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Effects of *Cassia alata* Root Extract on Smooth Muscle Activity

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Authors' contributions

This work was carried out in collaboration between all authors. The first stage of this work emanated from the M.Sc. thesis of author TAA. Author TAA designed the study, performed and managed the literature search/experimental process, while authors TAA and IOJ jointly carried out the statistical analysis. Author TAA wrote the protocol and the first draft of the manuscript. Author JA was the main supervisor while author OEO co-supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Ethno Pharmacological Relevance: The root of *Cassia alata* had been preferentially used early this century in the south-south Nigeria, especially in Old Calabar province as an abortifacient by women, for the termination of early pregnancy with apparent success. Some studies and investigations have been carried out on the seeds, leaves, barks and pods of Cassia species and in particular, the seeds, leaves and barks of *Cassia alata* have been studied to a greater extent but a few study had been reported on the pharmacological and toxicology properties of *Cassia alata.* roots.

Aims of the Study: This study investigated the acute toxicity effect (i.p. LD_{50}) of ethanolic

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Cassia alata (RCAE) root extract in albino mice only; also the ethanolic and aqueous roots extracts on smooth muscle activity in rat and rabbit. It also proffer into the possible mechanism of its action by comparism to known standard agonists and antagonists.

Materials and Methods: The acute toxicity effect (i.p. LD_{50}) of *Cassia alata* roots was investigated using forty two (42) Albino mice of both sexes (23-31 g). The mortality in each group was assessed twenty four (24) hours each day, and for three (3) consecutive days adding up to 72 hours after administration of the roots extract. In the second experiment, the rat and rabbit were assessed for the uterine smooth muscle activities using the extract and comparism standard agonists and antagonists.

Results: The LD_{50} of the treated mice intraperitoneally was 263±25 mg/kg. The influence of the ethanolic *Cassia alata* root extract (RCAE) on rat and rabbit smooth muscle preparations exhibited marked dose-dependent spasmodic effect on drug-induced contractions of the gastrointestinal tracts (GIT) and uterus/fallopian smooth muscle preparations tested. The log dose-response curves of Aceteylcholine and Histamine were shifted to the right in the presence of RCAE (8×10^{-4} g/ml) with increasing Kd_{50} (EC_{50}) values, but with decreasing amplitude ($P<0.01$). The stimulatory effect of the ethanolic and aqueous extract of *Cassia alata* root (8×10^{-4} g/ml) on the rabbit and rat GIT and uterus/fallopian tube smooth muscle was not attenuated by atropine (4.8×10^{-6} g/ml), propranolol (1.5×10^{-3} g/ml) or phentolamine (1×10^{-4} g/ml). But preadministration of Aminophylline (5×10^{-3} g/ml) significantly attenuated the contraction of RCAE (8×10^{-4} g/ml) induced spasms on the rat uterus ($P<0.05$-0.01). RCAE also exhibited significant spasmodic effect on the smooth muscle in response to increased Ca^{2+} concentration in a high Ca^{2+} free media. The microscopic examination of the histopathologic effect of RCAE revealed mild to moderate chronic inflammatory reaction on the uterine mucosa.

Conclusion: Our findings in this study indicate that, the ethanolic extract of the root of *Cassia alata* is moderately toxic with a lethality dose (LD_{50}) of 263±25 mg/kg and that *Cassia alata* root may contain pharmacologically active ingredients, which exhibits significant pharmacological contractility effects. *Cassia alata* roots extracts may be pharmacologically more potent than the leaf extract especially on the reproductive tract. *Cassia alata* root may also be a possible plant source of abortifacient and laxative drugs.

Keywords: Cassia alata; lethal dose [LD_{50}]; abortifacient; spasmodic; dose-response; histopathologic; contractility.

1. INTRODUCTION

The root of *Cassia alata* had been preferentially used early this century in the south-south Nigeria, especially in Old Calabar province as an abortifacient by women, for the termination of early pregnancy with apparent success. The crude extract of *Cassia alata* root was often applied as rectal enema to induce abortion. Furthermore*, Cassia alata* leaves and sometime the roots were ground to powder, and then mixed with local alcoholic, drink and then applied on the skin to decorate the skin by women during cultural ceremonies. The skin of the affected area often became inflamed and reddish, and erythematous: The overall effect is the inflammation of the affected skin, (oral communication gathered by Tologbonse [1]).

There are over 33 cassia species (leguminosae caesalpinodeae family) growing in Nigeria, especially in the Southern part. The plant *Cassia alata* is a herb commonly found in cool often grow up to 15 feet tall; and it has green alternate leaves, with even-pinnately leaflets. The flowers are on a long pedicle with yellow colour and bloom from the bottom to the end. They have 4-5 petals [1]. The plant is differently named in different countries. The names of some of the Nigeria Cassia species include *C. alata*, *C. fistula*, *C. hirsute*, *C. occidentalis*, *C. podocarpa*, *C. siamea, C. sieberiena, C. autifolia*, just to mention a few; *Cassia alata* is commonly called 'Udokaya' in South-South Nigeria precisely in Cross River and Akwa Ibom State [1,2]. *Cassia alata* locally known as dadmardan in .Bangladesh and India. Chinese: chi jia jue ming, Spanish: bajagua, mocote. It is also known as Candelbra Bush, or Candle tree in English It is a pan tropical shrub, native to tropical Americas. It is widely distributed from tropical America to India and Bangladesh [3]. Indonesia and Malaysia [4,5], Fiji [6].

