



**SCREENING OF PHARMACOLOGICAL EFFECT OF METHANOL EXTRACT OF
LEAVES OF LIBYAN *CAPPARIS SPINOSA* SUBSP *ORIENTALIS* (DUH.) JAFRI ON
VITAL ISOLATED ORGANS**

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ABSTRACT

The present study was undertaken to evaluate the effect of the methanol extract of leaves of *Capparis spinosa* on vital isolated organs (Rabbit aorta strip, Rabbit jejunum, Rat uterus and Frog rectus abdominus muscle) with use of standard methods. **Results:** The results revealed that methanol extract of leaves of *Capparis spinosa* has weak cholinergic effect on isolated rabbit jejunum which completely antagonized with 10ng/ml Atropine. Also the result showed that the extract has a potentiating effect to the reference Nor-epinephrine at low doses up 400µg/ml which completely reversed to Nor-epinephrine antagonizing effect at high doses of $\geq 1600\mu\text{g/ml}$. **Conclusion:** Methanol extract of leaves of *Capparis spinosa* has weak cholinergic effect on isolated rabbit smooth muscle, Nor-epinephrine potentiating effect at low doses and antagonizing effect on Nor-epinephrine at higher doses on isolated rabbit Aorta. The plant has no effect on tested isolated uterus and frog abdominus muscle.

KEYWORDS: *Capparis spinosa*, Pharmacological effects, Isolated vital organs.

1. INTRODUCTION

The world health organization defined the herbal medicine as plant derived material or preparation contains either raw or processed ingredients from one or more plants which when administered to man or animals exert a sort of pharmacological action on them (WHO, 1993). Natural products originated from plant, animal, and minerals have been the basis of treatment of human disease and believed to be resources of new drugs. It was estimated that over 50% of modern clinical drugs have natural products' origin (Adnan *et al.*, 2014). The importance of Traditional Medicine as a source of Primary Health Care was first officially recognized in 1978 by the World Health Organization in the primary health care and the tradition use of medicinal plants, as a basis for the maintenance of good health in most developing countries (Rukangira, 2001). Hendawy *et al.* (2010) reported that, herbal medicines have an important value in the developing countries for their spiritual and sociocultural use and also for their medicinal value in tribal and rural. In the mid-90s, it is estimated that receipts of more than US\$2.5 billion have resulted from the sales of herbal medicines. According to the world

health organization (2001), about 70-80% of world population uses herbal medicines for their therapeutic effects. More than 3.3 billion people in the less developed countries utilize medicinal Plants as backbone of traditional medicine because they consider as rich resources of therapeutic bioactive phytochemicals such as phenolic compounds and flavonoids which have been reported to have positive impact on health and cancer prevention. These phytochemicals can be used in novel drugs development and synthesis (Sasidharan *et al.*, 2011, Singh, 2105). *Capparis spinosa* is a perennial spiny bush that bears rounded, fleshy leaves and big white to pinkish-white flowers. It is native to the Mediterranean region and growing wild on walls or in rocky coastal areas (Manikandaselvi *et al.*, 2016). This plant has a lot of traditional and medical use. The whole plant was used for rheumatism. Roots were used as diuretic, astringent, and tonic. Bark of root, which has a bitter taste, was used as appetizer, astringent, tonic, anti-diarrheic and to treat hemorrhoids and spleen disease (Rahnavard and Razavi, 2016). It has been reported that *Capparis spinosa* has anticancer activity (Lam *et al.*, 2009, Al-Daraji, 2010), marked anti-inflammatory, anti-

arthritic activity (Al-Said *et al.*, 1988, Feng *et al.*, 2011, Bhojar, 2012), lipid lowering effect (Mishra *et al.*, 2012), antioxidant activity (Eltawaty *et al.*, 2018), decreased levels of liver function markers, creatinine and total bilirubin and improving the damaged liver tissue in a dose dependent manner (Al-khan *et al.*, 2012). Libyan people used Caper as anti-cancer in major and in wound healing in minor. Eltawaty *et al.* (2018) reported that methanol extract of leaves of *Capparis spinosa* has a pronouncing antibacterial activity against methicillin resistant *Staphylococcus aureus* especially with the limited treatment choices. Also Eltawaty *et al.* (2018) concluded that Libyan *Capparis spinosa* has good antioxidant activity, showed no significant changes on liver safety biomarkers; alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and they also reported that the lethal dose of the methanol extract of the plant leaves is more than 2000mg/kg. Accordingly, and to form a comprehensive understanding of the biological activity of *Capparis spinosa*, this study was undertaken to study the pharmacological effects of the plant extract on the vital isolated organ.

