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Full Length Research Paper

Studies on the use of *Zizyphus spina-christi* against pain in rats and mice

Bulus Adzu[1,2*] and Abdul Kaita Haruna[2]

[1]Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, P.M.B 21, Abuja, Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

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Zizyphus spina-christi **(Rhamnaceae) grows wild in tropical Africa and Asia and can be domesticated. It has folkloric usage in pain related ailments throughout these regions. In view of the claimed therapeutic potentials, investigation of the plant's root bark was initiated in our laboratory. The plant material was first sequentially extracted with hexane, chloroform, ethylacetate and methanol, and in this report, a fraction (numbered) ZS-4D from the methanol extract eluted with 70:30% (chloroform : methanol) using flash column chromatography was apparently traced to be responsible for its main analgesic, and in addition, anti-inflammatory activities. The fraction (25, 50 and 100 mg/kg, i.p.) was tested on chemical (acetic acid-induced writhing, formalin), mechanical (analgesy-meter) and thermal (tail-flick) analgesic tests with the aim of elucidating both central and peripherally mediated action in rats and mice. Its anti-inflammatory action against egg albumin-induced hind paw oedema was also tested in rats. Results show that the fraction has some levels of dose related effect on all the models except the tail-flick test in which the activity was not statistically significant.**

Key words: *Zizyphus spina-christi*, Fraction ZS-4D, pain, inflammation.

INTRODUCTION

Traditional herbal remedies have a long and popular usage in treatment of pain related ailments (Almeida et al., 2001). Interestingly, the World Health Organization have recommended the integration of traditional medicines proved to be useful into national health care programmes (WHO, 1976), set up guidelines for its study (WHO, 1991; 2001) and defined its role under what it termed 'Traditional Medicine/Complementary and Alternative Medicine (TM/CAM)' by developing a strategy to address issues of policy, safety, efficacy, quality, access and rational use (WHO Traditional Medicine Strategy 2002-2005). Traditional medicine practice is most popular in developing countries (Elisabetsky, 1991) and unique in its own way because it takes a holistic view of the patient's situation (Jager, 2005). Several bioactive agents in modern pharmacopoeia were derived from products initially used in traditional medicine (Farnsworth, 1985; Astin, 1998; De Smet, 1998; De Silva, 2005). In view of the popular and widespread use of herbal products used in such practice, important technical aspects such as standardization and quality control need development (Fansworth, 1980; Bonati, 1991; De Smet, 1991; Elisabetsky and Shanley, 1994; Patwardhan, 2005), since some are suspected to be interacting with synthetic drugs (Ernst, 2000).

Zizyphus spina-christi Willd (Rhamnaceae) is a plant that grows wild in Asia and tropical Africa. The plant is originally of the Middle East south of the Euphrates and spread to Saharan Oases across Africa into the Sahel (Burkill, 1997). It has several physiological and morphological characteristics (Keay et al., 1964) that assures its ability to adopt to arid environment. It derived its name from kinnara, a sweet edible fruit produced from Krists' thorn tree, one of the prickly/thorny shrubs found in Palestine believed to have been used for Christ's crown of thorns (Arndt, 2000). The plant has versed medicinal and nutritional values (Dalzell, 1937; Hutchens, 1973; Duke, 1985; Al-Yahya, 1986; Eden Foundation, 1992;

*Corresponding authors E-mail: bulusadzu@yahoo.com.

Burkill, 1997; Al-Khalifa and Al-Arify, 1999). It was reported to exhibit antibacterial, anticancer, antidiabetic, anti-nociceptive, antihypertensive, antidiarrhoeal and CNS effect from previous studies (Fleurentin and Pelt, 1982; Friedman et al., 1986; Tanira et al., 1988; Shah et al., 1989; Hussain and Deeni, 1991; Glombitza., 1994; Ali-Shtayeh et al., 1998; Ali et al., 2001; Shahat et al., 2001; Adzu et al., 2001, 2002a, 2003; Lev and Amar, 2002; Nazif, 2002; Abdel-Zaher et al., 2005; Adamu et al., 2005; Abdel-Wahhab et al., 2007). Some useful phytochemicals that include flavonoid, tannins, lipids, terpenes, alkaloids, steroids and carbohydrates have been isolated from the plant (Grindley, 1948; Aynehchi and Mahoodian, 1973; Tschesche et al., 1974; Ikram and Tomlinson, 1976; Tawfik et al., 1978; Rizk, 1982; Ali et al., 1985; Al-Yahya, 1986; Shah et al., 1986; Abdel Galil and El-Jissry, 1991; Mahran et al., 1994; Weinges and Schick, 1995; Shahat et al., 2001).

Among the popular use of *Zizyphus spina-christi* in traditional medicine is in the treatment of pain and inflammatory related ailments across regions in which the plant grows. This includes Saudi Arabia (Shah et al., 1989; Al-Said, 1993), Oman (Ghazanfar and Al-Sabahi, 1993), Israel (Friedman et al., 1986), northern Nigeria (Adzu et al., 2001) and a related specie (*Z. jujuba*) used in Japan (Watanabe et al., 1973; Shibata and Fukushi, 1975; Morishita et al., 1987). Of recent, we initiated fractionalization and pharmacological evaluation of useful components of the plant's root bark in line with the 31st WHO Assembly involving inventory, evaluation and standardization of traditional medicines (Farnsworth, 1980). A previous study (Adzu et al., 2001), which suggested potent anti-nociceptive activity prompted this work. In this report, we presented the potentials of a fraction (numbered ZS-4D) against pain and inflammatory models in rats and mice. The root bark was first sequentially extracted with four solvents along increasing polarity to give hexane (ZS-1), chloroform (ZS-2), ethylacetate (ZS-3) and methanol (ZS-4) extracts using a soxhlet extractor (Quickfit, England). The extracts were preliminary tested via monitoring of analgesic activity and methanol extract (ZS-4) gave most potent effect. The methanol extract was fractionated using flash column chromatography (Still et al., 1978), and the fraction (ZS-4D) eluted with 70:30% (chloroform: methanol) was evaluated using chemical (acetic acid-induced writhing and formalin test), thermal (tail-flick) and mechanical (analgesy-meter) pain models. Its anti-inflammatory potential was assayed using egg albumin-induced oedema as the *in vivo* model of inflammation. The phytochemical constituent as well as the safety of the methanol extract was also tested.