Some studies and investigations have been carried out on the seeds, leaves, barks and pods

of Cassia species and in particular, the seeds, leaves and barks of *Cassia alata* have been studied to a greater extent. Kirtikar et al. [3] reported decoction of flowers, bark and wood are used to treat skin diseases such as pruritis, eczema, itching, bronchitis and asthma. The leaf extract has been reported to have antimicrobial activity [7], and there are reports by Bokemo et al. on use in Leprosy, [4] but a few studies had been reported on the roots of *Cassia alata. Cassia alata* is one of the species that is now increasingly being used by herbalists as laxative, abortifacient; and in the treatment of various skin and respiratory diseases. They are also claimed to possess other medicinal properties, hence their use by traditional healer in folk medicine [1,8,9].

The laxative activity of senna and related species has been documented. The antimicrobial properties of most of the Cassia species growing in Nigeria have been studied [9]. According to Nostro et al. [10] medicinal plants play a dominant role in the treatment of human diseases from the twilight of the human civilization. Obsession on modern medicinal system leads people to an alternative approach to improve and maintain good health is increased tremendously by using medicinal herb over last centuries; many of the modern days important drugs and processed medicines are of plant origin [11]. The plant sap can act against microorganisms by preventing the growth of microbial colony [12]. *C. alata* are used for the treatment of uterus disorders as reviewed by Fernand et al. [13,14]. *C. alata* are widely distributed in the tropics [15].

The leaves of *C. alata* have been qualitatively analyzed for the presents of primarily five pharmacologically active anthraquinones: rhein, aloe-emodin, chrysophanol, emodin, and physcion [16], as well as the flavonoid kaempferol [17]. Rhein and chrysophanol are known to be present in the roots. [18], in addition to two other quinone pigments. [19], These anthroquinone derivatives are well known to exhibit a variety of biological activities [20], such as antimicrobial [18], cytotoxic [21], etc.

Rahman evaluated Brine Shrimp Toxicity of leaf and seed extracts of *Cassia* alata linn and their antibacterial potency; seed extract explored potent cytotoxicity similar to that of standard gallic acid (LC_{50} = 4.53 µg mL^{-1}) [4]. Some of the plant extracts also increase lethality of the cells due to their known cytotoxic effect.

Cassia alata has been used mostly as abortifacient and as laxative by Nigerians especially in the Southern part particularly in South-South States without realization of its full potential as a therapeutic agent or it capacity as a toxic compound. On the other hand, so much pharmacological and medicinal research has been carried out on some cassia species leguminosae- caesalpinodeae family. However, a detailed or comprehensive biological evaluations of the different morphorlogical parts especially the roots of *Cassia alata* growing in Nigeria is scanty; particularly, the roots has not been fully investigated; thus, the same level of interest has not been targeted to the *Cassia alata roots*, which has been used locally as abortifacient. Hence, the present study was done to investigate it acute toxicity effects (i.p. LD_{50}) in mice and pharmacological action on smooth muscle tissue in rabbit and rat.

2. MATERIALS AND METHODS

2.1 Plants

Fresh roots of *Cassia alata* were collected from a private farm in Calabar South local government area of Cross River State and also from Itak Ikot-Akap, in Ikono local government area of Akwa Ibom State in the month of August 2010. The plants were identified and authenticated by a taxonomist at the University of Calabar botanical garden as *Cassia alata* by its morphological characters, including numerical value of its stomatal index; it was also confirmed as *Cassia alata* by Dr. (Mrs) M. Bassey of the Department of Botany, Faculty of science, University of Uyo; and deposited at the Herbarium unit of the Department in the faculty of science, university of Uyo, Nigeria with Voucher No: 1059.

Enough quantity of *Cassia alata roots* was dried and grounded to powder. A quantity of the ground sample 100 g was weighed and soaked extracted with 250 ml distilled water at 100°C for 8-10 hours. Also, another 100 g of the dried root powdered was also extracted with ethanol at temperature 40-60°C with about 250 ml of the solvent (ethanol) for 72 hours. On evaporation of the ethanol at 40°C using a rotary vaccum evaporator a brownish semi-solid mass 128 g was obtained. The percentage yield of the extract was 21.3% (w/w yield)

2.1.1 Animals

Wistar mice (23-31 g) of both sexes were use for the lethality study. Twelve (12) female rats (140-200 g) were purchase from the animal house of the department of pharmacology university of Calabar. Also nine female rabbits were bought from farm house of the ministry of agriculture at Ikot Ekpene and then transfer and adapted to the animal house of the department of pharmacology, University of Calabar, Calabar. The animals were all healthy with a weight range of 1.05 kg-1.88 kg. These animals were fed with succulent leguminous leaves plant e.g potato leaves, centrosema and all of them were allowed free access to drinking water. All the animals were kept in a control condition of temperature (22°C), with good ventilation. They were all shielded from direct sunlight, and the environment were kept clean to ensure good sanitary condition; thus, the "principle of laboratory animal care" (National Institute of Health-NIH publication No. 85-23) guidelines and procedures were followed in this study. The Ethical Committee of the postgraduate Committee, college of basic medical sciences, university of Calabar, approved the research work.

