

Study of Antimicrobial Activities of *Guiera senegalensis* Leaves

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Abstract: In the current study, the claimed antimicrobial activity of Sudanese medical plant, *Guiera senegalensis* leaves studies. This involved preparation and use of methanolic extract of *Guiera senegalensis* leaves. The study conducted on Guinea pig and rat with induced inhibition and slight contraction. The extract showed dose dependant inhibition on force and rate of contraction of isolated Guinea pig atrium. In isolated rat uterus preparation the extract showed slight contraction which blocked by (2 mg mL⁻¹) cyproheptidine.

Key words: *Guiera senegalensis*, antimicrobial activity, methanolic extract, cyproheptidine, inhibition

INTRODUCTION

Guiera senegalensis is plant widely distributed in Africa. It was investigated for its biological activities as an anti-malarial (Benoit *et al.*, 1996; Azas *et al.*, 2002; Ancolio *et al.*, 2002) and antiviral (Silva and Gomes, 2003; Aniagu *et al.*, 2005).

In Sudan and Nigeria using this plant traditionally as a febrifuge, antileprosy and as an infusion for the treatment of diabetes mellitus. Also is used in the experiment did not show any significant toxicity (Diouf *et al.*, 2000).

MATERIALS AND METHODS

Plant material: The plant materials *Guiera senegalensis* (leaves), the samples were collected and deposited in the herbarium of medicinal institute. The leaves were cleaned carefully, dried in shade and coarsely powdered.

Animals:

- Wister Albino rats
- Rabbits: Local strain

- Guinea pigs: Laboratory Animal House, Department of Pharmacology and Toxicology, Medicinal and Aromatic Institute, National Research, Ministry of Science and Technology
- Frogs: Local strain

Method of extraction: The plant material was extracted by using methanol and chloroform in a soxhlet apparatus. The chloroform extract was filtered and evaporated under reduced pressure using rota-vap and kept a side. The extracted plant material was dried in shade, re-packed in the soxhlet and exhaustively extracted with methanol. The methanolic extract was filtered and evaporated to dryness under reduced pressure. The methanolic extract, thus obtained was weighted and kept into a refrigerator for different experimental research. The percentage yield for *Guiera senegalensis* was 41.6%.

Experimental; methods of preparation of isolated tissue

Preparation of isolated rabbit jejunum: This experiment was carried out following the used by Kitchen (1984). Rabbit of local strains were used. The animal was killed by dislocating the neck and exsanguinated. The abdomen was opened and then the proximal part of

intestine-jejunum-was dissected out, whatever the tissue was transferred to a Petri dish containing tyrode solution at room temperature. The tissue was cut in small pieces (2 cm each) cleaned from connective tissues and tied to an areater, transferred to an organ bath (25 mL) containing an aereated tyrode solution at 37°C, however the top of the tissue was tied to an isotonic transducer and allowed to equilibrate for 45 min under 0.5 g tension. The response of the tissue to the extracts were recorded using Harvard oscillographic (Kitchen, 1984).

Preparation of isolated Guinea pig ileum: The preparation is based on the method of Magnus, 1904 (De Jalon *et al.*, 1945). The Guinea pig was killed by dislocated the neck and exsanguinated. The abdomen was opened and the caecum was exposed. The ileum was removed from the caecal end and cut into small segments (4 cm in length). The ileum was transferred to Petri dish containing tyrode solution and the fat and mesentery were trimmed away. The tissue was transferred and mounted into the organ bath (25 mL) and attached to the isotonic transducer connected to oscillograph recorder (Kichen, 1984).