MATERIALS AND METHODS

Plant material

Z. spina-christi materials were collected locally at Midlu, Adamawa State, Nigeria between April and May, 2004. It was authenticated at Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD #4108) was deposited at the herbarium of the Institute. The root bark of the plant was carefully removed, cleaned and dried under shade. The dried root was grounded into powder and 1.29 kg of the powdered material sequentially extracted to obtain hexane (ZS-1), chloroform (ZS-2), ethylacetate (ZS-3) and methanol (ZS-4) extracts. The combined extract of each solvent was concentrated and evaporated to dryness. It gave yields of 4.623, 27.834, 4.443 and 245 g for hexane, chloroform, ethylacetate and methanol extracts respectively. The total yield of the extracts was 21.86% w/w of crude starting material.

Phytochemical test

Phytochemical constituents present were screened using standard procedures (Harborne, 1998; Evans, 2002). TLC was carried out to determine spot zones which were viewed under UV light (254/365 nm Eagle Scientific Limited, UK).

Flash column chromatography

Twenty grams of the methanol extract (ZS-4) was mixed with 20 g of silica gel (Aldrich Chemicals; 230-400 Mesh) and loaded on top of a flash column (Still et al., 1978) containing another 150 g of silica gel. It was flush with hexane, and eluted in increasing polarity with hexane, combinations of chloroform and methanol, and methanol in multiples of 100 ml using an air pump (ABM, Germany). Each of the fractions consisting of 100 ml was individually collected. A total of 73 fractions were collected. Fractions detected to contain the same product using TLC were combined together, concentrated over water bath, and allowed to evaporate at room temperature. This results in 6 fractions (ZS-4A to ZS-4F). Fraction ZS-4D eluted with 70:30 (chloroform: methanol) which presented both analgesic and anti-inflammatory activity according to analgesic activity monitoring was thus fully investigated.

Vacuum column chromatography

The chromatography (O'Donnell et al., 2006) was carried out using Sinta funnel (Quickfit, England) attached to an air pump (ABM, Germany) to get enough of the fraction (ZS-4D) for pharmacological studies. Eighty grams of the methanol extract (ZS-4) was mix with 60 g of silica gel and flush with mixture of 75:25 (chloroform: methanol) followed by elution of 2l of the fraction of interest that is, 70:30 (chloroform:methanol). It was concentrated over water bath and allowed to dry at room temperature. This gave 13.01 g.

Animals

Swiss albino mice and Wistar rats of both sexes obtained from Animal Facility Centre, NIPRD, Abuja, were used for the study. They were housed in plastic cages with saw dust as beddings and given food and water *ad libitum*. The mice were used in accordance with NIH Guide for the Care and Use of Laboratory Animals; published by the US National Institute of Health-NIH Publication (No 83-23) revised (1985), ethical guidelines for investigation of experimental pain in conscious animal (Zimmermann, 1983) and NIPRD's in-house Standard Operation Procedures (NIPRD-SOPs) on laboratory animal usage.

Chemicals, drugs and test agents

All the chemicals used for the extraction, fractionalisation and chromatography (hexane, chloroform, ethylacetate and methanol) were of analytical grade and were purchased from Sigma-Aldrich sales representative in Nigeria (Zayo International Limited, Jos, Nigeria). Other chemicals used are formaldehyde, Triton-X and acetic acid (BDH, England). Three dose levels of the fractions (25, 50 and 100 mg/kg, i.p.) were used throughout the study. The negative control received normal saline (10 ml/kg, i.p.) while acetylsalicylic acid (ASA) (100 mg/kg, i.p.) (Osadepe and Okoye, 2003) served as the positive control. ASA was chosen for the fact that it has anti-nociceptive action independent of their anti-inflammatory effect which does not have any delay of onset of action (Capetola et al., 1983; Hunskaar et al., 1985, 1986, 1987). The fraction (ZS-4D) was completely soluble in water.

Acute toxicity test

The safety of the methanol extract (ZS-4) was tested using Lorke's method (1983). Briefly, the extract was administered in geometric doses of 10, 100, 1000 and 2000 mg/kg, i.p. to four groups of mice. Another mouse was given normal saline to serve as the control and all the mice were kept under same conditions and observed for toxic signs, and mortality within 24 h was noted as index of acute toxicity. LD_{50} was estimated from the square root of the lowest lethal dose and the highest non-lethal dose (Osadepe and Okoye, 2003; Vongtau et al., 2004).

Acetic acid-induced writhing

The test was carried out using the methods described by Koster et al. (1959) and Collier et al. (1968). Two sets (1 and 2) of five groups of mice (n = 5) were pretreated with ZS-4D, saline and ASA. Each mouse was injected with 0.7% of an aqueous solution of acetic acid (10 ml/kg, i.p.) after the predetermined time (30 and 60 min for sets 1 and 2 respectively) and placed in transparent observational cage. The number of writhes (abdominal constriction) exhibited by each mouse was cumulatively counted for 15 min after 5 min (lag time) of the aqueous acid injection (Loro et al., 1999) and the mean count of each group taken.

