http://dx.doi.org/10.4314/jpb.v9i1.3
Vol. 9 no. 1, pp. 14-19 (March 2012)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Effects of aqueous and ethanol extracts of the stem bark of *Zizyphus spina-christi* L. on isolated rabbit jejunum

Garba T. Mohammed[1*], Hassan B. Yesufu[2], Irfan Z Khan[2] and Fanna I. Abdulrahman[2]

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmacy; [2]Departmemt of Chemistry, University of Maiduguri P.M.B.1069, Maiduguri. Nigeria.

Received 23rd November 2011; Accepted 9th March 2012

Abstract
Zizyphus spina-christi commonly known as *Kurna* has often been prescribed by traditional medicine practitioners in Borno State for the control of gastro intestinal disorders. The study focused on investigating the curative potentials of the plant material on gastro intestinal motility. Fresh samples of the stem bark were collected and used for this study. The plant material were air-dried for two weeks and pulverized. Six hundred gram of the powdered plant was placed in a thimble and extracted with 2 liters of 95% ethanol (60-80ºC). Also, Six hundred gram of the powdered sample was extracted using 2 liters distilled water. The extract concentrates were subjected to preliminary phytochemical screening and then tested on rabbit jejunum. The stem bark extract of *Zizyphus spina-christi* demonstrated antagonism to acetylcholine induced contraction of rabbit jejunum. The effect was similar to atropine which is a known intestinal smooth muscle relaxant. The effect of acetylcholine on rabbit jejunum showed a dose dependent depolarization response between 10 µg/ml-160 µg/ml. Atropine was able to antagonize the effect of acetylcholine competitively at 10 µg/ml. At lower doses of acetylcholine, the stem bark water extract showed more antagonist effect. However, as the doses increased similar antagonism was observed for both extracts. The result obtained therefore justifies the traditional use of the plant in controlling abnormal intestinal motility.

Keywords: *Zizyphus spina-christi*; Aqueous extract; Ethanol extract; Rabbit jejunum; Intestinal motility

INTRODUCTION

Despite immense technological advancement in modern medicine, many people in the developing countries still rely on traditional healing practices and medicinal plants for their daily healthcare needs (Ojewole, 2004). In developing countries traditional healers provide health care services to their community by using vegetable, animal and mineral substances. The success of these healers cannot be ignored in view of the scientific evidence available for the efficacies of these plants (Sofowora, 1984). The World Health Organization (1977) indicated that researchers must not only identify the chemical constituents of medicinal plants/herbs, but also determine the biological activities of such plants. *Zizyphus spina-christi* (Kurna) has been used extensively for the treatment of ulcer, bronchitis, fever and as diuretic agent (Amin, 1991). The plant is popular among traditional medicine practitioners in Borno State of Nigeria for gastrointestinal diseases (Abdulrahman *et al.*, 1998). Acetylcholine and related parasympathomimetics are known

* Corresponding author. *E-mail*: mohammedgarbatom@gmail.com; *Tel*: +234 (0) 8057273192
ISSN 0189-8442

to stimulate gastrointestinal tract (GIT) of a variety of species through the muscarinic receptors. Acetylcholine being a neurotransmitter on neuro-effector junction and all autonomic ganglia produce both muscarinic and nicotinic effect (stimulation and then blockage of the autonomic ganglia (Eglen *et al*., 1994). In the other hand, the contractions of the smooth muscle including that of rabbit jejunum are dependent upon an increase in cytosolic free Ca^{2+} levels (Karaki *et al*., 1997) and the entry via voltage-dependent Ca^{2+} channels is one of the major mechanisms of Ca^{2+} influx for the initiation of smooth muscle contraction (Itoh *et al.*, 1984; Goto *et al.*, 1989; Shimizu *et al.*, 2000). Drugs such as dicyclomine (Bentyl) and hyoscyamine (Levsin) have been used as treatment for gastro intestinal infections. These drugs inhibit muscarinic cholinergic receptors in the enteric plexus and on smooth muscle. Thus providing relief of abdominal symptoms at low doses (Bertram, 2004). Indeed, it is now well reported that different plant extracts are used in different diseases especially in gastrointestinal transit disorders (Thiana *et al.*, 2008; Cortes *et al*, 2006; Bashir *et al*, 2006). Therefore, this work is aimed at investigating the cholinergic and spasmolytic effect of the stem bark extract of *Z. spina-christi*.