Isolated rat vas deferens preparation: The preparation is based on the method of Henderson and Hart. The rat killed by dislocated the neck and the abdomen was opened and the tests and vasa defermtia were exposed. Then, each vas deferens was identified where was free from epididymis and dissected as close to the junction with the urethra as possible. The tissue was transferred to Petri dish containing Mg⁺⁺ free krebs solution and then all connective tissue and blood vessels was carefully removed. A fine thread was tied at the tip of one end of the muscle for attachment to the isometric transducer; other end was tied to the tissue holder. The preparation was mounted in an organ bath (25 mL) containing aerated Krebs solution maintained at 37°C. The responses of the tissue was recorded using Harvard oscillograph recorder connected to isomertic transducer (Kitchen, 1984).

Isolated toad rectus abdomens muscle preparation: A frog was decapitated after stunning and pitching the animal using a pitching needle. The frog was placed, ventral side up, on a cork board and a cut was made in mind ventral line of the trunk. The skin was separated along this midline and the recti muscle, underneath was exposed and moistened with frog ringer solution. The two longitudinal cuts were made on either sides of the xiphoid cartilage and attachment on the line of recti muscle was followed to their attachments on the pubis. The recti muscles were transferred to Petri dish containing frog ringer solution at room temperature and separated from xiphoid cartilage. By making a longitudinal cut a long the linea alba the two muscles were separated, a thread was passed through one

muscle at both top and bottom, the bottom thread was attached to an isotonic transducer-stretching weight was added to ensure that the muscle rectums to its baseline after inducing contracture (Kitchen, 1984).

Isolated Guinea pig atria preparation: The Guinea pig was killed by dislocated the neck and exsanguinated. The thorax was opened immediately and heart was removed as rapidly as possible, then transferred to Petri dish containing ice-cold erated ringer locke solution. The fat was removed and the ventricles were dissected off. Threads were tied to the tip of each atrium and the tissue was mounted to attached to isometric transducer (Kitchen, 1984).

Isolated rat uterus preparation: Female wister albino rat weighing 190-116 g were injected with 0.1 mg kg⁻¹ stilbestrol (IM) 24 h before the experiment. The rate was killed by dislocated the neck and exsanguinated. The abdomen was opened; two uterine horns were exposed by pulling a side. The intestine, each horn was freed from fat and mesenteric tissues. Each horn was transferred to Petri dish containing De Jalon's. Longitudinal cut was made, the tissue was set up as a sheet of muscle, then a thread was attached to both end at both top and bottom. The bottom was attached to a tissue holder and to the top to isometric transduced (Ashraf, 2004). The fat was removed and the ventricles were dissected off. Threads were tied to the tip of each atrium and the tissue was mounted to attached to isometric transducer (De Jalon *et al.*, 1945).

RESULTS AND DISCUSSION

Effect of methanolic exteract of *Guiera senealnsis* on isolated rabbit jejunum preparation: The methanolic extract of *Guiera senegainsis* exhibited dose dependant two different effects on rabbit jejunum. The extract in a concentration up to 2 mg mL⁻¹ contracted the rabbit jejunum. Further concentrations of the extract (4 mg, 8 mg mL⁻¹) inhibited the jejunum (Fig. 1).

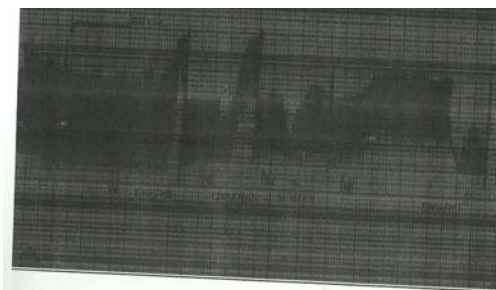


Fig. 1: Effect of methanolic exteract of *Guiera senealnsis* on isolated rabbit jejunum preparation

Effect of muscarinic blocker and adrenoceptor blocking drugs on *Guiera senegalensis* extract-induced effects on isolated rabbit jejunum preparation: Administration of the muscarinic blocker atropine (5 ng mL^{-1}) blocked the stimulant effect of the extract with subsequent significant relaxant effect. Administration of the non selective β blocker propranolol ($1 \text{ } \mu\text{g mL}^{-1}$) did not block the *Guiera senegalensis* methanolic extract induced relaxation of the jejunum. However, administration of the non-selective CL blocker Tolazoline ($2 \text{ } \mu\text{g mL}^{-1}$) partially blocked the relaxant effect induced by the extract (Fig. 2).

