the number of replicates for a given experiment. Data were analyzed by one-way ANOVA followed by Dunnet's post

hoc test for multiple comparisons. Results were considered significant at $p<0.05$

RESULTS

The oral LD_{50} of FPS and the crude methanol extract, from which FPS was obtained, were reported earlier to be 1274 ± 42.2 mg/kg and >5000 mg/kg respectively (Amos et al., 2002; Chindo et al., 2009). At higher doses, FPS caused a dose related decrease in locomotor activity, sedation and drowsiness (hypnosis), respiratory distress and subsequently death of the animals (Chindo et al., 2009).

FPS at doses of 25, 50 and 100 mg/kg showed dose-dependent anti-nociceptive activities in all the three experimental models of nociception studied. There was a significant ($p < 0.05$) reduction in responses to formalin-induced pain in rats in both phases I and II by FPS (25, 50, 100 mg/kg). Morphine (4 mg/kg) significantly ($P < 0.05$) attenuated the responses to formalin-induced pain in the 2 phases compared to control, while ASA at 150 mg/kg, showed higher activity in the late phase (phase II). FPS (25, 50, 100 mg/kg) significantly ($p < 0.05$) attenuated the number of acetic acid-induced writhes in mice. Acetylsalicylic acid significantly ($p<0.05$) inhibited acetic acid-induced writhes in mice. The percentile inhibition of early phase of formalin-induced nociception by FPS (25, 50, 100 mg/kg), aspirin and morphine are 35.7%, 50%, 64.3%, 14.3% and 71.4% respectively; In the late phase of formalin-induced nociception, the percentile inhibition observed following the administration of FPS (25, 50, 100 mg/kg), aspirin and morphine are 69.2%, 76.9%, 53.8%, 53.8% and 61.5%. The inhibition of acetic acid-induced writhes by FPS (25, 50, 100 mg/kg) and aspirin are 66.1%. 73.8%, 86.9% and 76.8% respectively at the 30 min after administration of test extract and aspirin; and 67.2%, 76.7%, 89.7% and 89% respectively after 60 min of test extract and aspirin administration.

In analgesymeter model that employ mechanically induced pain, FPS significantly ($p<0.05$) demonstrated antinociceptive activity in rats. Morphine expectedly exhibited a significant ($p<0.05$) antinociceptive effect in the analgesymeter model as well. FPS significantly inhibited albumin-induced oedema in rats over a period of 120 min duration. Effects were dose-dependent and comparable to the standard anti-inflammatory drug (ASA) used in this study.

DISCUSSION

The data presented in this study provide scientific evidence that saponins (FPS) obtained from crude methanol extract of *Ficus platyphylla* stem bark may contain biologically active principles that are relevant in the management of pain and inflammation. FPS showed significant and dose dependent antinociceptive activity against formalin, acetic acid and analgesy-meter models of pain, suggesting the presence of both central and peripheral pathway modulatory activities. In the formalin test there is a distinctive biphasic nociceptive response termed early and late phases. The early phase, which starts immediately after formalin injection and continues for 5 min, reflects centrally mediated pain while the late phase that begins 15 – 20 min after formalin injection and continue for approximately to 60 min duration, is due to inflammation with a release of serotonin, histamine, bradykinin and prostaglandins (Tjolsen et al., 1992) and at least to some degree, the sensitization of central nociceptive neurons (Coderre et al., 1990; Coderre and Melzack, 1992; Tjolsen et al., 1992). Consequently, centrally acting drugs such as opiates inhibit both early and late phases (Vogel and Vogel, 1997), whereas non-narcotic analgesics such as acetyl salicylic acid, 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). Suppression of both phases of nociception by FPS in this study suggests the presence of both central and