EXPERIMENTAL

Plant material. Fresh samples of the ripe stem bark of *Z. spina-christi* were collected in March, 2007 from Jiddari, Polo area of Maiduguri, Borno State. The plant specimen was identified by Prof. S.S. Sanusi of the Department of Biological sciences, University of Maiduguri. A voucher specimen was deposited at the herbarium.

Plant extraction. The stem bark was air-dried for two weeks and pulverized with mortar and pestle. Six hundred gram of the powdered plant was placed in a thimble and extracted with 95% ethanol (60-80^{0}C) using continuous soxhlet extraction method to exhaustion. The extract was concentrated *in vacuo* and a brown solid mass (29%w/w) obtained and labeled. For the aqueous extract, six hundred gram of the powdered sample was placed in a round bottom flask and extracted with one liter of distilled water. The aqueous layer was also concentrated *in vacuo* to yield a dark brown solid mass (18.36%w/w). The extracts were labeled and stored at 4^{o}C for further analysis.

Phytochemical screening. The crude ethanol and aqueous extracts were screened for the determination of constituents utilizing standard methods (Harborne, 1973; Evans, 2002).

Animal treatment. Experimental set up was similar in principle to that of Vane (1957). Each of the adult rabbits was sacrificed by stunning and decapitation. The abdomen was then opened and length of small intestine exteriorized and freed from the mesentery and cut out. A suitable length was cut and transferred into a container containing tyrode solution. Tyrode solution is a physiological salt whose preparation is isotonic with blood plasma and more suitable for intestinal tissues. The composition of the tyrode solution was: NaCl = 8g; KCl = 0.20g; $MgCl_2$ = 0.10g; $CaCl_2$ = 0.20g; $NaH_2PO_4.2H_20$ = 0.05g; $NaHCO_3$ = 1.00g; Glucose = 1.00g. The tyrode solution is placed in 50ml tissue bath and maintained at 37^{o}C. Drugs either standard or the extract was then added into the tissue bath as appropriate. The jejunum was cut into 2-3cm portions using a threaded needle, a piece of thread was attached to one of the segment and the thread was made into small loop and attached to the hook in the organ bath. The other piece of thread was attached to the frontal writing lever with a plastine. Adequate aeration was ensured and rhythmical contraction began within some few minutes. The amplitude started increasing gradually until the activity became fairly regular. It was then recorded to a moving

drum into which a graph paper was wrapped. At zero second kymograph was started, at 15 seconds the kymograph was stopped and the preparation washed. At 60 seconds the kymograph was started. With the above set up the spasmolytic effect of the following drug extract on the amplitude of contraction were investigated and response of the jejunum to acetylcholine and extract were recorded. The tracing of the extract on the tissue were recorded at the same dose of 3.2×10^{-3}g/ml against the effect of the acetylcholine of different doses of 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. Atropine at the dose of 10µg/ml was used against the effect of acetylcholine at the different doses above.

RESULTS AND DISCUSSION

The result of phytochemical screening revealed the presence of alkaloids, flavonoids saponins and glycosides which are potential pharmacological agents (Table 1).The result of the in-vivo study of the isolated rabbit jejunum indicated that the extract showed relaxation effect on the spontaneous contractions and spasmolytic effect since it significantly reduced the contraction induced by spasmogenes (ACh) on the smooth muscle of the jejunum. ACh caused an increase in jejunal basal tone (Fig 1). Atropine administered concurrently with acetylcholine significantly inhibited the contraction caused by the acetylcholine (Fig 2). Therefore, it was used as the standard for measuring the performance of the plant extract. The stem bark (aqueous and ethanol) administered concurrently with acetylcholine at concentration of 3.2×10^{-3}g/ml demonstrated significant inhibition similar to atropine. The amplitude and tone of spontaneous contraction were reduced in a concentration-dependent manner (Fig. 3 & 4). The inhibition of acetylcholine by the extract (3.2×10^{-3}g/ml), suggest that the extract probably contain substances that act through cholinergic mechanisms. It also suggest that the spasmolytic effect of *Z. Spina-christi* stem bark is probably due to Ca^{2+} voltage channel blockage since ACh induced jejunum contractions are mainly due to Ca^{2+} influx through voltage dependent channels (Bolton, 1996; Carl *et al.* 1996; Ghayur and Gilani, 2004; Gilani *et al.,* 2005). The observed relaxation exhibited by the extract was similar to the effect of *Ziziphus mauritiana* methanolic root extract (Dahiru *et al.,* 2006), methanolic leaf extract of *Securinega virosa* (Magaji *et al.*, 2007), and aqueous methanolic root bark extract of *Cochlospermum tintorium* A. Rich (Magaji *et al.*, 2010) on rabbit jejunum.