2.2 Drugs

Acetylcholine Chloride and histamine diphosphate were obtained from Sigma chemical co. (USA), propranolol from Macclesfield (Great Britain), oxytocin (GEOFMAN), Prostaglandin. The chemicals used were Ethanol (BDA), sodium chloride and glucose (M and B, England), calcium chloride (copharm): magnesium chloride (HOPKIN WILLIAMS, U.K). sodium bicarbonate (Sigma USA). All chemicals were of analytical grade and were dissolved in deionized distilled water at the required concentrations. Methylated spirit (M & B), Chloroform (BOH), Formalin, Stilbesterol were purchased locally and were of analytically grade.

2.3 Equipments

Dissecting set, dissecting board and Ohaus triple beam balance (USA), microchemical (top-loading) balance (USA). Organ bath apparatus (C.F PALMER LTD, ENGLAND), Aerator (Type r. 301. USA), slow-moving kymograph (C.F Palmer LTD England). Microscope (TOKYO, JAPAN), Triple beam balance (New Jersey, USA), Mettler balance P165 and (GALLENKAP , UK). Rotary evaporator (HERDOLPH W. GERMANY)

2.4 Acute Toxicity Test

Forty two (42) wistar mice of both sexes (23-31 g) were used for the study. They were randomly selected and assigned to 7 cages of 6 animals per cage (n=6). They were allowed for a week to adapt to their new environment. Each group then received one of the following doses 100,150, 200, 300, 400, 600 mg/kg body weight of the *Cassia alata* root extract via intraperitoneal route of administration (I.P). The maximum volume injected was 0.3ml from a stock solution of 0.1 g/2 ml (50 mg/ml). the last group, which was used as the control, received 0.3 ml of normal saline intraperitonially. After injection, mice in each group were then returned to their cages, and allowed free access to food and drinking water. The mortality in each groups were assessed 24 hours each day, and for 3 consecutive days adding up to 72 hours, after administration of the extract. Finally, the percentage mortality was converted to probits and plotted against the $\log^{10}$ of the dose of the extract [22] See Fig. 4.1.1.

LD_{50} = 263±25 mgkg

Abscissa scale: shows dose of extract in – logdose scales-3 unit represent -0.1 logdose Ordinate Scale: Response in Probit and the LD_{50} []

LD_{50} = 0.263 km/g, converted Probit 4 =0.182, Probit 6 = 0.355
2S = 0.355-0.182=0.173.
SE $ED_{50} = \frac{0.17}{\sqrt{2\ x\ 24}}$ = 0.025.
Thus LD_{50} = 0.263±0.025 mg^{-1} = 263±25 mg/kg

2.5 Effect of *Cassia alata* Root Extract on Smooth Muscle Uterine Integrity in Non Pregnant/Pregnant Rabbit

Nine (9) rabbits were used for this experiment there were 5 or 4 rabbits in each group respectively. In each group – the rabbits were further divided into 2 subgroups, which are to serve as control group and test group, respectively. In the first group A (with subgroups A1 and A2): the control group A1 with 2 rabbits, while test group - A2, also has 2 rabbits, the control receive no extract, and the uterus/fallopian tube was primed with stillbesterol, while the test group A2 received extract and were also primed. In this group A, all the animals were pregnant. In the second group B, the uteri of the rabbits in both the control group B1, and the test group B2 were primed

with stilbesterol. The control group, was without extract administration but the test groups was administered with extract. All the rabbits were non-pregnant in this second groups and were allowed free access to feed and drinking water. Each rabbit in the test group were administered with the crude extract (170 mg/kg/day/I.P). For 3 consecutive days the control groups were treated with 0.5 ml of stilbesterol only. The animals in group A and B were than sacrifice successfully with their control, after been starved for twelve hours. The pregnant rabbit (group A) were sacrifice on day 10 and 21 respectively (for 1st and 2nd triemester). The uteri were removed and fixed in 10% formalin, and subsequently studied in the laboratory, histologically. See Fig. 4.1.2 and Fig. 4.1.3 respectively.

2.6 Effect of *Cassia alata* Root Extract on Muscle Uterine Contraction in Rat and Rabbit

This involves the preparation of isolated tissues and experimentation using isolated tissue preparations. Adult Male/female rats and rabbits were used for this experiment.

The animals were sacrificed by stunning and exanguinated immediately. The abdominal region of each animal was opened by midline incisions and the jejunum, duodenum were quickly remove and placed in Tyrode solution. The pelvic region was also incised to reach the reproductive structures. Tubular segments of 2-3 cm long were cut from the small intestine and the same length from uterus/fallopian tube. Each piece was suspended in a 25 ml or 10 ml organ bath containing appropriate PSS. For the ileum, the 5 cm portion nearest to the ileocaecal junction was discarded and a suitable length (2-3 cm long) of the remaining portion of the uterus/fallopian tube was also utilized to studied the smooth muscles activity using different agonist and extract. See Fig 4.1.5 and Table 3.

2.6.1 Effect of *Cassia alata* root extract on and smooth muscle contractile responses

Contractile response test to acetycholine, prostagladine were conducted separately before the addition of *Cassia alata root extract.* The initial observations with agonist served as a control values, which were used to compare the effect extract on the agonist – induced contraction for graded dose – response relationships. The effect were observed for 0.5-2 minutes and was followed by 3-5 washings. Other antagonist were also added to the fluid bathing the tissues and the effects were also observed for 0.5-2 minutes and was followed by 3-5 washings. See Fig. 4.1.6 and the result were presented /discused below.