2. MATERIAL AND METHOD

2.1 Sample Preparation

Dimethyl sulfoxide; DMSO (SDFCI, India) was used as solvent to prepare a stock extract solution with concentration of 10mg/ml. the extract solution freshly prepared prior to addition into the tissue bath.

2.2 Preparation of physiological solutions

Krebs solution, Tyrode solution, D Jalon's solution and Frog solution are physiological solution prepared freshly for the screening of the pharmacological effects of tested extracts on the isolated, rabbit aortic strip, rabbit jejunum, rat uterus and frog rectus abdominus muscle organs respectively. The physiological solution prepared according to Ian Kitchen, 1984.

2.3 Animals

Animals obtained from the Experimental Animal House, Medicinal Aromatic Plants Research Institute, National Centre Research, Sudan. Animals were given standard feeding and tap water.

2.4 Isolated Rabbit Aortic preparation

A rabbit of local strain (1.75 kg) was used in this experiment. The preparation was based on the method adopted by Furchgott and Bhadrakom (1953). The rabbit was neck dislocated, sacrificed and exsanguination. The chest was opened, the internal viscera were pulled aside and the aorta had been exposed. The aorta was cut closed to the heart and dissected as fast as possible. Then after, the tissue was transferred to a petri dish containing aerated Krebs solution. The aorta was located over a large plastic cannula, surrounding fats and connective tissues were removed, then the aorta was cut spirally by curved scissor to produce a continuous strip. Threads had been tied to each end of the strip and one end was

attached to the tissue holder. The mounted tissue then was transferred to a 25 ml organ bath filled with oxygenated Krebs solution maintained at 37°C and the top thread was attached to Harvard isometric transducer connected to Harvard Universal Oscillograph recorder (Harvard Apparatus Limited UK). The preparation was allowed to stand for 45 minutes, under 2 g resting tension before addition of the reference drug (Nor-epinephrine; SIGMA ALDRICH) and the extract.

2.5 Isolated Rabbit jejunum Preparation

Rabbit of local strain weighing 2kg was used. The rabbit was sacrificed and the abdomen exposed. The first 2-3 cm of the jejunum was taken out and placed on Petri dish containing Tyrode solution at room temperature. The isolated 2-3 cm jejunum tissue was freed from fats and connective tissues and transferred to organ bath (25ml) containing aerated Tyrode solution which was maintained at 37°C. The tissues allowed to settle for 45 minute as adaptation time. The response of the extract was recorded with isotonic transducer connected to Oscillograph recorder (Harvard Apparatus Limited UK) with attenuation of speeds 0.25mm/sec. Under 1.5g tension (Ian kitchen. 1984)

2.6 Isolated Rat uterus Preparation

Female young Wister rats, weighing 120 gm was used in this study. The animal was brought into oestrus stage by subcutaneous administration of β -estradiol-3-benzoate (2.5 mg/kg) 24 h prior to the experiment. Preparation of the uterus was carried out according to the method described by De Jallon, (1945). The rat killed by a blow on the head and exsanguinated. The abdomen was opened and the two uterine horns were exposed by pulling aside the intestine. Each horn was freed carefully from surrounding fat and mesenteric attachments, cut out separately and transferred to a petri-dish containing De Jalon's solution. Each horn was cut open longitudinally to from a sheet of muscle instead of a narrow tube. A thread will be attached at each end of piece and the preparation will be mounted in a 25-ml organ bath containing aerated De Jalon's solution maintain at 37°C with one attached to a fixed pin and the other to an isometric transducer connect to Harvard oscillographic recorder with attenuation of speeds 0.1mm/sec. The preparation was allowed to equilibrate for 45 min, under 0.5 tensions before addition of the plant extract and the reference drugs.

2.7 Isolated Toad (Frog) rectus abdominal muscle preparation

The frog was decapitated after stunning and the animal was pithed using pithing needle. The frog then placed ventral side up on a cork board and a cut made in mid ventral of trunk. The skin separated along this midline and the recto muscles (which are underneath) was exposed and moistened with frog ringer solution. Two longitudinal cuts were made on either side of the xiphoid cartilage and the line of the recti muscles has been followed in their attachment to pubis. A transfer cut

through the xiphoid cartilage was made free from attachment to pubis, the recti muscle then transferred to Petri dish containing frog ringer solution at room temp. By making longitudinal cut along the linea alba, the two muscles were separated and the thread was passed through one muscle at both top and bottom. The bottom thread was attached to tissue holder. The mounted preparation transferred to the organ bath and the top thread was attached to an isotonic transducer and an additional stretching weight will be added to the resting tension to insure that the muscle returns to its base line after drug induced contracture. (Ian Kitchen 1984).

