

ORIGINAL ARTICLE**Effect of Solanum Nigrum on Uterus of Non-gravid Rats****Agoreyo Freddy O.^{1*}, Ohimai B. Rita¹, Omigie Magdalene J.²****OPEN ACCESS**

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ABSTRACT

BACKGROUND: *Solanum nigrum* is a widely used plant in oriental medicine where it is considered to be antioxidant, anti-inflammatory and diuretic. This study aimed to evaluate the effect of *Solanum Nigrum* on uterine contractions.

METHOD: Female Wistar albino rats were used for the study. They were housed in a single large cage in an atmospheric controlled environment. Twenty-four hours before every experiment, 0.2 mg/kg of diethylstilbesterol constituted in 1:1 ethanol/water solution, was administered intraperitoneally as a pre-treatment to the rats to induce oestrus. It was done for two weeks during the study period. The phytochemical analysis was carried out to test for the phytochemical constituent of the plant.

RESULT: The result showed that the extract inhibited the release of intracellular calcium ion. The effect of acetylcholine was significantly inhibited by the extract that is at 200mg/ml ($p>0.05$) and 300 mg/ml ($p<0.01$). The highest mean inhibitory effect of the extract observed on acetylcholine induced contractions was 90.54 ± 1.15 . The oxytocin induced contraction was significantly inhibited by the extract at 200 mg/ml ($p<0.05$) and at 300 mg/ml ($p<0.001$) doses respectively. The highest mean inhibitory effect of the extract observed on Oxytocin induced contractions was 41.10 ± 1.02 and was significantly stronger compared with acetylcholine induced contractions.

CONCLUSION: The aqueous extract of *Solanum nigrum* inhibited the activity of oxytocin on the uterus, and it may possess relaxant activity.

KEYWORDS: *Solanum Nigrum*, Oxytocin, Contraction, diethylstilbesterol

INTRODUCTION

Herbs have been reported to be widely used in traditional medicine (1). According to WHO, most traditional medicine practitioners live and work at the community level, which makes their treatment available and affordable to most people (2). *Solanum nigrum* is a medicinal plant well known for its therapeutic properties. It is also used for reclaiming degraded land (3). *S. nigrum* has also been extensively used traditionally to treat various ailments such as pain, inflammation and fever (4, 5). Phytochemical investigation of the whole plant shows that it contains alkaloids, flavonoids, tannins,

saponins, glycosides, proteins, carbohydrates, coumarins and phytosterols (6). Also, the fruits have been seen to possess beneficial activities such as antiulcer, antioxidant and antitumor promoting agent in rats (7). The fruit also possesses potential CNS-depressant action (8). It has also been reported that aerial parts of *Solanum nigrum* has antiulcer action (9). The uterus, also commonly known as the womb, is a hollow muscular organ of the female reproductive system that is responsible for the development of the embryo and the fetus during pregnancy. The muscular layer of the uterus (endometrium) is very useful during parturition when the fetus needs to be expelled out of the uterus (10). Drugs like acetylcholine and oxytocin aid its contraction while antagonists inhibit the contraction. The aim of this study was to further investigate the aqueous leave extract of *Solanum nigrum* whose pharmacological properties are little known, scientifically, in uterine contractions.

MATERIALS AND METHODS

Drugs: Oxytocin and Salbutamol were purchased from (Greenfield Pharmaceuticals, China), and atropine from (Martindale, England). All stock solutions of these drugs were made using physiological salt solutions (De jalon's solution) prior to use in in vitro experiments.

Preparation of plant extract: Fresh leave of *solanum nigrum* were collected in Benin, Edo State. The leaves were chopped into little chips and oven-dried at a temperature of 30 °C using a thermostat oven. The dried leaves were reduced to powder using a milling machine. The powder was weighed and 300g of it was macerated in 2 litres of distilled water for 24 hours. The aqueous extract was separated from the mixture using a white sieve, and the extract was concentrated using a water bath. The percentage yield of 20g was obtained.

Organoleptic test: Organoleptic test was done for proper identification of the sample. Properties such as colour, odour, taste and appearance were observed.

Animal treatment: Female Wister albino rats (180-250g) were obtained from the animal house

of the Department of Pharmacology University of Benin, Benin City, Nigeria. They were housed in a single large cage in an environmentally controlled room provided with a 12:12 hours light and a dark cycle for each 24 hours period at a temperature of $26 \pm 1^{\circ}\text{C}$. The animals were allowed to acclimatized for at least two weeks prior to the experiment. The rats were given free access to water and food.

