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Evaluation of the oxytocic and haematological effects of leaves of *Carica papaya* Linn (Caricaceae)

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Abstract

The aim of this study is to investigate the oxytocic activity of the methanol leaf extract of *Carica papaya* Linn (Caricaceae) and its effect on haematological parameters. Phytochemical analysis and acute toxicity also studied using standard methods. The *Carica papaya* leaves were extracted with 10 liters of methanol. The *Carica papaya* methanol leaf extract (CPMLE) was given at dose of 100, 200 and 400 mg/kg once daily. The oxytocic effect was done using in-vitro method of isolated rat uterus tissue. Oxytocin and acetylcholine used as standard drugs. The weight and blood samples were taken from animals every seven days for twenty- one days while being fed. Haematological study was done using in-vivo method on pregnant and non-pregnant Swiss albino rat. The studied doses of CPMLE cumulatively applied to the bath-fluid caused dose related, significant increase in baseline tone and induced spontaneous, rhythmic, myogenic contractions of the uterine muscle strips taken from stilbesterol-pretreated, non-pregnant female and pregnant rats. There was significant ($p < 0.05$) increase in mean haemoglobin concentration, red blood cell count and packed cell volume as compared to control receiving distilled water. There was a significant ($p < 0.05$) decrease in the white blood cell count in the test groups relative to that observed in the control group. The preliminary phytochemical analysis showed that CPMLE contained saponins, carbohydrates, alkaloids, glycosides etc. The extract caused no deaths up to 5000 mg/kg showing the extract is relatively safe. In conclusion, the results show that CPMLE do possess oxytocic and hematological effect.

Keywords: *Carica papaya*; Haematological Analysis; Phytochemical Studies; Oxytocic; Acute Toxicity Studies.

1. Introduction

Numerous herbs have been reportedly used historically by women to aid child delivery, stimulate menstrual flow or reduce fertility [1]. Oxytocics can be defined as “any drug that stimulates the smooth muscles of the uterus to contract. The administration of an oxytocic can initiate and enhance rhythmic uterine contraction at any time, but relatively high doses are required for such responses in early pregnancy. Oxytocic agents commonly used include oxytocin, certain prostaglandins, and the ergot alkaloids. These drugs are used to induce or augment labor at term, control postpartum hemorrhage, correct postpartum uterine atony, produce uterine contractions after cesarean section or other uterine surgery, and induce therapeutic abortion.

Oxytocin is normally produced in the hypothalamus and stored in the posterior pituitary gland. It plays a role in intimacy, sexual reproduction of both sexes, and during and after childbirth as well as social bonding. It is released in large amounts after distension of the cervix and uterus during labor and with stimulation of the nipples following childbirth. This helps with birth, maternal bonding, and lactation. Studies have looked at oxytocin's role in various behaviors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors. As a medication, it is

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used to cause contraction of the uterus, which is used to start labour, increase the speed of labor, and to stop bleeding following delivery [2].

Oxytocin is a nonapeptide hormone best known for its role in lactation and parturition. Oxytocin also plays an important physiological role in milk ejection. Stimulation of breasts through suckling and mechanical manipulation induces Oxytocin secretion, causing contraction of the myoepithelium that surrounds areolar channels in the mammary gland. The action forces milk from the alveolar channels into large collecting sinuses where it is available for the suckling infant [3].

The use of herbal medicine to alleviate problems associated with gynaecological conditions of menstruation and menopause, to support health during pregnancy and to facilitate childbirth is common amongst many traditional cultures. Many plants are known to possess oxytocic activity;

Aspilia mossambicensis [4], *Byrsocarpus coccineus* [5], *Citrus hystrix* [6], *Erythroxylum coca* [7], *Monechma ciliatum* [8], *Solanum americanum* [9], *Spondias mombin* [10], *Piper guineense* [11], *Prorocentrum lima* [12], *Montanoa tomentosa* [13].

Carica papaya Linn (Caricaceae) sometimes called pawpaw, is a giant herbaceous plant resembling a tree but not woody. Though the exact area of origin is unknown, the papaya is believed native to Tropical America, perhaps in Southern Mexico and neighbouring Central America. Successful commercial production today is primarily in Hawaii, Tropical Africa, The Philippines, India, Ceylon, Malaysia and Australia, apart from the widespread but smaller scale production in South Africa, and Latin America. In India, papaya is cultivated in Maharashtra, Bengal, Bihar, Haryana, Punjab, Delhi, Andhra Pradesh and Uttar Pradesh [14]. The flesh of the fruit varies from yellow to orange to red, and is thick and juicy, with a central cavity filled with many small black seeds [14].