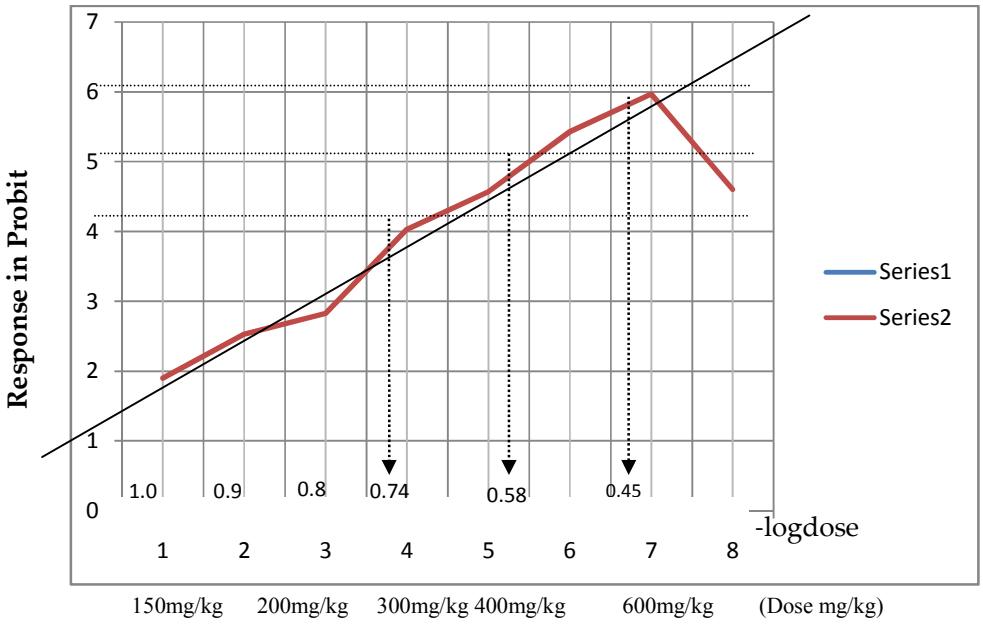

Fig. 4.1.1. Showing the plots of the lethality study in mices using the ethanolic extract of the root of cassia alata

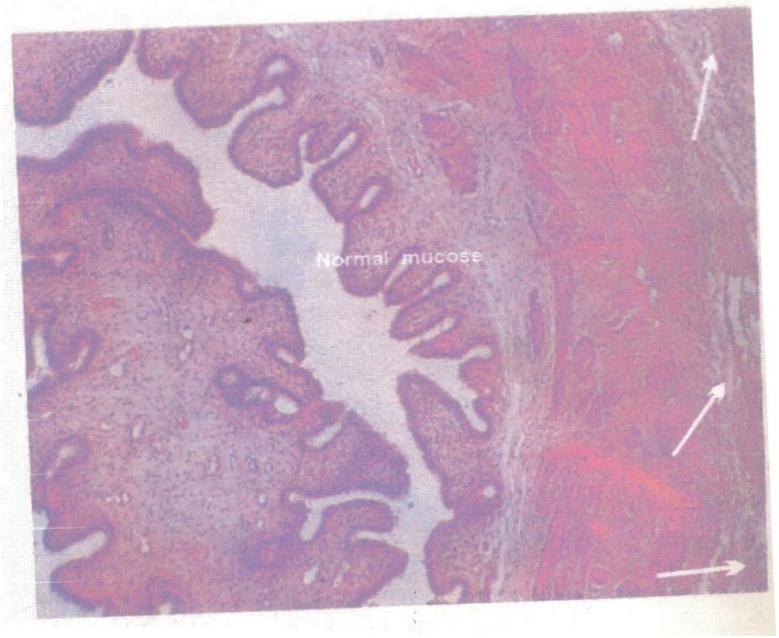

Fig. 4.1.2. Showing photomicrograph of area of mild desidual changes in pregnancy with normal muscle layers seen but the adventitial layer has severe acute inflammation (neutrophils proliferation) in rabbits at first trimester

2.7 Statistical Analysis

The research result were calculated as mean standard error of mean (SEM). Test of significance was done using analysis of variance (ANOVA), students t-test. Values of $P<0.05$ were considered statistically significant.

3. RESULTS

Results were presented as tables and figures and indicated appropriately.

The ethanolic extract of the root of *Cassia alata* shows moderate toxic effects in the wistar mice that were treated with RCAE intraperitoneally for three (3) days over a period of 72 hours. The Lethal dose (LD_{50}) that killed 50% of the treated mice is (263±25 mg/kg). See Fig. 4.1.1 for the calculated LD_{50}.