Phytochemical analysis: Qualitative phytochemicals analysis of the crude powder of the *Solanum nigrum* for alkaloid, saponin, tannins, flavonoides and protein. This was carried out using the method of Ogunyemi (11).

The effect of the aqueous extract of *Solanum nigrum* on OXY and ACH induced contractions:

Twenty four hours before every experiment, 0.2 mg/kg of diethylstilbestrol (DES) constituted in 1:1 ethanol/water solution was administered intraperitoneally as a pre treatment to the rats to induce oestrus. On the day of experiment, the rats were anaesthetized by chloroform inhalation in a gas chamber and sacrificed. The lower abdomen was dissected with the aid of a pair of scissors and forceps. The uterus was identified; the two horns were cut out and transferred into a Petri dish containing De-jalon's solution aerated with air. The horns were separated and free from fats and adhering blood vessel. Uterine segment measuring about 1.5 cm in length was cut out and threaded using needle and thread. A loop was formed at one end of the tissue and attached to the tissue holder. The other end was connected to an isometric transducer connected to the unirecorder model 7050 (Ugobasile, Italy). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection with a 0.76 g corresponding weight. The tissue was mounted in a 30 ml organ bath containing continuously aerated De-jalon's solution. The temperature of the organ bath was maintained at 32 °C by means of an external jacket through which warm water circulated, while the tension on the tissue was maintained at 7N. The tissue was allowed to equilibrate for 30 minutes. A preliminary test was done to ascertain the effect of the aqueous extract of *Solanum nigrum* extract on the uterus, and based on this study, the doses of

extract to administer were selected. (200mg/ml and 300mg/ml). A time cycle of 90 minutes and 30 seconds was allowed, 30 seconds of contact time and 90 minutes of relaxation time. The effects of antagonists (Atropine Sulphate and salbutamol) and the extract were investigated against the concentration response curves for ACH and OXY respectively. These blockers were administered separately, each allowed an equilibrium time of 10 minutes before subsequent administration of the extract.

Statistical analysis: Data were presented as mean \pm standard error of mean (SEM). The baseline obtained during equilibrium of 7N tensions was taken as zero centimeters, and the tension increase after the addition of the drugs was calculated with the run-up distance of the zero centimeters baseline.

RESULTS

The results showed that *Solanum nigrum* extracts contains saponins, alkaloids, flavonoids, tanins, phenols and proteins (Table 2). From the

organoleptic test (Table 1), the extract is dark green in colour, water soluble, with a non-characteristic smell and taste. Oxytocin elicited a dose dependent contraction of the uterus (Figure 1); this contraction was significantly inhibited by the extract. This inhibition may be dose-dependent because the level of significance at 300 mg/ml ($p < 0.001$) is higher than that at 200 mg/ml ($p < 0.05$).

Table 1: Organoleptic properties of extract (*Solanum nigrum*)

Characteristics	Features observed
Appearance	coarse powder
Colour	dark-green
Solubility	Water
Odour	Non- characteristic
Taste	Non-characteristic

Figure 2 indicates that the effect of acetylcholine was significantly inhibited by the extract that is at different doses of 200mg/ml ($p < 0.05$) and 300 mg/ml ($p < 0.001$).

Table 2: Result of phytochemical screening

TEST	OBSERVATION	INFERENCE
ALKALOID	It produced an Orange precipitate indicate	Alkaloid present
SAPONIN	Frothing which persists on warning was taken as preliminary evidence of the presence of saponins.	Saponin present
TANIN	Black precipitate indicate the presence	Tanin present
PHENOLS	Black precipitate indicate the presence	Phenols present
FLAVONOIDS	Red colour indicate the presence of Flavonoids,	Flavonoids present
PROTEINS	White precipitate is formed and the precipitate turns brick red after boiling.	Proteins present

*Alkaloid, saponin, tannin, phenols, flavonoids and protein were all present in the extract.

The results presented in Figure 1 show that oxytocin produced a dose dependent contraction of the uterus; this contraction was significantly inhibited by the extract. This inhibition was dose-

dependent such that at 200 mg/ml, and 300 mg/ml, the levels of significance were ($p < 0.05$) and ($p < 0.001$) respectively.