Table 1. Results of Preliminary phytochemical screening of the Stem bark extract of *Z. spina-christi.*

Constituents	SBE	SBA
Flavonoids	+	++
Alkaloids	+++	+
Saponins	+	+
Carbohydrates	++	++
Tannins	+	+
Steroidal nucleus	+	+

Key: SBE–Stem bark ethanol, SBA–Stem bark aqueous extract + = present – = Absent

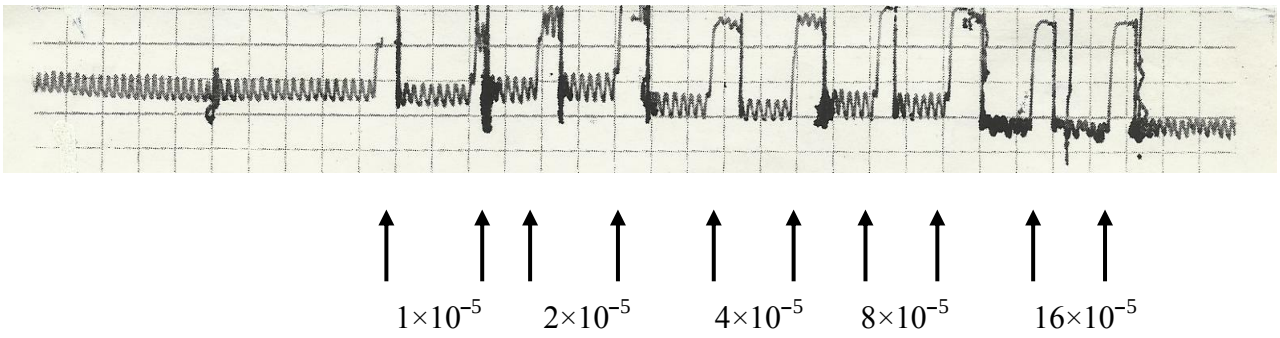

Fig 1. The effect of acetylcholine (ach) alone on isolated rabbit jejunum

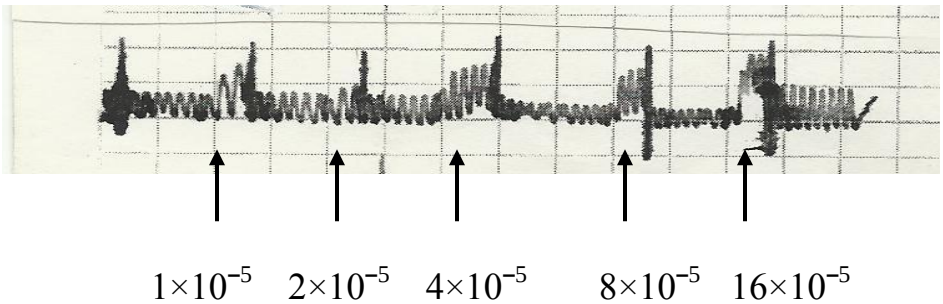

Fig 2. The effect of acetylcholine (ACh) and atropine on isolated rabbit jejunum

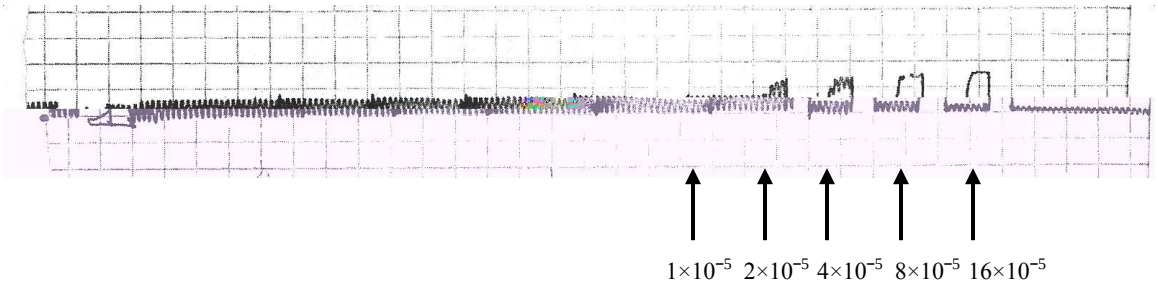

Fig. 3: The effect of concurrent administration of ach at various concentration and stem bark aqueous extract of 3.2×10^{-3}g/ml *Zizyphus spina-christi* on rabbit jejunum.