4. DISCUSSION

The roots of *Cassia alata* are used by herbalists as abortifacient to terminate early pregnancy and in parturition in the South-South Nigeria. The therapeutic effects have mainly been attributed to the pods and leafs and to a lesser extent, the bark and roots [9]. It is worthy of note that the present study was carried out to investigate the effect extract of *Cassia alata* root on smooth muscle activity. It was observed in this study that ethanolic extract of the root of *Cassia alata* was moderately toxic with a lethality dose (LD_{50}) of 263±25 mg/kg), which was neither high nor mild, but moderately low. This closely approximates the result of Pieme, et al on Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of senna alata; the results indicated that the medium lethal dose (LD50) was about 18.50 g/kg of body weight [23]. Lorke [24] stated that substances toxic at less than 1 mg/kg are considered highly toxic; and considering that the LD_{50} estimate of this root extract was fairly above this toxicity level thus, this root extract was moderately toxic. This acute lethality test showed that 50% of the total animal in the test group died after toxic exhibition of reactions which included defecation (watery), inflammation, reduction in motor activity, generalized weakness, some with serious eye inflammation (swollen) and a slight degree of sedation. The percentage death increased with increasing dose of the root extract. This was in agreement with the observation made by Villasenor et al. [25] their pharmacological studies showed that the extracts (*Cassia alata*) caused an immediate decrease in motor activity,

exophthalmus, hyperemia, micturition and diarrhea.

The effect of the root extract on uterine integrity after three (3) consecutive days of intraperitoneal injection of 170 mg/kg/day to the non-pregnant rabbit in the test groups reflect series of abdomalities ranging from mid-chronic endometritis of the uterus to moderate chronic inflammation of the mucosa. One of the tests groups results showed mild to moderate chronic inflammation of the mucosa (fallopian tube) with marked spilling over of chronic inflammatory cells into the Lumen. Close observation of the inflammatory effect of the extract on the non-pregnant uterus/fallopian tube, revealed that the effect is not as profound, when compared to the pregnant uterus/fallopian tube of the tested rabbits of group A2 which abort a fetus on the 20^{th} day before been sacrificed. Other animals in the control group A1 and B1 test group B2 exhibit nil abortion before been sacrificed. Suriname reported that root extracts from *C. alata* are used for the treatment of uterus disorders as reviewed by Fernand et al. [13].

Histology of sectioned tissue after treatment during the first triemester of pregnancy with 100 mg/kg/day/I.P. for 3 days, showed moderate muscosal epithelial necrosis with profound transmural chronic inflammation and oedema; proliferation of lymphocytes and plasma cells in the inflamed region was also marked out as seen in Fig. 4.1.4. The observed results is consistent with the report that white blood cells are the body's defence system; and neutrophils normally form the first line of defence in inflammatory processes, whereas monocytes and other white blood cell components form the second lines of body defence [26,27]. Thus, the present results therefore, proved that the root extract causes lesser damage to the non-pregnant uterus when compared to the pregnant uterus; at late pregnancy (third triemester) because the important organs are already formed, drugs (or extracts) will not cause the gross anatomical defects that occur when they are given in early pregnancy [27,28]. Palmer reported that *Cassia alata* leaf is used In parturition [29].

Furthermore the ethanolic extract (RCAE) and the aqueous extract of *Cassia alata* also shows some pharmacological actions on isolated GIT and reproductive system smooth activities. Administration of low concentration produced significant increase in the height of contraction on already contracting GIT smooth muscles; this was evidence nearly in all the test responses of RCAE on the GIT smooth muscle and in the reproductive systems isolated tissue (See Tables 1, 2 and 3).

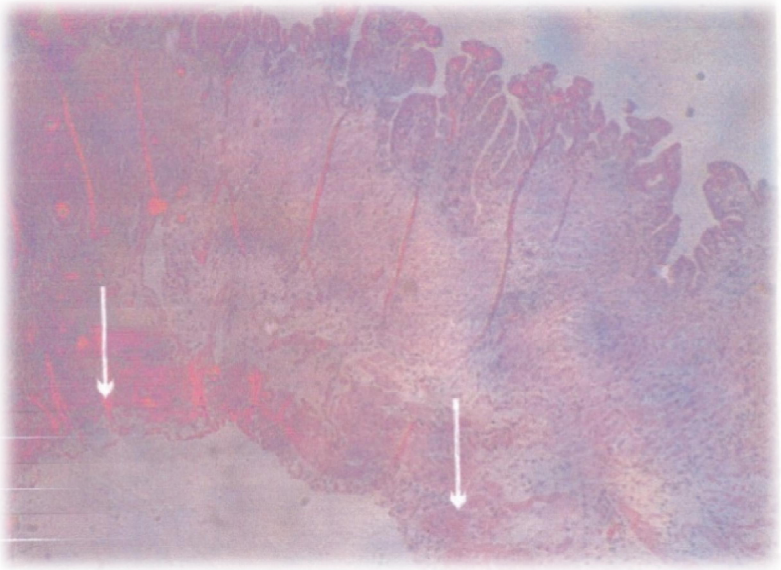

Fig. 4.1.3. Photomicrograph showing mild endometritis and mild to moderate chronic inflammation of mucosa in non-pregnant uterus

Table 1. Effect of water extract of *Cassia alata* root on pregnant rat uterus responses of graded concentration of prostaglandin

