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Effects of Methanolic Extract of the bark of *Khaya Senegalensis* on Various Isolated Tissues Preparations

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Abstract: ***In this study the methanolic extract of the bark of Sudanese plant Khaya senegalensis was investigated for its effects on isolated rabbit jejunum, rat uterus, rabbit aortic strip,frog rectus abdominis and guinea pig atria. When methanolic extract of bark of Khaya senegalensis was added to isolated rabbit intestine it inhibited the spontaneous contracting rabbit jejunum and this activity was blocked by phentolamine, implicating α-adrenergic stimulant activity, when added to isolated rat uterus it stimulated the rat uterus and this activity was blocked by cyproheptadine, implicating serotonin like activity. It had no effect on rabbit aortic strip, frog- rectus abdominis and guinea- pig atria.***

Keywords: Methanolic extract, *Khaya senegalensis*, isolated tissues preparations, rabbit, drugs

1. Introduction

Medicinal plants represent one of essential sources in our country. Sudan is a large country and has excellent geographical localization and medicinal plants are widely spread in many areas. In Sudan in folk medicine watery maceration of the bark of *Khaya senegalensis* is used in treatment of malaria, hepatitis, dysentery and sinusitis. Also leaves of plants were used to treat dermatological disorders, abdominal diseases and trachoma. It is commonly used for wound healing and malaria (1).

The use of plants and herbs for medicinal purposes spread overall the world. Therefore, a high consumption of medicinal plants is clearly observed in the developing Islamic and non-Islamic countries. Other reasons are appearance of various adverse effects of synthetic chemical drugs and universal existence of these plants and their low expenses compared to synthetic drugs (2).

1.2. Plant description:

1.2.1. *Khaya senegalensis*

Khaya senegalensis (Desr.) A. Juss.: The plant belongs to the family *Meliaceae* and locally known as Mahogany tree (Senegal Mahogany) .Other species: *K. grandifoliola*, *K. anthotheca* and *K. ivorensis*.

1.2.2. Preparations of plants material

1.2.2.1. *Khaya senegalensis*

Plant was obtained from Elobied, North Kordofan, Western Sudan.

1.3. Objectives

To study the effects of plant extract on isolated tissues preparations to determine its pharmacological effects.

2. Material

2.1. Animals

Female albino rats were obtained from animal house of faculty of Pharmacy, University of Khartoum & National Centre for research, Khartoum, Sudan. African guinea- pigs were obtained from National Centre for Research, Khartoum, Sudan. Rabbits were purchased from Omdurman market and frogs from Shambat area near the Nile. All animals were kept at room temperature (26 ±1°C) and freely accessed to food and water.

2.2. Drugs

Drugs and agents used in this study are as follows: Carboxy methyl cellulose, cyproheptadine, Sodium chloride, Sodium bicarbonate, potassium chloride, calcium chloride, D-glucose, magnesium sulphate, phentolamine, propranolol, physostigmine and d-tubocurarine.

3. Methods

3.1. Preparation of Drugs

The drug solutions were freshly prepared daily, 1% carboxy methyl cellulos in normal saline.

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3.2 Preparations of Plants Material

3.2.1. *Khaya senegalensis*:
Plant was obtained from Elobied, North Kordofan, Western Sudan.

3.2.2. Preparation of extracts:
3.2.2.1. Methanolic extract:
100gm of the dried small pieces of plant homogenized with one liter of 80% methanol. The mixture was filtered with Whatman No.1 filter paper and the filtrate was dried to a solid mass under air at room temperature.

3.2.3. General phytochemical tests for the major components of the tested plants:
The following phytochemical screening tests of the chemical constituent were conducted according to the methods established by (3).