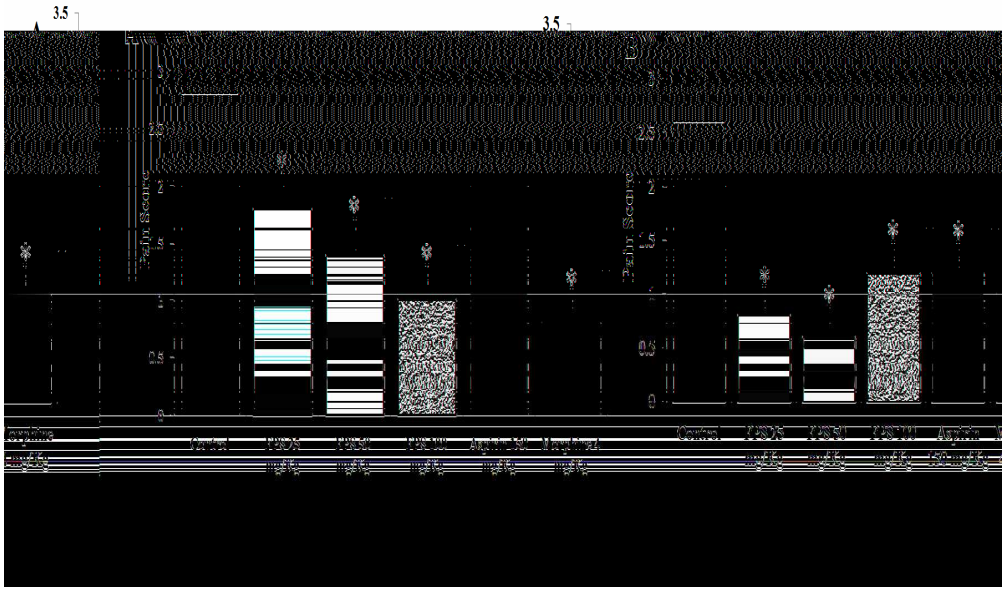

Figure 1: Effect of FPS on the early phase of formalin-induced pain in rats. (A) Pain score of the early phase of formalin-induced nociception and (B) Pain score of the late phase of formalin-induced nociception in rats. *significantly different from control ($p<0.05$, n=6).

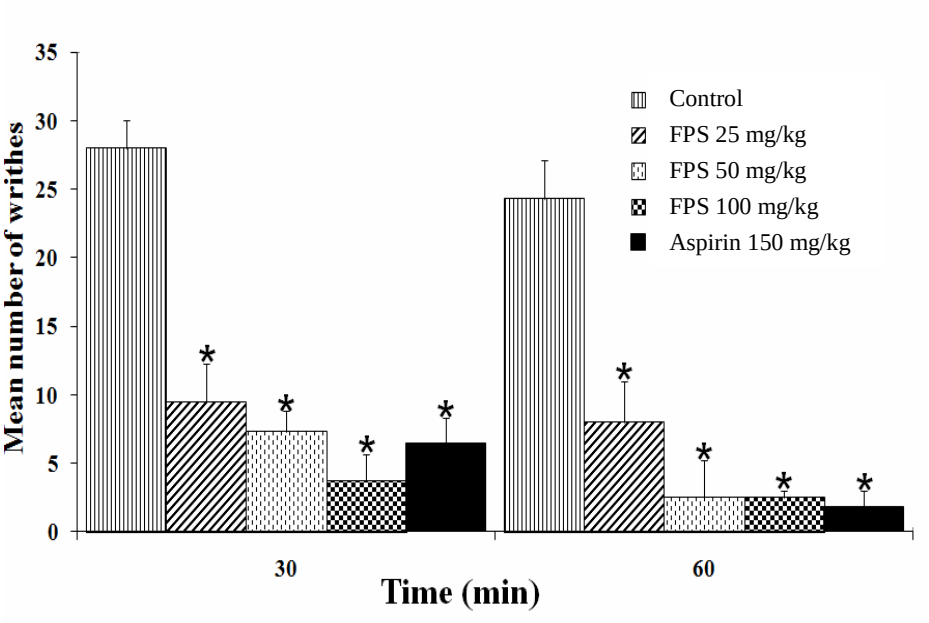

Figure 2: Effects of FPS on acetic acid-induced writhes in mice. (A) Figures represent Mean number writhes ± SEM. * significantly different from control ($p<0.05$, n=6).

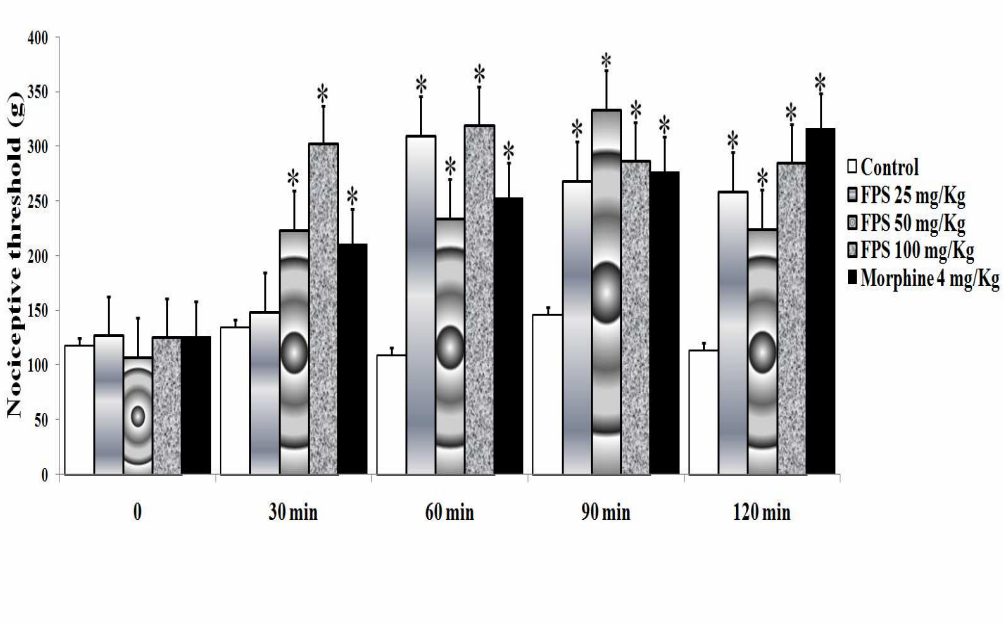

Figure 3: Effects of FPS on pain threshold in analgesy-meter tests in rats.
*significantly different from control ($p<0.05$, n=6).