Formalin test

Dubuission and Dennis (1977) method modified by Tjolsen et al. (1992) was adopted. Five groups of rats (n = 5) were pretreated with either the fraction, saline or ASA. They were injected s.c. with 50 µl of 2.5% solution formalin into the sub-plantar surface of rat left hind paw 30 min after the treatment. Severity of pain was rated in two distinct phases for 1 h; the first phase (0 – 10 min) taking every two min and late phase (15 – 60 min) every 5 min using 3 prominent pain-induced behaviour in the following scoring manner: (0) normal weight baring on the injected paw; (1) light resting on the paw on the floor; (2) elevation of the injected paw and (3) for licking, biting or grooming of the injected paw. The mean (±SEM) of the readings were recorded as the pain score.

Analgesy-meter

The experiment was carried out using Ugo Basile Analgesy-Meter (No. 7200) on normal rats as described by Vongtau et al. (2004). Twenty five rats were grouped into five (n = 5) and treated with saline, ZS-4D or ASA. The rat paw was placed on a plinth under a cone-shaped pusher of the instrument and increased pressure applied to the middle dorsum of the rat's left hind paw (Chipkin et al., 1983; Kitchen, 1984; Nguelefack et al., 2004). Stimulus was terminated and force threshold readings in grams (Anseloni et al., 2003) taken as soon as nociceptive response were elicited by the rats (Iwalewa et al., 2003; Srinivasan et al., 2003). Readings were taking at 0, 15, 30 and 60 min after treatment.

Tail-flick test

The study (D'Amour and Smith, 1941), as subsequently modified for rats using hot water bath (Janssen et al., 1963; Asongalem et al., 2004a, 2004b; Rabanal et al., 2005; Sanchez-Mateo et al., 2006) was adopted with minor modification for our local laboratory settings. The test was carried out by immersing the terminal 2 cm of the rat tail in hot water contained in a 500 ml beaker maintained at 55 ± 1°C using a hot plate (Ugo Basile, Socrel DS-37). A thermometer was placed inside the water to monitor the temperature reading. Twenty five rats that shows response (withdrawing the tail) within 3s were selected and grouped into five (n = 5). The rats were then treated with either ZS-4D, saline or ASA and their responses taken at 30 and 60 min after treatment.

Anti-inflammatory activity

In this procedure, fresh undiluted egg albumin was used as *in vivo* model of acute inflammation in rats (Akah et al., 1993). The rats were divided into five groups (n = 5) and treated with saline, ZS-4D or ASA. Oedema was then induced 30 min later in the rats by subplantar injection of 0.1 ml of raw egg-albumin to the left hind paw and oedema volume measured with a LETICA (Spain) digital plethsmometer (LE 7500) calibrated with 0.1% Triton X-100. Readings were taken 5 min later in an interval of 20 min for a total of 120 min after treatment (Adzu et al., 2002b).

Data analysis

Results were expressed as mean ± standard error of mean (SEM). Student t-test was used to analyze level of statistical significance between groups and Analysis of Variance (ANOVA) among groups. All level of significance were set at $p < 0.05$; $F [(4, 24) = 2.78]$.

RESULTS

The methanol extract of the extract (ZS-4) gave positive test for balsams, carbohydrates, saponins and tannins. The extract produced 100% lethality in doses higher than 900 and 0% in doses below 700 mg/kg, i.p. in the second phase of dosing. The LD_{50} was thus established to be 793.74 mg/kg, i.p. Tremor, then sedation was observed as the acute sign of toxicity. Convulsion preceded death in a mouse among the group that received 200 mg/kg, i.p. of the extract.

The tested fraction (ZS-4D) exhibited significant $F [(4, 24) = 3.8; p < 0.05]$ inhibition of the abdominal writhing (writhing/15 min) induced by acetic acid dose dependently. The potency was observed at both 30 and 60 min pre-treatment (Table 1). Results of the formalin test shows that the fraction inhibited the formalin noxious stimulation on both the aphasic (first) and the tonic (second) phases of the test. More of the inhibition was obser-

Table 1. Effect of *Zizyphus spina-christi* ZS-4D fraction on acetic acid-induced writhing (mean ± SEM/15 min) in mice.

Treatment	Dose (mg/kg, i.p.)	30 min	60 min
Control	-	39.0 ± 3.4	36.2 ± 2.9
ZS-4D	25	31.4 ± 2.2	32.8 ± 2.7
	50	21.0 ± 1.9	19.4 ± 2.0
	100	15.0 ± 2.1	13.0 ± 1.2
ASA	100	4.6 ± 0.8	2.8 ± 0.8

Table 2. Effect of *Zizyphus spina-christi* ZS-4D fraction on formalin induced noxious stimulus score[a] (mean ± SEM) test in rats.

Treatment	Dose (mg/kg, i.p.)	First phase (0-10 min)	Second phase (15-60 min)
Control	-	2.6 ± 0.2	2.6 ± 0.1
ZS-4D	25	2.2 ± 0.2	2.1 ± 0.2
	50	2.0 ± 0.3	1.9 ± 0.2
	100	1.4 ± 0.2	1.4 ± 0.1
ASA	100	2.0± 0.3	0.9 ± 0.1

[a]Maximum score = 3.

ved in the late phase for the ASA group (Table 2). The fraction was also potent in the analgesy-meter test, with the animals showing an increased threshold to the pressure when compared with the control (Table 3). The fraction however failed to exhibit significant effect in the tail-flick test (Table 4).