Control responses				Test responses of H_2O RCAE (G/ml)			
FBC of prostaglandin(g/ml)	-log	*Maximum height (mm)	% of max	FBC of RCAE	-log	* Maximum height (mm)	% of max
$1.6x10^{-10}$	9.8	24.6±0.7	90±2.3	$4x10^{-8}$	7.4	25.2± 0.1	92±0.3
$1.6x10^{-9}$	8.8	26.2±0.5	96±1.5	$8x10^{-7}$	6.1	26.8±0.2	98±0.5
$1.6 x10^{-8}$	7.8	26.4±0.6	96±2.5	$8x10^{-6}$	5.1	27.2±0.1	99±0.6
$1.6 x10^{-7}$	6.8	26.5±0.0	97±0.0	$8x10^{-5}$	4.1	26.9±0.2	98±0.9
$1.6 x10^{-6}$	5.8	26.5±0.2	97±0.5	$8x10^{-4}$	3.1	27.4±0.2	100±0.7

- *Control response: graded concentration of concentration of prostaglandin (mg/ml),*
- *Test response: responses concentration of Cassia alata in g/ml*

* $\overline{X}$ *±SEM of 4 values; maximum height = 27.4±0.2 by ERCAE*

Table 2. Effect of water extract of *Cassia alata* root on rabbit jejunum compared to responses of graded concentration of acetylcholine (ACH)

Control responses				Test responses of H_2O RCAE (g/ml)			
FBC of Ach (M)	-log	* Maximum height (mm)	% of max	FBC of RCAE	-log	* Maximum height (mm)	% of max
$2.2x10^{-9}$	8.7	11.5±0.8	45±3.1	$8x10^{-7}$	6.1	4.8±0.1	19±0.3
$2.2x10^{-8}$	7.7	12.9±0.3	51±1.0	$8x10^{-6}$	5.1	5±0.1	20±0.1
$2.2x10^{-7}$	6.7	12.7±0.4	50±1.6	$8x10^{-5}$	4.1	6±0.1	24±0.0
$2.2x10^{-6}$	5.7	22.5±0.9	88±3.3	$8x10^{-4}$	3.1	14±0.2	55±0.9
$2.2x10^{-5}$	4.7	25.4±0.3	100±1.2	$1.2x10^{-3}$	2.9	15.5±0.2	61±0.8

- *Control response: graded concentration of acetylcholine (M)*
- *Test response: graded concentration of RCAE aqueous extract in g/ml*
- $\overline{X}$ *±SEM of 4 values*
- *FBC = Final Bath concentration*
- *SEM = standard error of mean*

Table 3. Influence of water/ethanolic extracts of RCAE on EC_{50} and E_{max} with or without addition of different agonists in tissue preparation

Tissue	Agonist	Pretreatment with CAE g/ml	EC_{50} (M)	E_{max} %
Rat Jejunum	Acetylcholine ($22x10^{-4}$ – $2.2 x 10^{-5}$)	$8x10^{-4}$ (H_2O Extract)	$2.2 x 10^{-9}$ $1.8 x 10^{-7}$	100 ±1.3% 67±3.4%
Rabbit Jejunum	Acetylcholine ($22x10^{-9}$ – $2.2 x 10^{-5}$ M)	-	$2.0 x 10^{-8}$	100±1.2%
Rabbit Jejunum	Histamine ($22x10^{-8}$–$2.2 x 10^{-4}$ M)	$8 x 10^{-4}$ (H_2O)	$1.2 x 10^{-5}$ $3.2 x 10^{-5}$	100 ±3.3% 86±1.2%
Rabbit Jejunum	CAE (H_2O Extract) ($8x10^{-7}$ – $1.2 x 10^{-3}$ g/ml)	-	$6.8 x 10^{-4}$	61± 0.8%
Rat uterus (non-pregnant)	Oxytocin ($8x10^{-11}$–$8 x 10^{-7}$ g/ml)	-	$4.4 x 10^{-10}$	100±6%
Rat uterus (non-pregnant)	RCAE (Ethanol Extract)	-	$8.8 x 10^{-6}$ g/ml	94±7%
Rat uterus (non-pregnant)	RCAE (H_2O Extract)	-	$6.x 10^{-5}$g/ml	100±22%
Rabbit Jejunum compared to rabbit uterus	RCAE (H_2O Extract)	-	$5.0x 10^{-4}$g/ml	71± 0.4%

EC_{50} = Effective minimum concentration that produces responses in 50% of test or control groups.
E_{max}% = Percentage of maximum height (amplitude) of contraction.
RCAEg/ml = In the presence of Cassia alata extract

The root also possess intrinsic pharmacological action on uterine smooth muscles and could be a useful oxytocics agent like oxytocin in the enhancement of labour during maternal delivery. Anthroquinone derivatives are well known to exhibit a variety of biological activities [20], RCAE also exhibited significant spasmodic effect on contraction of smooth muscle in response to increased ca^{2+} concentration in a high ca^{2+} free media, this also indicated that *Cassia alata* may be acting by interfering with calcium ion mobilization during smooth muscle depolarization and contraction. The mechanism of action and the active ingredients in *Cassia alata* responsible for some of its effects may be related to the alkaloid content. Alkaloids are known to stimulate specific receptors present in the GIT smooth muscles [30]. The availability of calcium ion is a basic determinant for smooth muscle contraction [31,32], The ethanolic root extract induced contraction response at dose range (4×10^{-8} - 4×10^{-4} g/ml) closely approximated that of oxytocin at the dose range (8×10^{-11} - 8×10^{-7} g/ml) on non-pregnant rat uterus.

The aqueous extract in the same dose range of (4×10^{-8} - 8×10^{-4} g/ml) closely approximated the contractile effect of that of prostaglandin (1.6×10^{-10} – $1.6x\ 10^{-6}$ g/ml) on the pregnant rat uterus. *Cassia alata* does not activate cholinergic muscarinic receptor.