3.2.4. Pharmacological effects of plants extracts:

3.2.4.1. Isolated rat uterus preparation:
The preparation is based on the method of (4). A female rat was injected with 0.1 mg/kg stilboesterol intramuscularly 24 hours before the experiment to induce an estrus cycle. The rat was killed by dislocating the neck and the animal was exsanguinated. The abdomen was opened and the two uterine horns were exposed by pulling aside the intestine. They are often easily distinguishable by their pink colouration. Each horn was freed from surrounding fat and mesenteric attachments, and each was cut out separately and transferred to a Petri dish containing De Jalon Ringer and transferred to an isolated organ bath and attached to the transducer, and left for 30 minutes to equilibrate.

3.2.4.2. Isolated rabbit jejunum preparation:
The jejunum was Isolated according to the method used by (5). The abdomen was exposed and opened; the jejunum was identified, by following it back to the stomach. A suitable length was cut and transferred to a dish containing Tyrode's solution was tied to an isotonic transducer connected to recorder (Harvard, America).

3.2.4.3. Determination of the cholinergic effect of the plant extract on nicotinic receptors using the frog rectus abdominis muscle:
A frog was decapitated after stunning and the animal pithed using a pithing needle. The frog was placed on a corkboard and made cut in mid ventral line of the trunk. The skin separated along this mid line and exposed.
The recti muscles transferred to a Petri dish containing Ringer solution and separated into two. The mounted preparation transferred to the organ bath and the top thread attached to the recorder (6).

3.2.4.4. Preparation of guinea- pig atria:
The preparation was carried following the method of (6). A guinea-pig weighing 400-550gm was killed by dislocating its neck. The thorax was opened and the heart removed as rapidly as possible and transferred to a Petri dish containing aerated ice cold Ringer solution. Ventricles were dissected off; threads were tied to tip of each atrium. One thread was fixed to a tissue holder and the tissue was transferred to an organ bath at 30°C. The upper thread was attached to an isotonic transducer connected to an oscilliaographic recorder Bioscience MD2.

3.2.4.5. Rabbit aorta strip preparation:
The rabbit aorta strip preparation was made accordingly to the method of (7). Rabbit was killed by a blow in the neck. The chest was opened, the diaphragm was removed, the heart and the lung were dissected out to expose the aorta. Aorta was removed by gentle dissection; the aortic tissue was transferred to a Petri dish with oxygenated Kreb's solution.The preparation was attached one to the holder and the end connected to isometric transducer connected to recorder (Harvard, America).

4. Result

4.1. Phytochemistry

Khaya senegalensis was found to be positive for flavonoids, tannins, triterpenoids and saponins and negative for unsaturated sterols, alkaloids and coumarins.

4.2. Effect of plants extracts and drugs on isolated tissue preparation:

4.2.1. Effect of methanolic extract of the bark of *Khaya senegalensis* (K) on isolated rabbit jejunum
Rabbit jejunum has myogenic contractions; relaxation of these contractions is produced by sympathomimetic or direct muscle relaxants. Figure (1) showed the influence of extract on contracting rabbit jejunum. The extract (K) produced inhibitory effect at a dose of 2 mg/ml. This effect was not blocked by 4 µg/ml of propranolol and blocked by2 µg/ml of phentolamine.

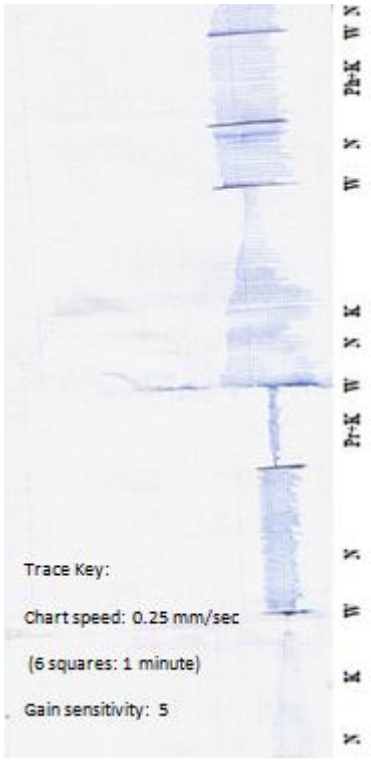

Figure 1: Effect of methanolic extract of the bark of *Khaya senegalensis* (K) in a dose of 2 mg/ml on isolated rabbit

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jejunum, the extract produced inhibitory effect which was not blocked by propranolol (Pr = 4 μg/ml) and blocked by phentolamine (Ph= 2 μg/ml); (N: normal, W: wash).