The pre-treated fraction as well as ASA produced significant F [(4, 24) = 2.9; $p < 0.05$] activity against the inflammation processes induced by egg-albumin, which was maintained throughout the 120 min duration of the experiment. In contrast, there is a consisted increase in the hind paw oedema in the control (untreated) group (Table 5).

DISCUSSION

We have shown in previous study that the crude aqueous extract of *Z. spina-christi* exhibited anti-nociceptive potency with both central and peripheral effect (Adzu et al., 2001). The plant has been implicated in the folkloric treatment of inflammatory and pain related ailments (Friedman et al., 1986; Shah et al., 1989; Al-Said, 1993; Ghazanfar and Al-Sabahi, 1993). In this study, we fractionated the extracts of the plant material and trace the main analgesic and in addition, an anti-inflammatory activity to either fraction (numbered) ZS-4D eluted with 70:30 (chloroform : methanol) along or in combination with components of methanol extract of the root bark. Although there are some advantages to the medical use of extracts as oppose to isolated entities (Phillipson, 2001), there is the need to get to the closest of which component is responsible for the observed effect (Farnsworth, 1980). Such search for analgesic agents have been a priority of some pharmaceutical industries for some time now (Mattison et al., 1988), and plant sources are one of the logical and productive strategies, especially for the fact that important analgesic prototypes like salicyclic acid and morphine were originally derived from plant kingdom (Elisabetsky et al., 1995). The experimental models were chosen in such a way that the existence of both central and peripherally mediated pain relief could be measured.

The results show that the fraction exhibited potent analgesic activity against aqueous acetic acid-induced writhing. The acid produces abdominal writhing response in animals through chemosensitive nociceptors by increasing fluids of PGE_2 and $PGF_{2\alpha}$ (Daraedt et al., 1980) and sympathetic nervous system mediators (Duarte et al., 1988; Barsato et al., 2000) leading to production of algogenic substances at the peritoneal receptors (Bentley et al., 1983). The test is a sensitive procedure in detecting analgesic effects of medicinal substances and can detect antinociceptive effect that may appear inactive in other methods (Collier et al., 1968; Bently et al., 1981). However, the test cannot indicate the exact mechanism of analgesic effect of test substances (Stai et al., 1995) with false positive occurring with agents like sedatives (Elisabetsky et al., 1995). The fraction gave a similar effect on the formalin test inhibiting both the first and the second phase. Formalin test is biphasic, and measures pain of both neurogenic (first phase) and of inflammatory origin (second phase). The first phase (0 – 10 min) being a result of direct stimulation of nociceptors measures centrally mediated effects and is insensitive to anti-inflammatory agents while the second phase (15 – 60 min) which is qualitatively different from

Table 3. Effect of *Zizyphus spina-christi* ZS-4D fraction on analgesy-meter in rats.

Treatment	Dose (mg/kg, i.p.)	Threshold (mean ± SEM X 20 g)		
		0 min	30 min	60 min
Control	-	4.0 ± 0.5	3.3 ±0.5	3.8 ± 0.8
ZS-4D	25	3.2 ± 0.3	6.9 ± 0.4	8.3 ± 0.7
	50	3.9 ± 0.4	8.7 ±0.6	11.3 ± 0.9
	100	2.8 ± 0.5	12.2 ± 1.01	5.4 ± 1.4
ASA	100	3.0 ± 0.8	7.2 ± 2.0	7.6 ± 1.6

Table 4. Effect of *Zizyphus spina-christi* ZS-4D fraction on thermal stimulus stimulus-induced tail-flick test (withdrawal time (s)) in rats (mean ± SEM).

Treatment	Dose (mg/kg, i.p.)	30 min	60 min
Control	-	3.8 ± 0.7	4.2 ± 0.3
ZS-4D	25	5.0 ± 0.7	5.4 ± 0.8
	50	6.2 ± 0.6	6.6 ± 0.9
	100	6.4 ± 0.7	5.6 ± 0.9
ASA	100	5.8 ± 1.4	5.3 ± 0.8

Table 5. Effect of *Zizyphus spina-christi* ZS-4D fraction on egg albumin-induced paw oedema in rats.

Treatment	Dose (mg/kg,i.p.)	Experimental time (min)					
		20	40	60	80	100	120
Control		0.134±0.04	0.158±0.04	0.188±0.03	0.208±0.03	0.222±0.03	0.244±0.03
ZS-4D	25	0.160±0.05	0.154±0.06	0.135±0.05	0.134±0.05	0.130±0.05	0.114±0.05
	50	0.136±0.04	0.130±0.04	0.100±0.04	0.116±0.03	0.124±0.03	0.108±0.04
	100	0.140±0.04	0.120±0.04	0.118±0.04	0.106±0.04	0.114±0.04	0.102±0.03
ASA	100	0.132±0.04	0.122±0.04	0.112±0.04	0.110±0.04	0.116±0.04	0.098±0.03

Values in parenthesis are paw oedema volume (cm^3).

the first phase is dependent on peripheral inflammation and changes in central procession due to chemical mediators release from damaged cells that stimulate nociception and thus induced pain (Hunskaar and Hole, 1987). In general, the test measures the response to a long lasting nociceptive stimulus similar to clinical pain (Tjolsen et al., 1992) and is recommended as a tool in basic pain research for studying the mechanisms of analgesic agents (Hunskaar and Hole, 1987) because of its connection to tissue injury (Tjolsen et al., 1992). Agents that act primarily on the CNS inhibit both phases equally while peripherally acting drugs inhibit the late phase. The ability of the fraction to inhibit both phases of the formalin test indicates its involvement in both central and peripherally mediated activity, probably by prostaglandin synthesis inhibition, as well as central inhibition mechanism.