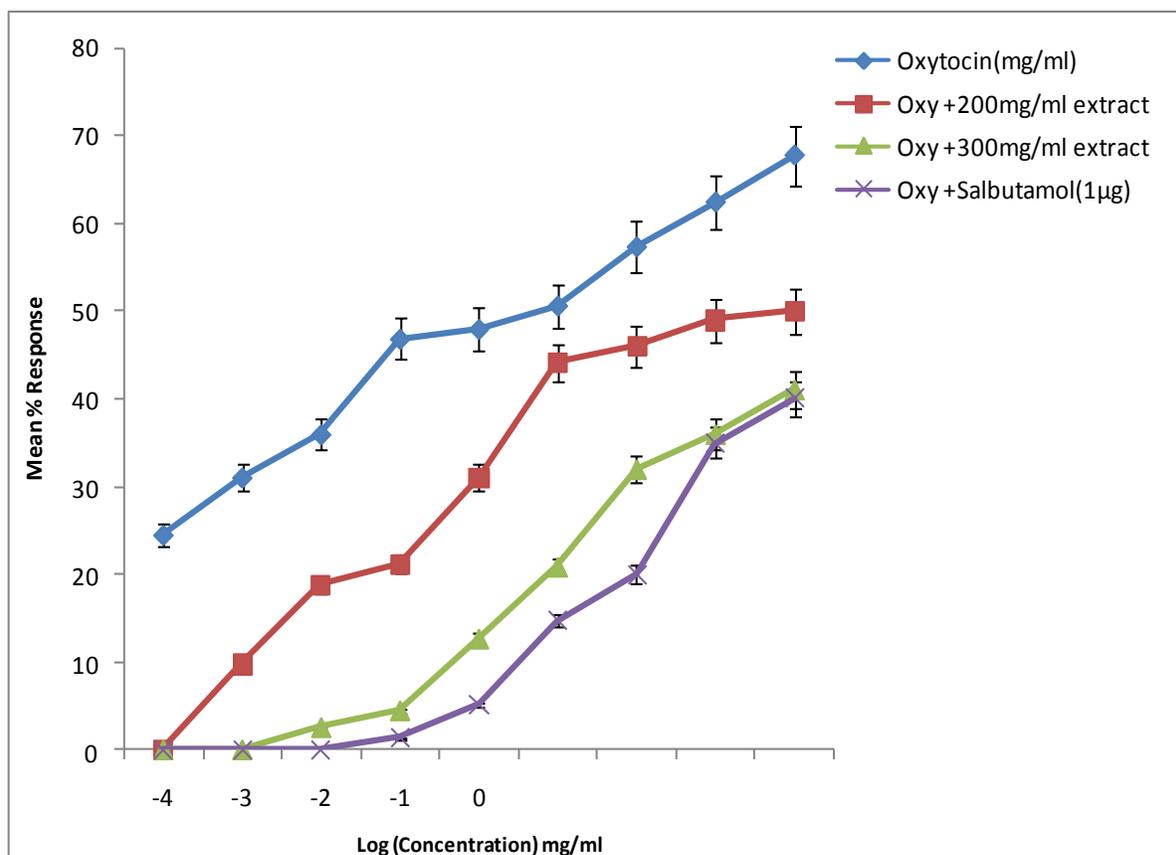


Figure 1: The effect of the aqueous extract of *Solanum nigrum* and salbutamol on oxytocin induced contractions of rat uterus. Values are mean \pm SEM. ($n = 5$ per experiment). Significantly different from oxytocin induced contractions alone

Oxy – oxytocin alone

Oxy + EXT (200) - Oxytocin + 200 mg/ml of extract

Oxy + EXT (300) – Oxytocin +300 mg/ml of extract

Oxy + Sal (1µg) – Oxytocin + Salbutamol (1µg).

The results presented in figure 2 indicate that the effect of acetylcholine was significantly

inhibited by the extract that is at 200mg/ml ($p > 0.05$) and 300 mg/ml ($p < 0.01$).