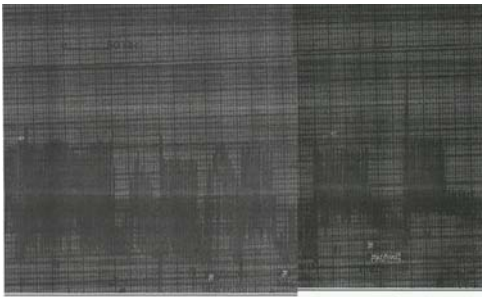


Fig. 2: Effect of muscarinic blocker and adrenoceptor blocking drugs on *Guiera senegalensis* extract-induced effects on isolated rabbit jejunum preparation

The effect of *Guiera senegalensis* extract on isolated Guinea pig ileum preparation: The extract in a dose up to 2 mg mL^{-1} did not neither contract nor relax the smooth muscle of the ileum (Fig. 3).

The effect of *Guiera senegalensis* methanolic extract on isolated rat vas deferens preparation: Increasing doses of the methanolic extract of *Guiera senegalensis* ($1.6\text{-}2 \text{ mg mL}^{-1}$) were without activity on this preparation (Fig. 4).

The effect of *Guiera senegalensis* on isolated Toad rectus abdominis preparation: Different doses of acetylcholine was added to identify the sub-maximum dose which ($2 \text{ } \mu\text{g mL}^{-1}$). In this preparation, *Guiera senegalensis* methanolic extract exhibited no activity on toad rectus abdominis muscle preparation (Fig. 5).

Effect of *Guiera senegalensis* methanolic extract on acetylcholine induced contracture on isolated toad rectus abdominis muscle preparation: Prior addition of *Guiera senegalensis* methanolic extract (8 mg mL^{-1}) inhibited the contracture induced by acetylcholine ($2 \text{ } \mu\text{g mL}^{-1}$). When the dose of *Guiera senegalensis* methanolic extract

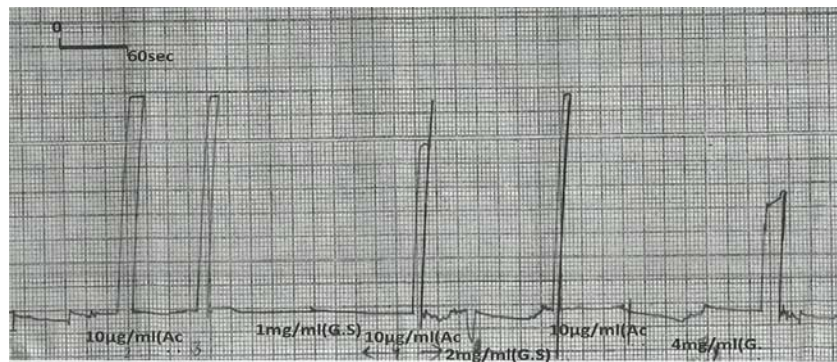


Fig. 3: The effect of *Guiera senegalensis* extract on isolated Guinea pig ileum preparation

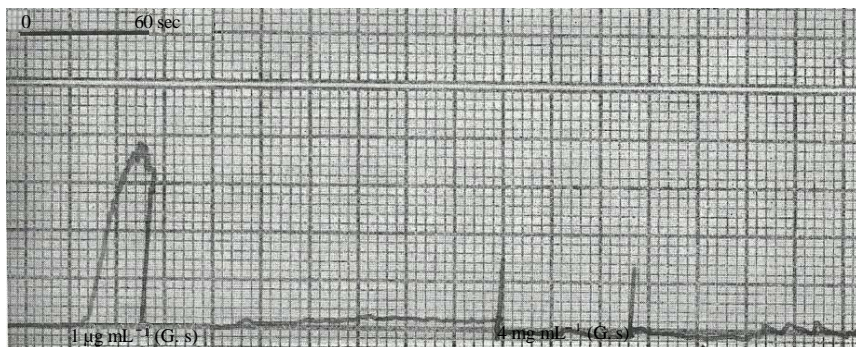


Fig. 4: The effect of *Guiera senegalensis* methanolic extract on isolated rat vas deferens preparation