This observation is further collaborated by the fraction's ability to give a potent test on the mechanical model using analgesy-meter. The meter exerts force at a constantly increased rate on rat paw monitored by a pointer moving along a linear scale. It is a modification (Vongtau et al., 2004) of the Randall-Selitto (1957) test for measuring analgesic activity based on the principle that inflammation increases the sensitivity to nociception and this sensitivity is susceptible to modification by analgesics (Vogel and Vogel, 1997). The fact that the fraction increases the threshold of the intact paw strengthens the evidence of peripheral and centrally mediated activity (Vogel and Vogel, 1997; Vongtau et al., 2004). The fraction however failed to exhibit potent effect on the tail-flick test. The test is a standard method for investigating nociception and analgesic (D'Amour and Smith, 1941) that measures the response to a brief, noxious stimulus which appears to be spinal reflex that is modulated by supraspinal inhibitory mechanism. The test is selective for centrally acting analgesics (Ramabadran et al., 1989) indicative of morphine like effect (Domer, 1990) and also like NSAIDs by inhibiting cycloxygenase in peripheral tissues thereby interfering with the mechanism of transduction in primary afferent nociceptors (Fields, 1987). The lack of potent effect on this test can therefore be deduced that the central effect observed in other

models is devoid of specific morphine-like activity. This central action is presently not completely understood, but a preliminary work earlier done on the crude aqueous extract, shows that it has CNS activity that is sedative in nature (Adzu et al., 2002a). This might have been responsible for the central effects seen in the formalin and analgesy-meter tests.

The fraction exhibited anti-inflammatory effect by inhibiting oedema induced by egg albumin in rat paw. The test is used as an *in vivo* model of inflammation to screen agents for anti-inflammatory effect (Akah et al., 1993; Okoli et al., 2007) especially if inflammation wasn't intended to be sustained for long (Williamson et al., 1996; Osadebe and Okoye et al., 2003). This may also be an immense advantage of the fraction since inflammatory diseases are currently treated with steroidal and NSAIDs, often associated with adverse effects (Fung and Kirschenbaum, 1999) hence the need for safe compounds. Inflammation, a common occurrence in infective condition is a complex array of responses of tissue to injury (Vane and Bolting, 1995) which is usually associated with pain as a secondary process, resulting from the release of algesic mediators (Hunskaar and Hole, 1987) with therapy often directed at the inflammation process. The anti-inflammatory effect of the fraction therefore was perhaps elicited through prostaglandins synthesis, the most frequently encountered mechanism of action amongst anti-inflammatory drugs (Vane, 1971) that exert their effect through spectrum of different mode of actions (Samuelson et al., 1978). Conclusively, these observations provide evidence for analgesic and anti-inflammatory activity of the fraction (ZS-4D) as the primary component of the plant material that exhibit pain relief action. It is difficult (at this stage) to pin down which of the phytochemical component is responsible for the activity of the fraction because of the little correlation between anti-inflammatory activity and the chemical nature of agents (Sertie et al., 1990). On the mechanism, the fraction exhibited activity similar to NSAIDs in a complementary manner, most likely through an intertwine action of blocking the stimulus propagation in the pain nervous fibers or decrease in the production of prostaglandins responsible for pain mediation (Matheus et al., 2005). This shows, with some certainty, that the anti-inflammatory component may in addition involve the observed peripheral analgesic activity (Spessoto et al., 2003) and vice versa. Whereas all these explains the basis of the use of plant in the treatment of pain related cases, the fraction is worth probing for development into phytodrug with the hope that it will be in agreement with the folkloric experience of its crude usage.

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REFERENCES

Abdel-Galil FM, El-Jissry MA (1991). Cyclopeptide alkaloids from *Zizyphus spina-christi*. Phytochem. 30: 1348-1349.

Abdel-Wahhab MA, Omara EA, Abdel-Gali MM, Hassan NS, Nada SA, Saeed A, Elsayed MM (2007). *Zizyphus spina-christi* extract protects against aflotoxin B1-initiated Hepatic carcinogenicity. Afr. J. Trad. Compl. Alt. Med. 4: 248-256.

Abdel-Zaher AO, Salim S, Assaf MH, Abdel-Hady RH (2005). Antidiabetic and toxicity of *Zizyphus spina-christi* leaves. J. Ethnopharmacol. 101: 129-138.

Adamu HM, Abayeh OJ, Agbo MO, Abdullahi AL, Uba A, Dukku HU, Wufem BM (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. J. Ethnopharmacol. 99: 1-4.

Adzu B, Amos S, Wambebe C, Gamaniel K (2001). Antinociceptive activity of the aqueous extract of *Zizyphus spina-christi* root bark extracts. Fitoterapia 72: 344-350.

Adzu B, Amos S, Dzarma S, Wambebe C, Gamaniel K (2002a). Effect of *Zizyphus spina-chriti* Willd aqueous extract on the central nervous system in mice. J. Ethnopharmacol. 79: 13-16.

Adzu B, Amos S, Dzarma S, Muazzam I, Gamaniel K (2002b). Pharmacological evidence favouring the folkloric use of *Diospyros mespiliformis* Hochst in the relief of pain and fever. J. Ethnopharmacol. 82:191-195.

Adzu B, Amos S, Amizan MB, Gamaniel K (2003). Evaluation of the antidiarrhoeal effects of *Zizyphus spina-christi* in rats. Acta Tropica 87: 245-250.

Akah PA, Okogun JI, Ekpendu TO (1993). Antioedema and analgesic actions of *Diodia scandans* extracts in rats and mice. Phytother. Res. 7: 317-319.