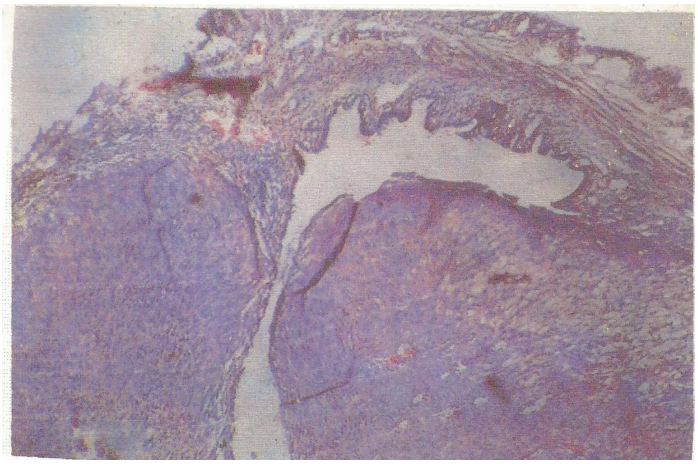

Fig. 4.1.4a

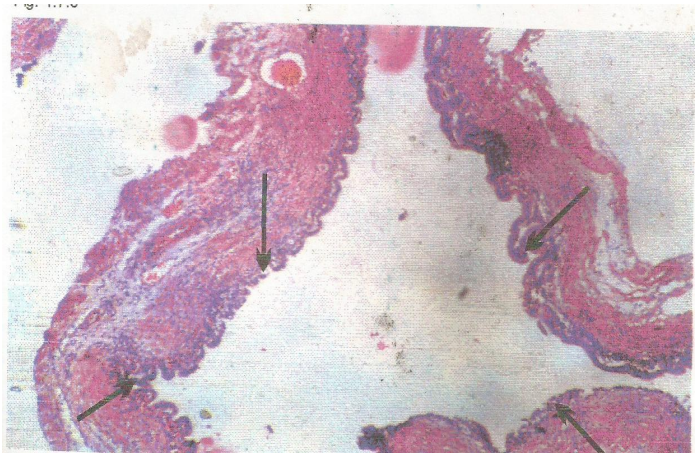

Fig. 4.1.4b

Fig. 4.1.4. 4.1.4a. above shows the photomicrographs of the control normal uterus in pregnant rabbit (first trimester),with changes associated with normal pregnancy; while 4.1.4b with arrows showing the photomicrographs of the area of mild mucosa inflammation with necrosis of innermost tissue in second trimester rabbits, due to toxic effect of the *Cassia alata* extract