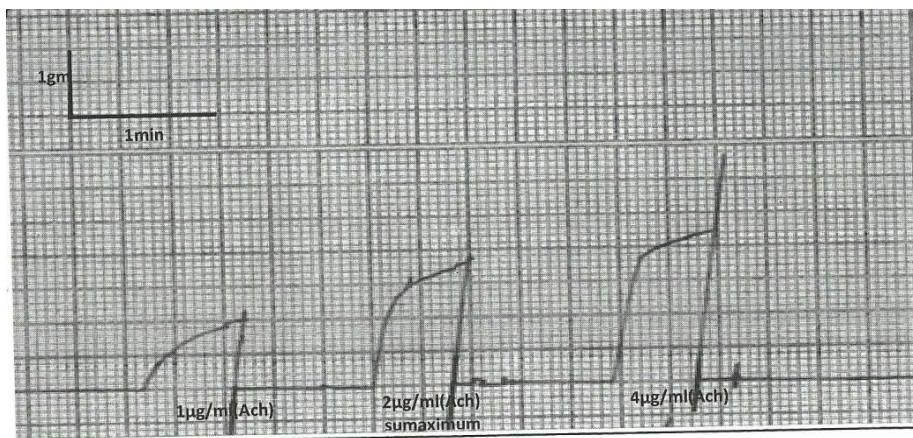


Fig. 5: The effect of *Guiera senegalensis* on isolated toad rectus abdominis preparation

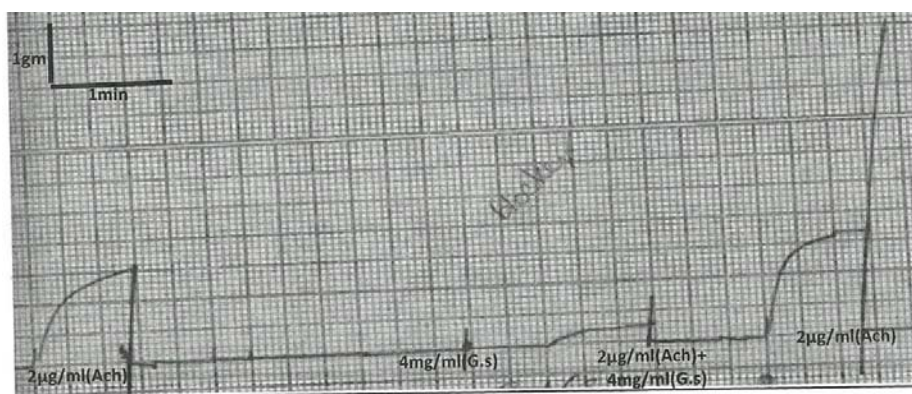


Fig. 6: Effect of *Guiera senegalensis* methanolic extract on acetylcholine induced contracture on isolated toad rectus abdominis muscle preparation

increased up to 1.6 mg mL^{-1} completely blocked acetylcholine-induced contracture on the striated muscle (Fig. 6).

The effect of neostigmine on the antagonistic effect of *Guiera senegalensis* on acetylcholine-induced contracture on isolated toad rectus abdominis muscle preparation: Administration of increasing doses of the anti-cholinesterase neostigmine up to 20 µg mL^{-1} followed by the extract (8 mg mL^{-1}) then 5 min latter acetylcholine (2 mg mL^{-1}), only partially reversed the *Guiera senegalensis* extract inhibitory effect on acetylcholine-induced contracture on toad rectus abdominis (Fig. 7).