Al-Khalifa AS, Al-Arify IA (1999). Physiochemical characteristic and poll spectrum of some Saudi honeys. Food Chem. 67: 21-25.

Al-Said MS (1993). Traditional medicinal plants of Saudi Arabia. Amer. J.Chinese Med. 21:291-298.

Al-Yahya M (1986). Phytochemical studies of the plants used in traditional medicine in Saudi Arabia. Fitoterapia 57: 179-182.

Ali AA, El-Shanawany MA, Mesbah MK (1985). Phytochemical study of the leaves of *Zizyphus spina-christi* L. Willd. Bull. Pharm. Sci. Assiut University, Egypt 8: 1-11.

Ali NAA, Julich W-D, Kusnick C, Lindequist U (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol. 74: 173-179.

Ali-Shtayeh MS, Yaghmour RM-R, Faidi YR, Salem K (1998). Antimicrobial activity of 20 plants used in folkloric medicines in the Palestine area. J. Ethnopharmacol. 60: 265-271.

Almeida RN, Navarro DS, Barbosa-Filho JM (2001). Plants with central analgesic activity. Phytomed. 8:310-322.

Anseloni VCZ, Ennis M, Lidow MS (2003). Optimization of the mechanical nociceptive threshold testing with the Randall-Sellitto assay. J.Neuroscie. Methods 131:93-97.

Arndt SK (2001). Ecosystems Research Group.http://chemsrro.pph.univie.ac.at/ska/ziplant.htm

Asongalem EA, Foyet HS, Ngogang J, Folefoc GN, Dimo T, Kamchouing P (2004a). Analgesic and anti-inflammatory activities of *Ergeron floribundus*. J. Ethnopharmacol. 91: 301-308.

Asongalem EA, Foyet HS, Ekobo S, Dimo T, Kamchouing P (2004b). Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. J. Ethnopharmacol. 95: 63-68.

Astin JA (1998). Why patients use alternative medicine: results of national study. Amer. Med. Assoc. 279: 1548-1553.

Aynehchi Y, Mahoodian M (1973). Chemical examination of *Zizyphus spina-christi* (L) Willd. Acta Pharma Suecica 10: 515-517.