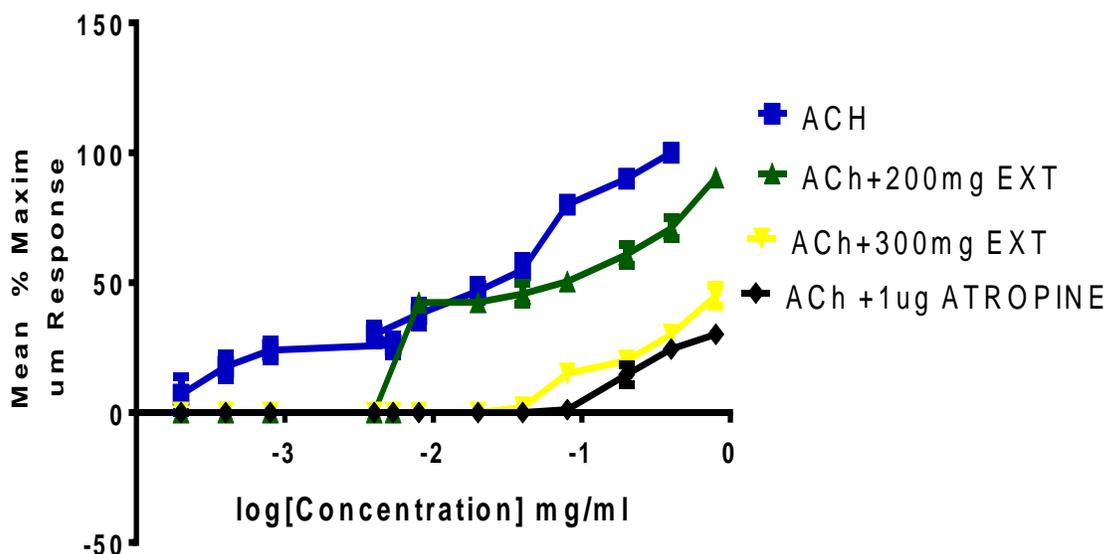


Figure 2: The effect of the aqueous extract of *Solanum nigrum* and atropine on acetylcholine induced contraction of rat uterus

ACh – Acetylcholine alone

ACh + EXT (200) – 200 mg / ml of Extract + Acetylcholine

ACh + EXT (300) – 300 mg / ml of Extract + Acetylcholine

ACh + Atrp (1 μ g) - 1 μ g of Atropine + Acetylcholine

Values are mean \pm SEM. (n = 5 experiments).

Significantly different from acetylcholine induced contractions alone.

DISCUSSION

Smooth muscle inhibition or relaxation by an agent synthetic or non synthetic are mediated by various mechanisms such as potassium channel opening or channel blockade, or by receptor antagonism such as antimuscarinic, antihistaminic, (12) or blockade of adrenoceptors (13). Oxytocin produced a dose-dependent contraction on the uterus; the highest response produced by oxytocin at 0.8 I.U (1.144mg/ml) gives an E_{max} of 40.1 ± 0.43 . The presence of the extract significantly inhibited the contractile effect of oxytocin ($p < 0.001$), and the effect of the extract may be dose-dependent since a higher level of significance is seen at 300 mg/ml ($p < 0.001$) compared to 200 mg/ml ($p < 0.05$). Salbutamol also significantly inhibited the effect of oxytocin. Salbutamol is a known beta-adrenergic stimulant (14). It causes smooth muscle cell relaxation by activating Gs proteins, and their G α s subunit

stimulates adenylyl cyclase. This leads to an increase in the level of cyclic adenosine monophosphate (cAMP), which activates protein kinase A. Both of these changes inactivate myosin light-chain kinase and activate myosin light-chain phosphatase that cause inhibition (15). The inhibitory effect of salbutamol compared with the extract was less which implies that at a high dose, the extract appears to be more potent than salbutamol. Oxytocin binds to specific receptors to increase intracellular calcium (Ca^{2+}) by inhibiting calcium extrusion and suppression of calcium ATPase through the opening of calcium channels and stimulation of inositol -1, 4, 5- triphosphate to release internally stored calcium (16). The action of oxytocin is used to raise intracellular calcium level by facilitating the activation of voltage gated ion channels during the process of excitation. This suggests that the extract inhibits oxytocin receptor directly or indirectly. The effect of the extract and Atropine on acetylcholine induced contractions

were significant ($p < 0.001$). A muscarinic agonist like Acetylcholine binds to its receptors (M2, M3 in the uterus) resulting in the activation of the inositol triphosphate (IP3) and diacylglycerol (DAG) cascade. Both IP3 and DAG have been implicated in the opening of the smooth muscle calcium channels and release of calcium from the endoplasmic and sarcoplasmic reticulum (17). These physiological processes mediate acetylcholine induced uterine contractions. The highest response produced by atropine was at 0.8 mg/ml with an E_{max} of 38.09 ± 1.25 . Atropine is a muscarinic receptor antagonist that inhibits the muscarinic effect of Acetylcholine; which suggests that the mechanism of the inhibitory effect of the extract may be through antimuscarinic receptors. The inhibitory effect of the extract was observed to be more significant on oxytocin induced contractions 41.10 ± 1.02 compared with acetylcholine induced contractions 90.54 ± 1.15 . This implies that the extract has more affinity for oxytocin receptors.

In conclusion, the aqueous extract of *Solanum nigrum* significantly inhibited oxytocin, and Acetylcholine contraction of the uterus of the non-pregnant rat. This effect was more significant in oxytocin contraction than on acetylcholine contraction. Thus, the aqueous extract possesses uterine relaxant activity. The extract of *Solanum nigrum* can thus be synthesized and analyzed therapeutically to establish safe dose for the management of preterm contraction in pregnant women.

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