RESULTS AND DISCUSSION

Very little data have been found concerning the pharmacological effects of *Capparis spinosa* plant on the vital isolated organs. A weak cholinergic effect in a dose dependent manner on the isolated rabbit jejunum has been exerted from methanol leaves extracts of *Capparis spinosa* in this study. This cholinergic effect was antagonized with 10ng/ml atropine (Figure 1). This result agreed with Yang *et al.*, (2008) who reported that *Capparis spinosa* plant has many extensive pharmacological effects including stimulation of smooth muscles. Also Benzidane *et al.* (2013) and Nabavi *et al.* (2016) also reported that the plant *Capparis spinosa* gave contractile effects on the smooth muscle as this study showed but their results revealed from aqueous extract of the plant leaves while this study was concerned with the effect of methanol leaves extract.

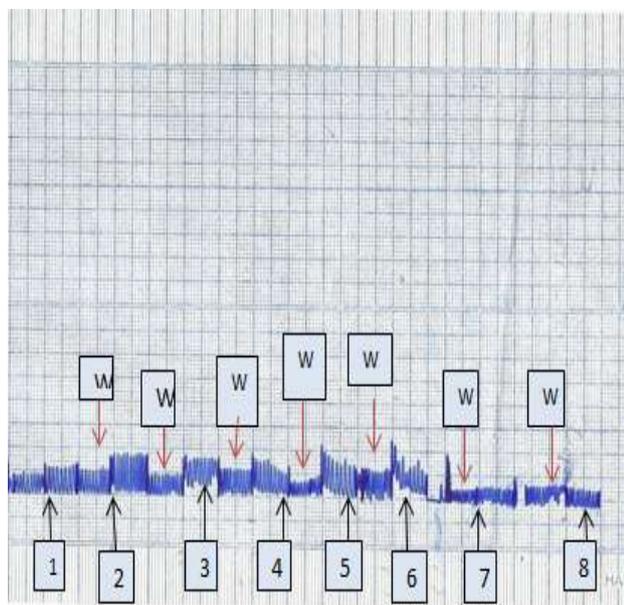


Figure (1): Pharmacological effect of *Capparis spinosa* leaves extract on Rabbit Jejunum W = Wash 1 = 200µg/ml plant extract 2 = 400µg/ml plant extract 3 = 800µg/ml plant extract 4 = 100mg/ml plant extract 5 = 200mg/ml plant extract 6 = 400mg/ml plant extract 7 = 100mg/ml plant extract + 10ng/ml Atropine 8 = 400mg/ml extract + 10ng/ml Atropine.

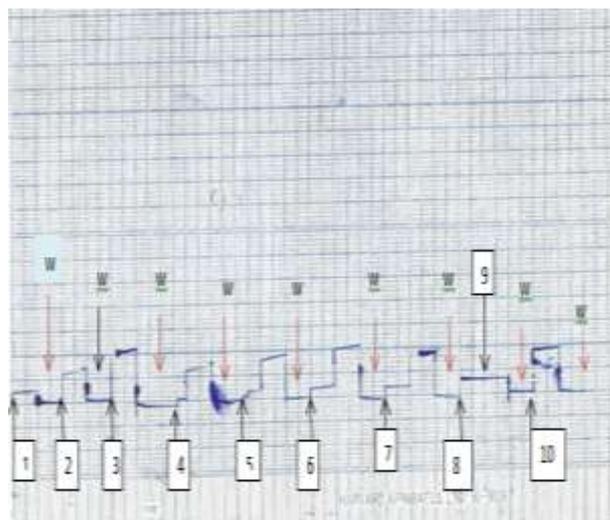


Figure (2): Pharmacological effect of *Capparis spinosa* methanol leaves extract on isolated rabbit aortic strip W = Wash 1 = 1ng/ml Nor-epinephrine 2 = 2ng/ml Nor-epinephrine 3 = 4ng/ml Nor-epinephrine 4 = 100µg/ml plant extract + 2ng/ml Nor-epinephrine 5 = 200µg/ml plant extract + 2ng/ml Nor-epinephrine 6 = 400µg/ml plant extract + 2ng/ml Nor-epinephrine 7 = 800µg/ml plant extract + 2ng/ml Nor-epinephrine 8 = 1600µg/ml plant extract + 2ng/ml Nor-epinephrine 9 = 3.2mg/ml plant extract + 2ng/ml Nor-epinephrine 10 = 2ng/ml Nor-epinephrine