Bently GA, Newton SH, Starr J (1981). Evidence for an action of morph-

ine and the enkephalins on sensory nerve endings in the mouse peritoneum. Brit. J. Pharmacol.73: 325-332.
Bonati A (1991). How and why should we standardize phytopharmaceutical drugs for clinical validation? J. Ethnopharmacol. 32: 195-198.
Borsato MLC, Grael CFF, Souza GEP, Lopes NP (2000). Analgesic activity of the lignans from *Lychnophora ericoides*. Phytochem. 55:809-813.
Burkill HM (1997). The Useful plants of West Tropical Africa. Royal Botanic Gardens, Kew. 4: 493-496.
Capetola RJ, Rosenthale ME, Dubinsky B, McGuire JL (1983). Peripheral antialgesics: a review. J.Clin. Pharmacol. 23: 545-556.
Chipkin RE, Latranyi MB, Iorio LC, Barnett A (1983). Determination of analgesic drug efficacies by modification of the Randall and Sellitto rat yeast paw test. J. Pharmacol. Methods 10: 223-229.
Collier HO, Dinneen LC, Johnson CA, Schneider C (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. Brit. J. Pharmacol. 32: 295-310.
D'Amour FE, Smith DL (1941). A method for determining loss of pain sensation J. Pharmacol. Exper. Therap. 72: 74-79.
Dalziel JM (1937). The Useful Plants of West Africa. Crown Overseas Agent for the Colonies. London, p. 300.
Deraedt R, Jouquey S, Delevallee F, Flahut M (1980). Release of prostaglandins E and F in an algogenic reaction and its inhibition. Europ. J. Pharmacol. 61: 17-24.
De Silva T (2005). Industrial utilization of medical plants in developing countries. Chemical Institutes Branches. Industrial Sectors and Environment Division, United Nation Industrial Development Organisation. pp. 1-11, http://www.fao.org/docrep/W7261e07.htm.
De Smet PAGM (1991). Is there danger in using traditional remedies? J. Ethnopharmacol. 32: 43-50.
De Smet PAGM (1998). Traditional Pharmacology and Medicine in Africa: Ethnopharmacological themes in sub-saharan art and utensils. J. Ethnopharmacol. 63: 1-179.
Domer F (1990). Characterization of the analgesic activity of Ketorolac in mice. Europ. J. Pharmacol. 177: 127-135.
Duarte JDG, Nakamura M, Ferreira SH (1988). Participation of the sympathetic system in acetic acid induced writhing in mice. Braz. J. Med. Res. 21: 341-343.
Dubuission D, Dennis SG (1977). The formalin test: a quantitative study of the analgesic effect of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4: 161-174.
Duke JA (1985). CRC Handbook of Medicinal Herbs. Florida, USA: CRC Press, Inc.
Eden Foundation (1992). Nutritional study on *Ziziphus spina-christi.* (www.eden-foundation.org/project/articles-nutritionspina.html).
Elisabetsky E, Amadar TA, Albuquerque RR, Nunes DS, Calvalho ACT (1995). Analgesic activity of *Psychotria colorata* (Willd. Ex R. & S.) Muell. Arg. Alkaloids. J. Ethnopharmacol. 48: 77-83.
Ernst E (2000). Possible interactions between synthetic and herbal medicinal product: Part 1: a systematic review of the indirect evidence. Perfusion 13: 4-15.
Evans WC (ed.) (2002). Trease and Evans Pharmacognosy. 15th ed. Habid, W.B Saunders, Edinburgh.
Farnsworth NR (1980). The development of pharmacology and chemical research for application to traditional medicine in developing countries. J. Ethnopharmacol. 2:173-181.
Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z (1985). Medicinal Plants in therapy. Bull. World Health Organ. 63: 226-230.
Fields HL (1987). Analgesic Drugs. In: Day W (Ed.), Pain. MacGraw-Hill, USA. p. 272.
Fleurentin J, Pelt MJ (1982). Repertory of drugs and medicinal plants of Yemen. J. Ethnopharmacol. 6: 86-108.
Fung HB, Kirshenbaum HL (1999). Selective cycloxygenase-2 inhibitors for treatment of arthritis. Clin. Therap. 21: 1131-1157.
Ghazanfar SA, Al-Shabahi MA (1993). Medicinal Plants of northern and central Oman (Arabia). Econ. Botanicals 47: 89-98.
Glombitza KW, Mahran GH, Mirhom YW, Michel KG, Motawi TK (1994). Hypoglycemic and antihyperglycemic effects of *Zizyphus spina-christi* in rats. Planta Medica 60: 244-247.
Grindley DD (1948). Investigation of some new Sudan seed oils. J. Soc. Chem. Indus. 67: 230-231.
Harborne JB (1998). Phytochemical methods: A Guide to Modern Techniques of Analysis. 3rd ed. Chapman and Hall, London.
Hunskaar S, Fasmer OB, Hole K (1985). Acetylsalicyclic acid, paracetamol and morphine inhibit behavioural responses to intrathecally administered substance P or capsaicin. Life Sci. 37:1835-1841.
Hunskaar S, Berge OG, Hole K (1986). Dissociation between antinociceptive and anti-inflammatory effects of acethysalicyclic acid and indomethacin in the formalin test. Pain 25: 125-132.
Hunskaar S, Hole K (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30: 103-114.
Hussain HSN, Deeni YY (1991). Plants in Kano ethnomedicine; screening for antimicrobial activity and alkaloids. Int. J. Pharmacog. 29:51-56.
Hutchen AR (1973). Indian Herbology of North America: Hero; Ontario, Canada, p. 382.
Ikram M, Tomlinson H (1976). Chemical constituents of *Zizyphus spina-christi*. Planta Medica 29: 289-290.
Iwalewa EO, Iwalewa OJ, Adeboye JO (2003). Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* Less leaf. J. Ethnopharmacol. 86: 229-234.
Jager AK (2005). Is traditional medicine better off 25 years later? J. Ethnopharmacol. 100: 3-4.
Janssen PAJ, Niemegeers CJE, Dony JGH (1963). The inhibitory effect of fentanyl and other morphine-like analgesic on the warm water induced tail withdrawal reflex in rats. Arzneim-Forsch Drug Res. 6: 502-507.
Keay RMJ, Onochie CFA, Stanfied DP (1964). Nigerian trees. Vol II. Department of Forestry, Ibadan, Nigeria. p. 225-226.
Kitchen I (1984). Modification of an analgesy meter for paw-pressure antinociceptive testing in neonatal rats. J. Pharmacol. Methods 12: 255-258.
Koster R, Anderson M, De Beer EJ (1959). Acetic acid for analgesic screening. Federation Proceeding 18: 412-417.
Lev E, Amar Z (2000). Ethnopharmacological survey of traditional drugs sold in Israel at the end of 20th century. J. Ethnopharmacol. 72: 191-205.
Levine JD, Lau W, Kwait G, Goetzl EJ (1984). Leukotriene B4 produces hyperalgesia that is dependent on the polymorphonuclear leucocytes. Science 225: 743-745.
Lorke D (1983). A new approach for acute toxicity testing. Arch.Toxicol. 54: 275-287.
Loro JF, del Rio I, Perez-Santana L (1999). Preliminary studies of analgesic and anti-inflammatory properties of *Opuntia dillenii* aqueous extract. J. Ethnopharmacol. 67: 213-218.
Mahran GEDH, Glombitza KW, Mirrhom YW, Hartmann R, Michel CG (1996). Novel saponins from *Zizyphus spina-christi* growing in Egypt. Planta Medica 62: 163-165.
Matheus ME, Berrondo LF, Veieitis EC, Menezes FS, Fernandes PD (2005). Evaluation of the antinociceptive properties from *Brillantaisia palisotil* Lindau stems extracts. J. Ethnopharmacol. 102: 377-381.
Mattison N, Trimble AG, Lasagna L (1988). New drug development in the United States, 1963 through 1984. Clin. Pharmacol. Theurap. 43: 290-301.
Morishita S, Mishima Y, Hirai Y, Saito T, Shoji M (1987). Pharmacological studies of water extract of *Zizyphus* seed and the *Zizyphus* seed containing drug. Gen. Pharmacol. 18: 637-641.
Nazif NM (2002). Phytoconstituents of *Zizyphus spina-christi* L. fruits and their microbial activity. Food Chem. 76: 77-81.
Nguelefack TB, Folio A.L, Wacho P, Wansi SL, Dimo T, Kamanyi A (2004). Analgesic properties of the aqueous and ethanol extracts of the leaves of *Kalanchoe crenata* (Crassulaceae). Phytother. Res. 18: 385-388.
O'Donnell G, Bucar F, Gibbons S (2006). Phytochemistry and antimycobacterial activity of *Chlorophytum inornatum*. Phytochemistry 67:178-182.
Okoli CO, Akah PA, Mwafor SV, Anisiobi AI, Ibegbunam IN, Erojikwe O (2007). Antiinflammatory activity of hexane leaf extract of *Aspilia africana* C.D Adams. J. Ethnopharmacol. 109: 219-225.
Osadepe PO, Okoye FBC (2003). Anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. J. Ethnopharmacol. 89:19-24.
Patwardhan B (2005). Ethnopharmacology and drug development. J. Ethnopharmacol. 100:50-52.