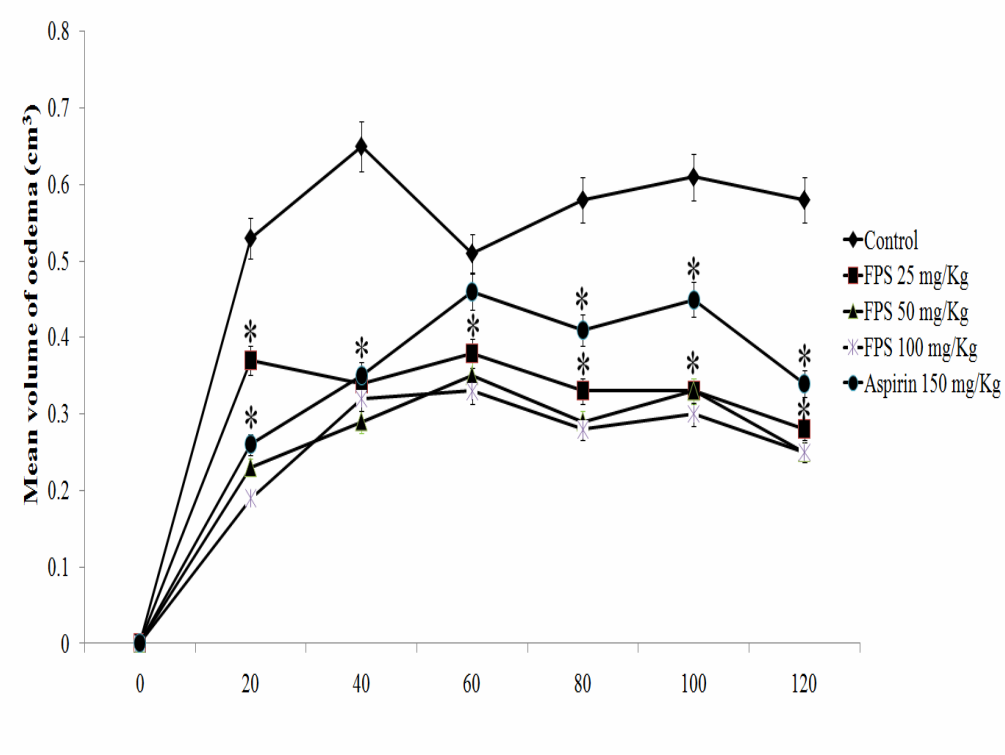

Figure 4: Anti-inflammatory effects of FPS in rats.
* significantly different from control ($p<0.05$, n=6).

peripheral activities (Chan, et al., 2000; Tjolson et al., 1992).

FPS significantly attenuated acetic acid induced pain in mice dose-dependently, which is consistent with previous findings on the crude methanol extracts (Amos et al., 2002). The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics (Gene et al., 1998). This model is able to detect antinociceptive activities of compounds at dose levels that may appear inactive in other models of pain (Collier et al., 1968; Bentley et al., 1981). Local peritoneal receptors, increased levels of prostaglandins, bradykinins, serotonin, substance P and leukotrienes in peritoneal fluids are postulated to be partly involved in the acetic acid-induced abdominal constriction response (Bentley et al., 1983; Levini et al., 1984; Dhara et al., 2000). The significant reduction in acetic acid-induced writhes by FPS suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of prostaglandins and other related endogenous substances.

The analgesy-meter antinociceptive test is useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level (Vongtau et al., 2004). The significant increase in pain threshold produced by FPS in the analgesymeter test suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Bensreti and Sewel, 1983; Headley and Oshaughnessy, 1985; Wigdor and Wilcox, 1987; Pasero et al., 1999; Salawu et al., 2008). The analgesic effect produced by this extract therefore may be via central mechanisms involving opiate, dopaminergic, descending noradrenergic and serotonergic systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain.

The results of the anti-inflammatory studies suggest that FPS possess anti-inflammatory principles of high potency. There are several mechanisms involved in the anti-inflammatory activity of drugs. They include the endogenous release of glucocorticoids, interaction with prostaglandin biosynthesis, interaction with tachykinins or other inflammatory mediators (Barnes et al., 1990; Vongtau et al., 2004). It is difficult to ascertain from this data, the exact mechanism by which FPS achieved anti-inflammatory activity. However, the anti-inflammatory effects are comparable to those of non-steroidal anti-inflammatory drugs, particularly the salicylates and their derivatives.

In conclusion, we have presented scientific evidence that saponins are involved in the analgesic and anti-inflammatory effects earlier observed in our studies (Amos et al., 2002) on the methanol extract of *Ficus platyphylla* stem bark, thus supporting the isolation and development of the saponin components of this medicinal plant as analgesic and anti-inflammatory agents.

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