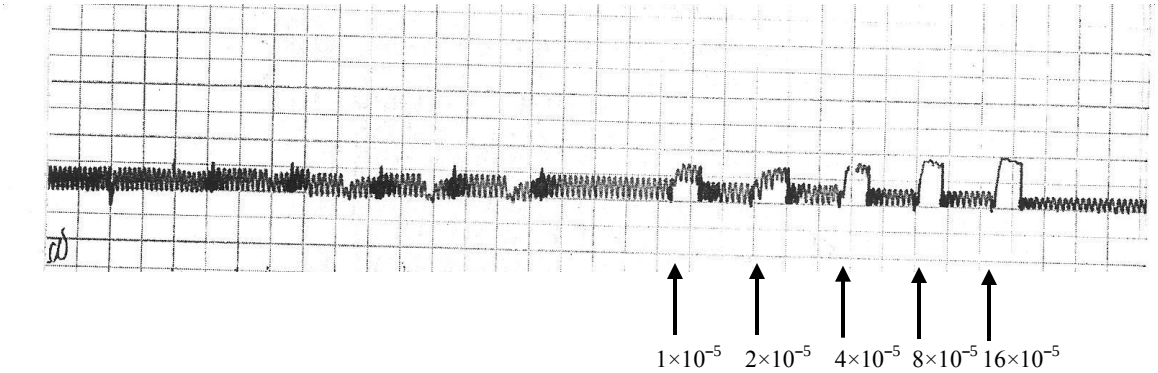

Fig 4. The effect of concurrent administration of ach at various concentration and stem bark ethanol extract of 3.2×10^{-3}g/ml *Zizyphus spina-christi* on rabbit jejunum.

Early studies have reported that the activity of medicinal plants on gastro-intestinal disorders may be due to alkaloids, saponins, tannins, flavonoids and reducing sugars (Galvez *et al.*, 1991; Galvez, 1993., Rao and Gurfinkel, 2000). The inhibitory activity of flavonoids on intestinal motility in a dose related manner was earlier reported (Dicarlo, *et al.*, 1994), flavonoids possess anti-oxidant activity which is presumed to be responsible for the inhibitory effect on several enzymes including those involved in arachidonic acid metabolism (Rao *et al.*, 2006).

In conclusion, this study showed that the stem bark extracts of *Zizyphus spina-christi* was able to antagonize acetylcholine induced contraction though less potent compared to atropine. Therefore, it has the potential of being used for the treatment of colic spasm and abnormal intestinal motility.

ACKNOWLEDGEMENT

We extend our gratitude to Prof. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri for identifying the plant and Mr. Mamza Yusuf of the Kidney centre, University of Maiduguri Teaching Hospital, Maiduguri.

REFERENCES

Abdulrahman, F., Ogarawu, V.C and Akinniyi, J.A (1998). Phytochemistry of some psycotropic plants in Borno State. A review. *Annals of Borno,* 15:165-173.

Amin, G. (1991). Popular medicinal plants of Iran. Ministry of Health Publication, Tehran. Vol.1, P.67.

Arunlakshana, O and Schild, H.O (1959). Some quantitative uses of antagonist. *Br. J. Pharmacol.* 14, 48-59.

Bashir, S., Janbaz, K.H., Jabeen, Q. and Gilani, A.H. (2006). Studies on spasmolytic activities of *Calendula officinalis flowers. Phytother. Res.,* 20: 906-910.

Bertram, G.K. (2004).Basic and Clinical Pharmacology (international edition), McGraw Hill, U.S.A. Pp. 1046-1057.

Bolton, T.B. and Imaizumi, Y. (1996). Spontaneous transient outward currents in smooth muscle cells and interstitial cells of blood vessels. *Cell Calcium,* 35: 643-657.

Carl, A., Lee, H.K., Sanders, K.M. (1996). Regulation of ion channels in smooth muscles by calcium. *Am. J. Physiol.,* 271: 9-34.

Cortes, A.K., Delgadillo, A.J., Hurtado, M. Dormingues-Ramirez, A.M., Medina, J.R. and Aoki, K. (2006). The antispasmodic activity of *Buddleja scordioides and Buddleja perfoliata* on isolated intestinal preparations. *Biol. Pharm. Bull*., 29:1186-1190.

Dahiru, D., Sini, J.M and John-Africa, L. (2006). Antidiarrhoeal activity of *Ziziphus mauritiana* root extract in rodents. *African Journal of Biotechnology,* Vol. 5 (10):941-945.