Randall LO, Selitto JJ (1957). A method for measurement of analgesic activity on inflamed tissue. Arch. Intern. Pharmacodynamics 111: 409-419.
Ramabadran K, Bansinath M, Turndorf H, Puig MM (1989). Tail immersion test for the evaluation of a nociceptive reaction in mice. methodological consideration. J. Pharmacol. Methods 21: 21-31.
Rabanal RM, Bonkanka CX, Hernandez-Perez M, Sanchez-Mateo CC (2005). Analgesic and topical anti-inflammatory activity of *Hypericum canariense* L. and *Hypericum glandulosum* Ait. J. Ethnopharmacol. 96:591-596.
Rizk AM (1982). Constituents of plants growing in Qatar. I. A chemical survey of 60 plants. Fitoterapia 53: 35-44.
Samuelsson B, Goldyne M,Granstrom E, Hamderg M, Hammastrom S, Malmsten C (1978). Prostaglandins and tromboxanes. Ann. Rev. Biochem. 47: 997-1029.
Sanchez-Mateo CC, Bonkanka CX, Hernandez-Perez M, Rabanal RM (2006). Evaluation of analgesic and topical anti-inflammatory effects of *Hypericum reflexum* L. fil. J. Ethnopharmacol. 107: 1-6.
Shah AH, Ageel, AM, Tariq M, Mossa JS, Al-Yahya MA (1986). Chemical constituents of the stem bark of *Zizyphus spina-christi*. Fitoterapia 57: 452-454.
Shah AH, Qureshi S, Tariqu M, Ageel AM (1989). Toxicity studies of six plants used in the traditional Arab system of medicine. Phytother. Res. 3: 25-29.
Shahat AA, Pieters L, Apers S, Nazif NM, Abdel Azim NS, Berghe DV, Vlietinck, AJ (2001). Chemical and biological investigations of *Zizyphus spina-christi* L. Phytother. Res. 15:593-507.
Shibata M, Fukushima M (1975). Acute toxicity and sedative action of *Zizyphus* seeds. Yakugaku Zashi 95: 465-469.
Spessoto MA, Ferreira DS, Crotti AEM, Silva MLA, Cunha WR (2003). Evaluation of the analgesic activity of extracts of *Miconia rubiginosa* (Melastomataceae).Phytomed. 10: 606-609.
Stai HY, Chen YF, Wu TS (1995). Anti-inflammatory and analgesic activities of extract from roots of *Angelica pubescens*. Planta Medica 61: 2-8.
Sertie JA, Basile AC, Panizza S, Matida AK, Zelink R (1990). Anti-inflammatory activity and sub-acute toxicity of artemetin. Planta Medica 56:36-40.
Srinivasan K, Muruganandan S, Lal J, Chandra S, Tandan SK, Raviprakash V, Kumar D (2003). Antinociceptive and antipyretic activities of *Pongamia pinnata* leaves. Phytother. Res. 17: 259-264.
Still WC, Kahn M, Mitra A (1978). Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. 43: 2923-2925.
Tanira MOM, Ageel AM, Tario M, Mohsin A, Shah AH (1988). Evaluation of some pharmacological, microbiological and physical properties of *Zizyphus spina-christi*. Int. J. Crude Drug Res. 26: 56-60.
Tawfik NI, El-Tawil BAH, El-Refai AH, Khalaf AA, Khalil AM (1978). Constituents of local plants. Part I. Chemical Investigation on some cultivated Saudi Arabian plants. Qual. Plants Foods Hum. Nutr. 28: 203-210.
Tjolsen A, Gerge O-G, Hunskaar S, Rosland JH, Hole K (1992). The formalin test: an evaluation of method. Pain 51: 3-17.
Tschesche R, Khokhar I, Spilles C, von Radloff M (1974). Peptide alkaloids from *Ziziphus spina-christi*. Phytochem. 13: 1633.
Vane JR (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature New Biol. 231: 232-235.
Vane JR, Bolting RM (1995). New insight into the mode of action of anti-inflammatory drugs. Inflam. Res. 44: 1-10.
Vogel HG, Vogel WH (Eds.) (1997). Analgesic, anti-inflammatory and antipyretic activity. In: Drug Discovery and Evaluation: Pharmacological Assays. Springer-Verlaag, Berlin Heidelderg, pp. 360-418.
Vongtau HO, Abbah J, Mosugu O, Chindo BA, Ngazal IE, Salawu AO, Kwanashie, HO, Gamaniel KS (2004). Antinociceptive profile of the methanolic extract of *Neorautanania mitis* root in rats and mice. J. Ethnopharmacol. 92: 317-324.
Watanabe I, Saito H, Takagi K (1973). Pharmacological studies of *Zizyphus* seed. Jap. J. Pharmacol. 23: 563-571.
Weinges K, Schick H (1995). Dodecaacetylprodelphinidin B3 from the dried leaves of *Ziziphus spina-christi*. Phytochem. 38: 505-507.
WHO (1976). Resolution World Health Organisation pp. 29-72.
WHO (1991). Guidelines for assessment of herbal medicines, programme of traditional medicine, CH-1211. Geneva, p. 21.
WHO (2001). Protection of traditional medicines/ ICRIER working paper 66, pp. 1-36.
Williamson EM, Okpako DT, Evans FJ (1996). Selection, Preparation and Pharmacological Evaluation of Plant Material. Johnaiky and Sons Ltd, London; pp. 131-154.
Zimmermann M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16: 109-110.