4.2. 2. Effect of methanolic extract of the bark of *Khaya senegalensis* (K) on isolated non contracting rat uterus

The uterus is innervated by sympathetic nerves and possibly a parasympathetic supply, which runs to the wall of the tissues. The response of the uterus to drugs varies from species to species. Figure (2) showed the effect of extract. 5-hydroxy-tryptamine (2μg/ml) stimulated the isolated uterus preparation, the extract (K) 2 and 4 mg/ml showed stimulant effect. This effect was blocked by 2 μg/ml cyproheptadine.

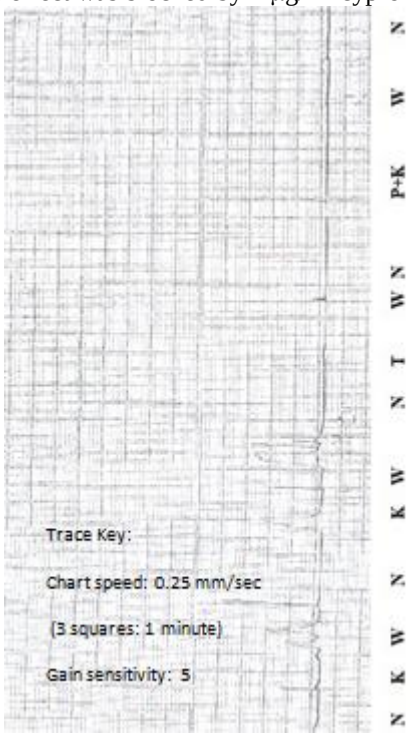

Figure 2: Effect of methanolic extract of the bark of *Khaya senegalensis* (K) on isolated non contracting rat uterus in a dose of (2 and 4 mg/ml); 5-hydroxy-tryptamine (T) was administered to produce standard peak of contraction in a dose of (2μg/ml). The extract showed stimulant effect which was blocked by cyproheptadine (P) in a dose of (2 μg/ml); (N: normal, W: wash).

4.2.3. Effect of methanolic extract of the bark of *Khaya senegalensis* (K) on isolated frog-rectus abdominis muscle:

The frog-rectus abdominis muscle contains both multiply innervated and focally innervated fibers, but the characteristic of response are typically of multiply innervated fibers. In figure (3) Acetylcholine (2 μg/ml) stimulated the frog rectus abdominis muscle preparations. Addition of extract (K) 2, 4 and 8 mg/ml produced no effect; at a dose of 4 mg/ml the extract (K) blocked partially the effect of acetylcholine, in comparison with the effect of 1 μg/ml of d-tubocurarine. In presence of 20 μg/ml of physostigmine and 2 μg/ml