The effect of *Guiera senegalensis* methanolic extract on Guinea pig atrium: The methanolic extract of *Guiera senegalensis* ($1\text{-}2 \text{ mg mL}^{-1}$) produced dose dependant inhibition significant ($p < 0.05$) on the force of contraction

on Guinea pig atrium preparation. Also, the extract inhibited the rate of contraction (Fig. 8 and Table 1).

Effect of atropine sulphate on *Guiera senegalensis* induced inotropic and chronotropic effects on isolated Guinea pig atrium preparation: Administration of atropine sulphate ($10\text{-}20 \text{ ng mL}^{-1}$) was without effect on neither negative inotropic nor the negative chronotropic activities of the methanolic extract of *Guiera senegalensis* (1 mg mL^{-1}) on isolated Guinea pig atrium (Fig. 9). *Guiera senegalensis* induced inhibition of the Guinea pig atrium studied by using Student t-test, $p < 0.05$ and the inhibition is significant.

Effect of *Guiera senegalensis* methanolic extract on isolated rat uterus preparation

Non-contracting rat uterus preparation: Slight contraction on rat uterus preparation was shown by

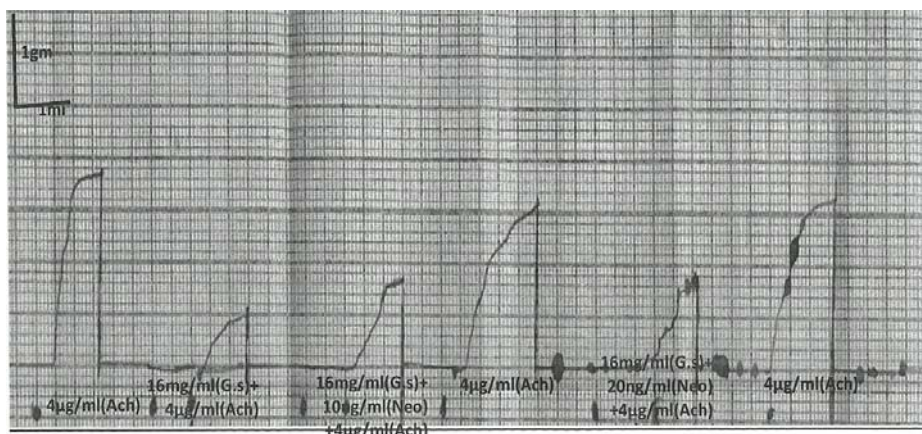


Fig. 7: The effect of neostigmine on the antagonistic effect of *Guiera senegalensis* on acetylcholine-induced contracture on isolated toad rectus abdominis muscle preparation

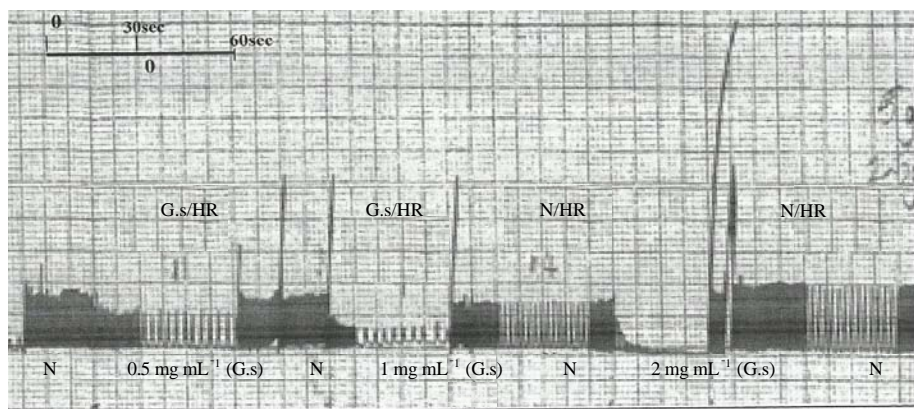


Fig. 8: The effect of *Guiera senegalensis* (G.s) methanolic extract on isolated Guinea pig atrium; F.S/HR = G.s-induced inhibition on heart rat; N/HR-normal heart rat