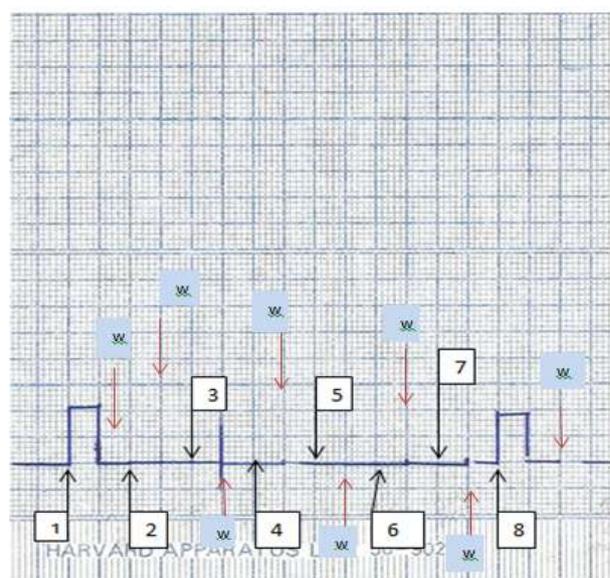


Figure (3): Pharmacological effect of *Capparis spinosa* methanol leaves extract on isolated Frog rectus abdominus muscle.

1 = 500ng Acetylcholine 2 = 100mcg/ml extract 3 = 200mcg/ml extract 4 = 400mcg/ml extract 5 = 100mg/ml extract 6 = 200mg/ml extract 7 = 400mg/ml extract 8 = 500ng Acetylcholine

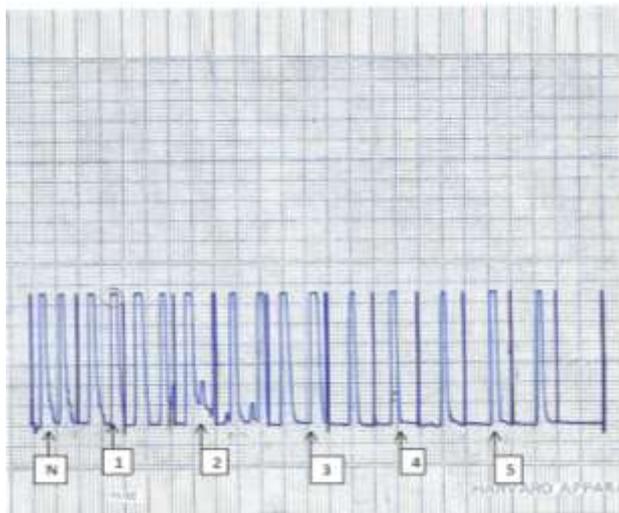


Figure (4): Pharmacological effect of *Capparis spinosa* methanol leaves extract on isolated rat uterus.

N = Normal 1 = 200 μ g/ml plant extract 2= 400 μ g/ml plant extract 3 = 1mg/ml plant extract 4 = 2mg/ml plant extract 5 = 4mg/ml plant

In this study methanol leaves extract of *Capparis spinosa* at low doses 100-400 μ g/ml exerted a dose dependent potentiation to the reference Nor-epinephrine on isolated aorta but at high dose of 800 μ g/ml showed less potentiation and at higher doses (1.6 – 3.2 mg/ml) the extract completely antagonized the reference nor-epinephrine 2ng/ml (Figure 2). It is important to mention here that this study concerned with crud extract and the GC/MS phytochemical analysis of this crud extract done by Eltawaty *et al.*, (2018) concluded that the methanol leaves extract of *Capparis spinosa*, comprises of 33 bioactive compounds and it is possible that some compounds contained will mask or antagonized others at different concentrations. This result is not in line with Al-Snafi, (2015) result which claimed that *Capparis spinosa* produced a rapid relaxation when added during the plateau phase of aorta contraction induced by noradrenaline. This may contributed to the different extract type, since this study studied methanol extract while Al-Snafi, (2015) review referred the action to the aqueous extract of *Capparis spinosa*. This result suggested that the extract may be used as hypotensive agent at low doses up to 400 μ g/ml.

This study showed no effect has shown on tested frog abdominus muscles (Figure 3). No data found clarify the effect of *Capparis spinosa* on isolated frog abdominus rectus muscle. Also no data found explain the effects *Capparis spinosa* on isolated uterus organ, but this study proved that even though rat uterus previously brought to oestrous stage but not effected by the tested *Capparis spinosa* extract, the result which suggested that the tested extract can be used during pregnancy for its proved antibacterial activity against methicillin resistant *Staphylococcus aureus* to treat the organism infection and for its antioxidant activity to promote health without fear of causing abortion for pregnant cases.

CONCLUSION

Methanol extract of leaves of *Capparis spinosa* has weak cholinergic effect on isolated smooth muscle, Nor-epinephrine potentiating effect at low doses up to 400 μ g/ml and antagonizing effect on Nor-epinephrine at high doses \geq 1600 μ g/ml on isolated Aorta. The plant has no effect on tested isolated uterus and frog abdominus muscle.

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