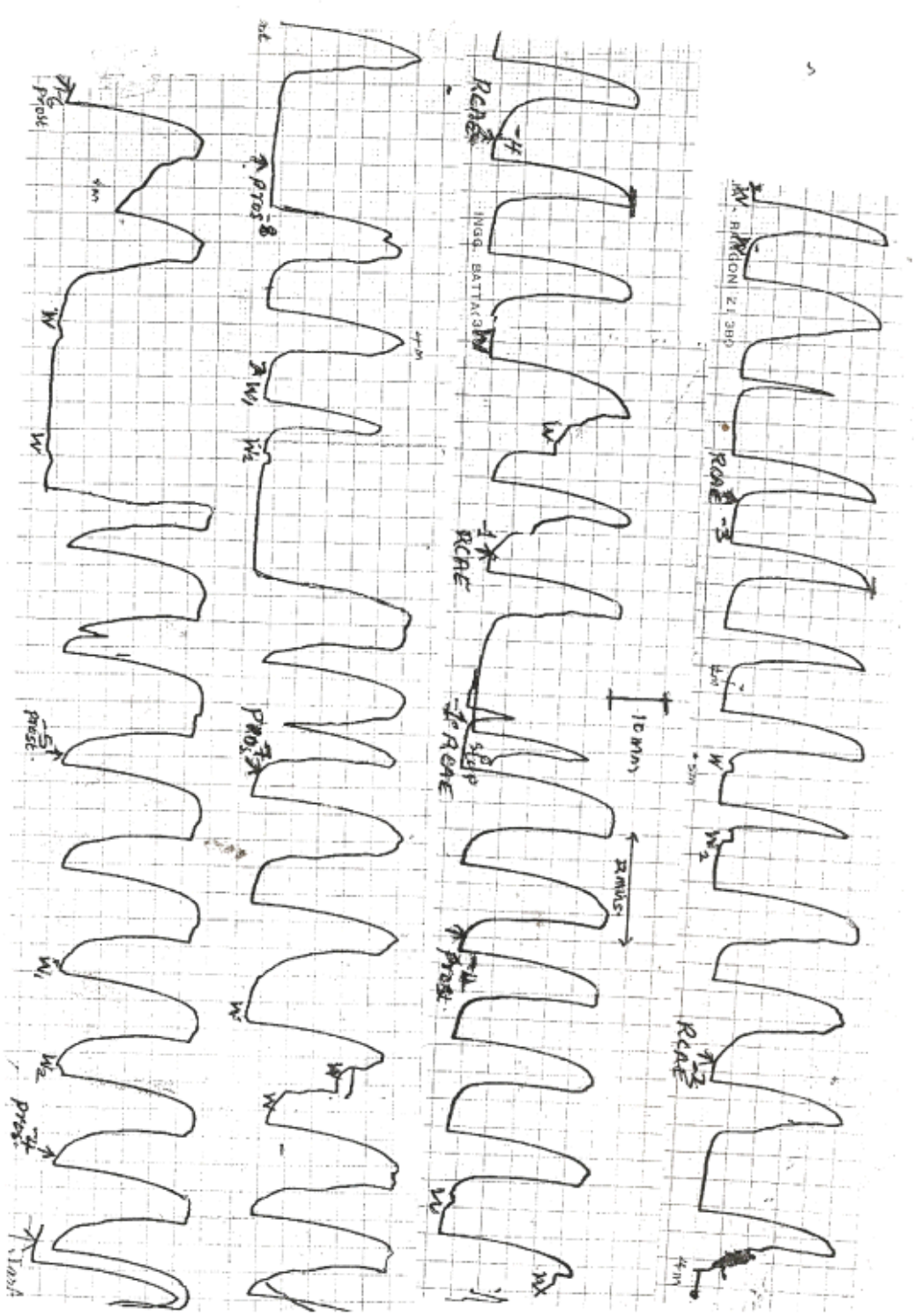

Fig. 4.1.5. Tracing of the effect of root extract (RCAE) in pregnant uterus compared with prostaglandin graded concentration on rat uterus. prostaglandin^{-8} to prostaglandin^{-4} represent (1.6 x 10^{-10} – 1.6 x 10^{-6} g/ml); RCAE^{-4} to $^{-2}$ represent(8 x 10^{-7} to 8 x 10^{-3} g/ml)

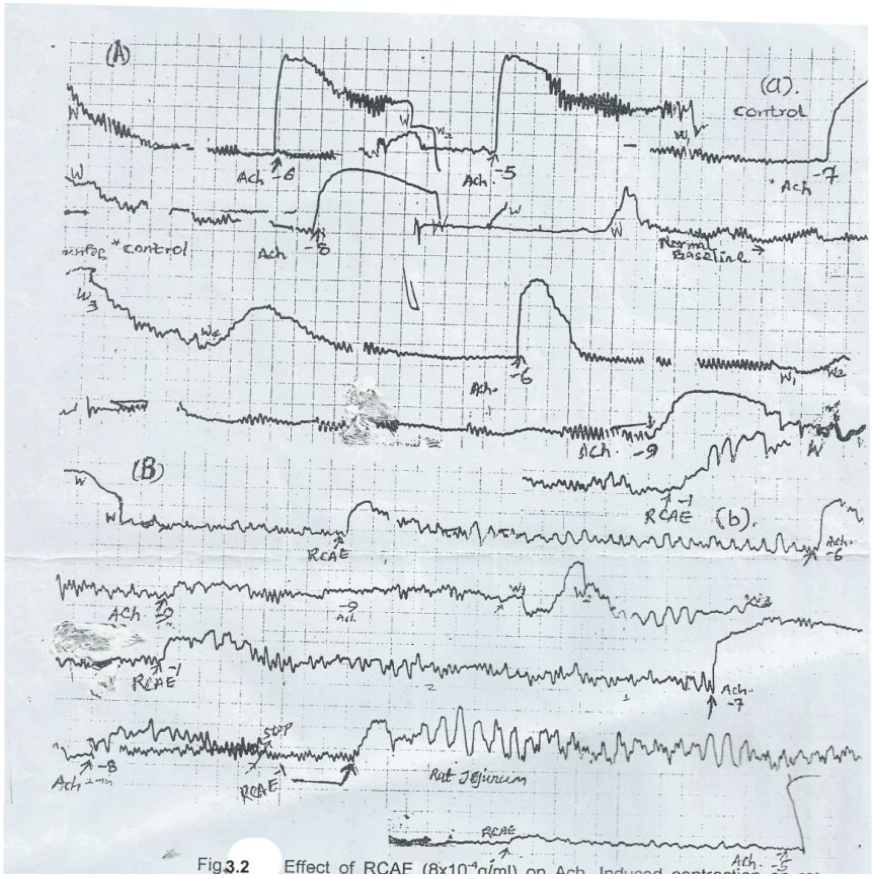

Fig 3.2 Effect of RCAE (8x10^{-4}g/ml) on Ach. Induced contraction on rat jejunum (a) control contractile responses to graded concentration (concs.) of ach. (b) contractile responses to graded concs of ach. On the present of RCAE (8x10^{-4} g/ml). (graphical recordings of some of the experiments. Ach. 5-9 represent exopenous addition of acetylcholine (2.2x10^{-5} -2.2x10^{-9}M) in the ascending order to the bath fluid respectively

5. CONCLUSION

Our finding therefore suggest that *Cassia alata* root extract is moderately toxic with LD_{50} of 263±25 mg/km. The root also possess intrinsic pharmacological action on smooth muscles and could be a useful oxytocics agent like oxytocin and prostaglandin in the enhancement of labour during maternal delivery and could be a possible plant source of necessary medical abortifacient and laxative drugs especially in life threatening situation; the use of *Cassia alata* in parturition may have a slow but far reaching toxic effect on the reproductive tract and possibly other organ system in man when taken in moderate to high doses.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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