Table 1: The effect of *Guiera senegalensis* (G.s) methanolic extract on isolated Guinea pig atrium

Doses (mg mL ⁻¹)	Contractility (g)		Rate/min	
	Treated	Control	Treated	Control
0.5	0.09±0.0150*	0.32±0.06	224±21.1*	336±13.9
1	0.06±0.0200*	0.32±0.06	200±8.0*	336±13.9
2	0.013±0.007*	0.32±0.06	200±8.0*	336±13.9

Data were expressed on mean±Standard Error of mean; *p≤0.05

Guiera senegalensis methanolic extract (1-2 mg mL⁻¹). This activity was reduced when the dose of the extract increased up to (4 mg mL⁻¹) as shown in Fig. 10.

Isolated contracting rat uterus preparation: *Guiera senegalensis* methanolic extract did not affect spontaneously contracting rat uterus (Fig. 11).

The effect of cyproheptadine on the extract-induced contraction on isolated rat uterus preparation:

Cyproheptadine (2 µg mL⁻¹) blocked completely *Guiera senegalensis* induced contraction on isolated non-contracting rat uterus preparation (Fig. 12).

Based on previous study by Ashraf (2004), the methanolic extract of *Guiera senegalensis* as most effective antimicrobial extracts.

The plant methanolic extract was prepared and screened on isolated tissue preparation to reveal its other pharmacological activities and to suggest other phytochemical tests that should be performed, as step forward for its formulation in acceptable dosage form and incorporation in modern medicine.

The methanolic extract of *Guiera senegalensis* exhibited a dose dependent two different effects on rabbit jejunum. In a dose up to 2 mg mL⁻¹ contracted the jejunum. Further doses of the extract 4-8 mg mL⁻¹ inhibited the jejunum. The muscarinic blocker atropine blocked the stimulants effect with subsequent significant

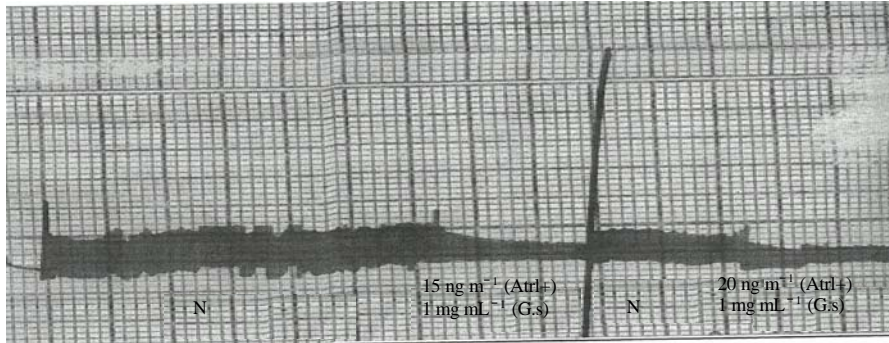


Fig. 9: Effect of atropine sulphate on *Guiera senegalensis* induced inhibition on isolated Guinea pig atrium preparation; Atr = Atropine