Dicarlo, G.D., Mascolo, N., Izzo, A.A., Capasso, F. (1994). Effect of quercertine on the gastrointestinal tracts in rats and mice. *Phytother Res.* 8:42-45

Eglen, R.M., Reddy, H., Watson, N., and John, R.A. (1994). Muscarinic acetylcholine receptor in

smooth muscle. *Trends in Pharmacol. Sci*. 15,114-119.

Evans, W.C. (2002). Trease and Evans' Pharmacognosy (15th edition) Elsevier company, Philadelphia, U.S.A. Pp. 191-418.

Galvez, A., Zarzuelo, M.E., Crespo, M.D. (1991). Antidiarrhoeic activity of *Scleroarya birrea* bark extract and its active tannin constituent in rats. *Phytotherapy Research*, 5:276-278.

Galvez, A., Zarzuelo, M.E., Crespo, M.D., Lorente, M., Ocete, A. and Jimenez, J. (1993). Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of active flavonoid constituent. *Planta Medica* 59,333-336.

Ghayur, M.N. and Gilani, A.H. (2004). Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. *Dig. Dis Sci.*, 50: 1889-1897.

Gilani, A.H., Jabeen, Q., Ghayur, M.N., Janbaz, K.H. and Aktar. (2005). Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. *J. Ethnopharmacol.*, 98: 127-135.

Goto, K., Kasuya, Y., Matsuki, N., Takuwa, Y. and Kurihara, H. (1989). Endothelin activates the dihydropyridine-sensitive, voltage dependent Ca^{2+} channel in vascular smooth muscle. Proc. Natl. Acad. Sci., 86:3915-3918.

Harborne, J.B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis . Chapman & Hall, London. Pp. 279-282.

Itoh, T., Kanmura, Y., Kuriyama, H and Suzuki, H. (1984). Nisoldipine-induced relaxation in intact and skinned smooth muscles of rabbit coronary arteries. Br. J. Pharmacol., 83: 243-258.

Karaki, H., Ozaki, H., Hori, M., Mitsui-Saito, M., Amano, K. (1997). Calcium movements, distribution and functions in smooth muscle. *Pharmacol. Rev*. 49: 157-230.

Magaji, M.G., Yaro, A.H., Mohammed, A., Zezi, A.U., Tanko, Y. and Bala, T.Y. (2007). Preliminary antidiarrhoeal activity of methanolic extracts of *Securinega virosa*. African *Journal of Biotechnology*, Vol.6 (24) : 2752-2757.

Magaji, M.G., Shehu, A., Musa, A.M., Sani, M.B. and Yaro, A.H. (2010). Pharmacological evidence on the folkloric use of *Cochlospermum tintorium*. *Int. J. Pure Appl. Sci.*, 4 (1): 14-20

Ojewole, J.A.O. (2004). Evaluation of the antidiabetic and anti-inflammatory properties of *Sclerocarya birrea*. Stem bark aqueous extract in mice and rats. Phytother. Res., 18:601-608.

Rao, A.V. and Gurfinkel, D.M. (2000). Bioactivity of saponins: Triterpenoids and steroidal glycosides. Freund Publishing house Ltd. Canada. 211-231.

Rao, N.V., Prakash, K.C and Kumar, S.M.S (2006). Pharmacological investigation of Cardiospermum halicacabum (Linn) in different animal models of diarrhoea. *Indian Journal of Pharmacology.* 38 (5): 346-349.

Shimizu, S., Yokoshiki, H.N. Sperelakis, H.N., and Paul, R.J. (2000). Role of voltage- dependent and Ca^{2+} activated K^{+} channels on the regulation of isometric force in porcine coronary artery. *J. Vasc. Res.*, 37: 16-25.

Sofowora, E.A. (1984). Medicinal Plants and Traditional Medicine in Africa (1st ed.) John Wiley and Sons Ltd. London. Pp.180-188.

Thiaina, P., Tungchareon, P., Wongnawa, M., Reanmongkol, W. and Subhadhiraskul, S. (2008). Uterine relaxant effects of Curcuma aeruginosa Roxb Rhizome extracts. *J. Ethnopharmacol.*, 121: 433-443.

Vane, J.R. (1957). A Sensitive Method for the assaying of 5- Hydroxyltryptamine. *Brit. J. Pharmacol.* 12, 344-349.

WHO (1977). Selection of Essential Drugs. Technical Report Series No. 615, Geneva.