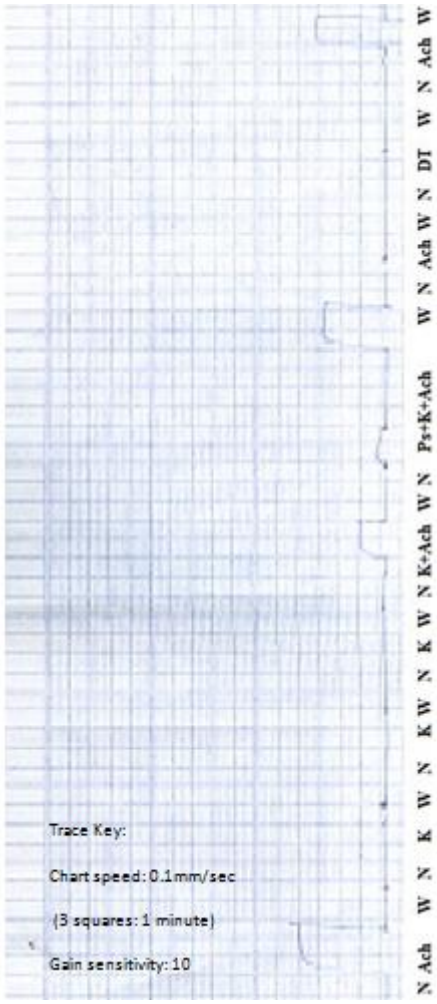

Figure 3: Effect of methanolic extract of the bark of *Khaya senegalensis* (K) on isolated frog-rectus abdominis muscle. The extract was added in a dose of (2, 4 and 8 mg/ml) produced no effect. Acetylcholine (Ach) was administered to achieve standard peak of contracture in a dose of (2 μg/ml). Acetylcholine added after extract in a dose of (4 mg/ml), the extract block partially the effect of acetylcholine, in comparison with the effect of d-tubocurarine (DT) (1 μg/ml). In presence of physostigmine (Ps) in a dose of (20 μg/ml) and acetylcholine (2 μg/ml) the extract diminished the effect of acetylcholine to some extent . (N: normal, W: wash)

4.2.4. Influence of methanolic extract of the bark of *Khaya senegalensis* (K) on rabbit aortic strip preparation:

Vascular blood vessels are innervated almost exclusively by the sympathetic branch of autonomic nervous system. The distribution of adrenoceptors at the post synaptic terminals of vasomotor nerves varies considerably from tissue to tissue. α-adrenoceptors mediate vasoconstriction and there is a predominance of these receptors in blood vessels serving the skin and viscera. β-adrenoceptors (mostly of the β_2 type) mediate vasodilatation and predominate in skeletal muscle blood vessels. Adrenaline 2 μg/ml stimulated the aortic strip preparation. Addition of the extract (K) 1, 2, and 4 mg/ml to aortic preparation induced no effect (Fig4).

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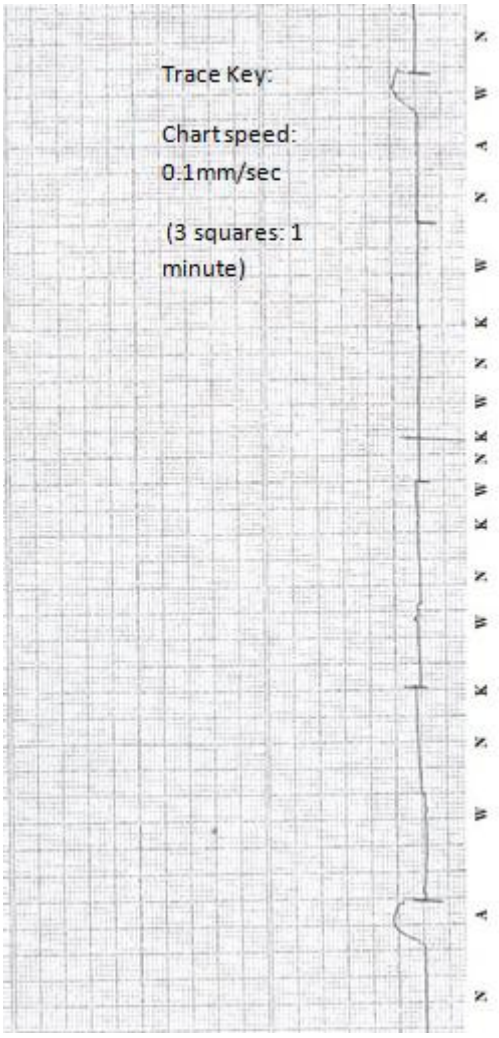

Figure 4: Influence of methanolic extract of the bark of *Khaya senegalensis* (K) on rabbit aortic strip. Adrenaline (A) in a dose of (2 μg/ml) was administered to produce standard contractile peak. The extract (K) was added in a dose of (1, 2, and 4 mg/ml) produced no effect. (N: Normal, W: wash).