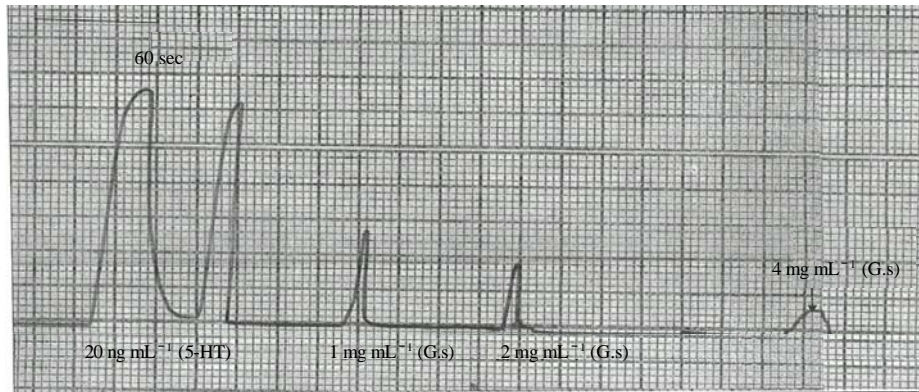


Fig. 10: Effect of *Guiera senegalensis* methanolic extract on isolated non-contracting rat uterus. 5-HT = Sertonin, G.s = *Guiera senegalensis*

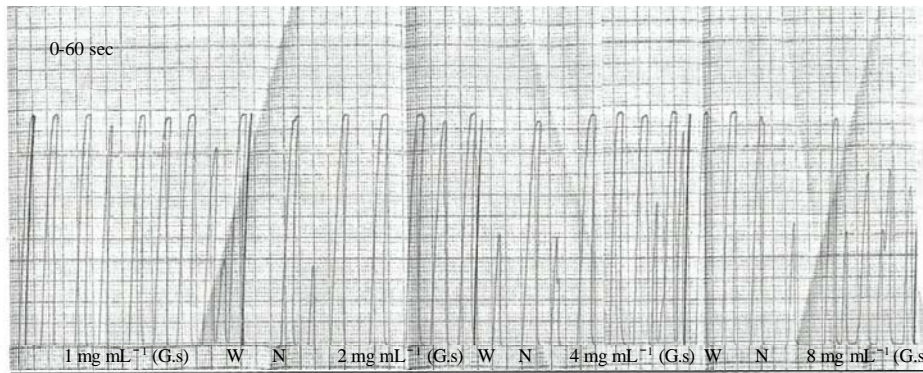


Fig. 11: The effect of *Guiera senegalensis* methanolic extract on isolated contracting rat uterus

relaxant effect. The non-selective β blocker propranolol did not block the extract relaxant effect but partially blocked by α -selective blocker tolazoline. These findings, suggested a weak muscarinic and adrenergic effects that predominated on rabbit jejunum. As mentioned in literature aqueous root extract *Guiera senegalensis* significantly reduced the intestinal transit time in mice and

gastric emptying delayed. This in line with high dose dependent effect on intestinal motility methanolic leaves extract of *Guiera senegalensis*. However, neither cholinergic nor the adrenergic effects was shown on the Guinea pig ileum and the isolated rat vas deferens when the innervations is exclusively adrenergic which suggested another component (s) of the extract that mask

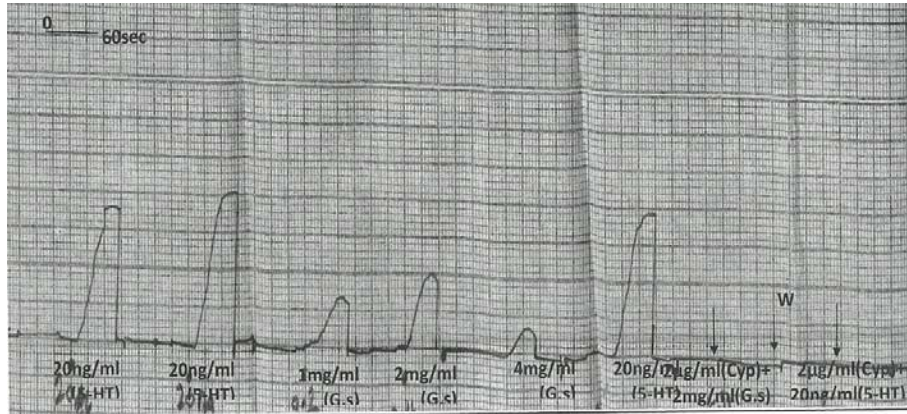


Fig. 12: The effect of cyprohepatadine on *Guiera senegalensis* induce contraction (G.s) on isolated rat uterus; 5-HT = Serotonine; Cypr = Cyproheptadine

the mentioned stimulants effects on the two smooth muscles (Kitchen, 1984). The extract was without stimulants or inhibitory activities on toad rectus abdominis preparation but it completely blocked acetylcholine induced contracture on the striated muscle preparation. However, the antagonistic effect of the extract on the muscle was reversed by the anticholinesterase, neostigmine (20 ng mL⁻¹) which suggested a non-depolarizing antagonistic action at the neuromuscular junction. This result encourages further investigations oriented towards isolation of a muscle relaxant component that might be of clinical value as pre-anaesthetic medication, since increased muscle tone is disadvantages during surgery and increases the metabolic demands which interferes with surgical procedures (Davies *et al.*, 2011). Similar research was carried by Roy *et al.* (2005) who reported the skeletal muscle relaxant effect of the alcoholic extract of *Chonemorpha macrophylla*, the effects were similar to the effects of succinylcholine but different from those of gallamine. Thus the *Guiera senegalensis* may inhibit the somatic nicotinic receptors. Also, the ethyl acetate Extract of *Spigella anthelmia* (EASa) with validated anthelmintic activity was evaluated for its acute toxicity and general effects in albino Swiss mice and for the neuromuscular relaxant activity in the frog sciaticgastrocnemius and rectus abdominis preparations. The extract induced a dose-related myotonia and muscular paralysis of rapid onset at higher doses (Fennell *et al.*, 2004).

Guiera senegalensis methanolic extract exhibited a dose dependent inhibition on force and rate of contraction on Guinea pig atrium preparation which refractory to atropine. This indicated that the extract inhibitory effect on the myocardium was not through the M2-receptor of the cardiac muscle and possibly by the

effect of another component of the extract that of direct myocardial depressing effect. Although, the extract showed cholinergic effect on rabbit jejunum but probably due to a selective effect on the muscarinic receptor M3-subtype of the intestinal muscle. The plants of a myocardial depressing effect are abundantly mentioned in literature.

Tetrandrine is one of the best characterized Ca²⁺ channel blocker of plant origin which is used as antihypertensive agent and was studied on guinea pig atrium which inhibited both contractility and rate of contraction this effect related with its blockage on L-type calcium channels (Yao and Jiang, 2002). Also, it has been reported that *Averrhoa carambola* folklorically used as anti-diabetes and anti-vomiting, in Guinea pig atrium its extract the contractile force in a concentration-dependent manner. Also, its promoted a negative chronotropic effect was antagonized by isoproterenol bitartrate (Vasconcelos *et al.*, 2005).

The extract slightly contracted the isolated non contracting rat uterus and the rat fundus strip. The effect of the extract on both preparations was completely blocked by the 5-HT antagonist cyproheptadine (Fig. 3). This blockade indicates that the extract may has a 5-HT like activity. Other plants were studied due to their 5-HT property such as the anti-stress action of the essential oils of lavender, rose and lemon. Lemon oil had the strongest antistress effect in three behavioral tasks, Elevated Plus-Maze task (EPM), a Forced Swimming Task (FST) and an Open Field Task (OFT) in mice (Komiya *et al.*, 2006).

CONCLUSION

In this study, concluded that the methanolic extract of *Guiera senegalensis* possesses non-depolarizing

neuromuscular junction blockers like effect which was confirmed by comparing the extract activity with dtubocurarine acceptability to reverse by anticholinesterase neostigmine on isolated toad rectus abdominis preparation. Also, the extract has a serotonin agonist-like activity which was blocked completely by cyproheptadine (non selective 5-HT blocker) on isolated rat non-contracting uterus preparation. This confirmation of, this activity was done by adding a same dose to isolated rat fundus strip preparation.

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