4.2.5. Influence of methanolic extract of the bark of *Khaya senegalensis* (K) on isolated guinea- pig atria:

The heart is innervated by both sympathetic and parasympathetic nerves. Atrial muscle is supplied by parasympathetic nerve, which arises from vagal axon. Addition of the extract (K) 1, 2, 4 and 8 mg/ml to the atria preparation produced no effect (Fig.5).

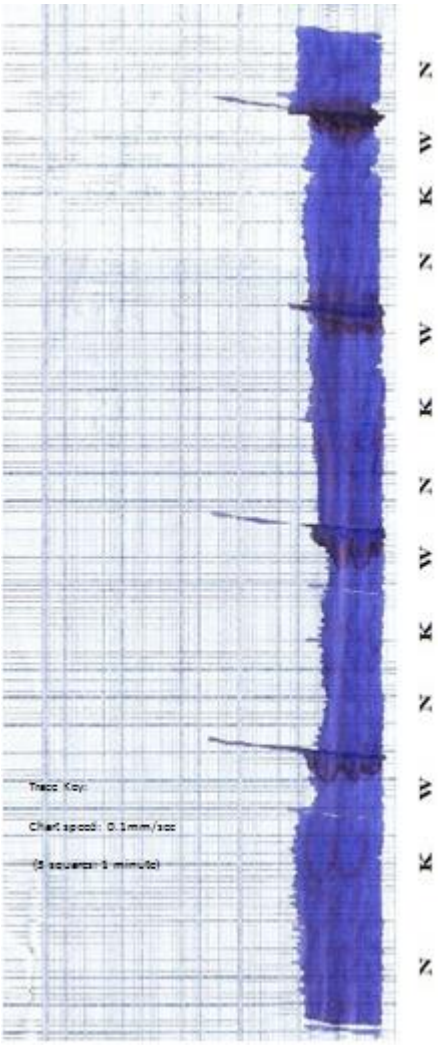

Figure 5: Influence of methanolic extract of the bark of ***Khaya senegalensis* (K) in a dose of (1, 2, 4 and 8 mg/ml) for 2 minutes on isolated guinea-pig atria, the extract produced no effect on the isolated tissue (N: normal, W : wash).**

5. Discussion

In the present work the methanolic extract of the bark of *Khaya senegalensis* when added to spontaneous contracting rabbit jejunum produced inhibitory effect which was blocked completely by phentolamine which implies α-receptor stimulant activity. This is in agreement with the result reported by (8) that was able to block the inhibitory action of methanolic extract of the bark of *Khaya senegalensis* on spontaneously contracting rabbit jejunum by tolazoline.

He also reported that the methanolic extract of the bark of *Khaya senegalensis* contained pharmacologically active component with a biphasic activity. The methanolic extract of the bark of *Khaya senegalensis* on isolated rabbit jejunum in a dose of 2.5mg/ml produced contraction, which was blocked partially by cyprohepatdine and in a dose of 10 mg/ml showed relaxation, which was blocked by tolazoline. When the methanolic extract of the bark of *Khaya senegalensis* added to isolated rat uterus (non-contracting and contracting), it exerts stimulant action which was blocked by cyproheptadine which implies 5-hydroxy tryptamine like action. This is supported by the findings of

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(9) who was able to block the stimulant action of methanolic extract of the roots of *Clerodendron capitatum* on the rat uterus by cyproheptadine.

When the methanolic extract of the bark of *Khaya senegalensis* was added to the rabbit aorta strip or guinea-pig atria or to the frog- rectus abdominis, no effects were produced.

Although the methanolic extracts of *Khaya senegalensis* exhibited α- stimulant effect, no effects was observed when add to isolated rabbit aorta.

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