Carica papaya Linn is one of the valuable plant used for various purposes in medicinal field. Leaves, fruit and seeds of are used as ethno medicine. Applying the lotion of the leaves stops bleeding and shrinks the haemorrhoids [15]. The tender leaves are used as spinach. Leaves are smoked and inhaled in place of tobacco for the relief in asthma. These are believed to be cardiotoxic and also promote sweating hence used in fever also. A fine paste of young leaves (5 - 6 g) is taken internally in severe cases of jaundice. The infusion of tender leaves is used for various urinary complaints and gonorrhoea [16]. Previous scientific investigations have shown that it has lots of activities. It normalizes the pulse rate in fever and acts as diuretic when administered as decoction [17]. It is attributed to exterminate cough and respiratory, liver and spleen related diseases as well as useful in loss of appetite and oedema [18]. Leaves of *Carica papaya* L. are used in severe jaundice [19] to expel guinea worm [20], as a poultice [21]; in fracture healing [22], constipation and indigestion [23]. It has been used to treat oral candidosis, malaria, dengue, yellow fever [24] and as a diuretic in dengue related anaemic [25; 26; 27; 28] etc. The seed extract shows hypoglycemic and hypolipidemic activity as well as antifungal and antibacterial activity. The aqueous seed extract has antihelminthic activity. The unripe seed is nephro-protective. The latex shows uterotonic activity [29].

The aim of this study is to investigate the oxytocic activity of the methanol leaf extract of *Carica papaya* and also observe its effect on the haematological parameters.

2. Material and methods

2.1. Collection and preparation of plant material

The fresh leaves of *Carica papaya* were collected from Hilltop village, Nsukka, Enugu state, Nigeria and identified by Mr. Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development (InterCEDD) Nsukka. The leaves were carefully separated, dried and grounded using Lab mill, serial no 4745, Christy and Norris Ltd, England. The ground leaf was weighed and stored in an air tight container till needed.

2.2. Animal

Adult swiss rats (110-150 g) and mice (13-25 g) of both sexes were obtained from the animal facility of the department of Pharmacology and Toxicology, University of Nigeria, Nsukka. They were housed in metal cages within the facility and allowed free access to standard livestock feed and water ad libitum. Approval for the use of animal subjects was secured from the Animal Research Ethics Committee, University of Nigeria, Nsukka. The animals were handled according to International protocol for handling laboratory animals as documented in the European Community guideline, revised Council Directive, 2010/63/EU.

2.3. Extraction

The powdered material was extracted using the process of cold maceration. About 1200 g of the ground leaves were weighed and soaked with 10 liters of 95% methanol in an air tight flask. The mixture was allowed to stand for 3 days with intermittent agitation to facilitate extraction. At the end of the third day, the sample was filtered using Whatman no.4 filter paper and the extracted material was evaporated using a rotary evaporator. After this procedure, an oily and gummy extract was obtained.

2.4. Phytochemical analysis

The preliminary phytochemical analysis was carried out to detect the presence of the active constituents present in the plant such as alkaloids, flavonoids, saponins, tannins, glycosides etc and was carried out based on procedure by Trease and Evans [30].

2.5. Acute toxicity studies (LD50)

The acute toxicity and lethality (LD50) of the *Carica papaya* methanol leaf extract (CPMLE) in mice (n=12) was estimated using the method described by Lorke [31]. The study was carried out in two stages. In stage one, 9 mice were placed in three groups (n=3) and treated with oral administration of 10, 100 and 1000 mg/kg of CPMLE (suspended in 3% tween 80) respectively and was observed for 24 hours. The number of dead animals and the death pattern in the first stage determines the doses used in the second stage (Lorke, 1983).

In stage two, a different batch of mice (n=1 per group) received 1600, 2900 and 5000 mg/kg of CPMLE respectively and were observed for 24 hours for deaths and the number of deaths were recorded [31].

2.6. Oxytocic studies

The experiment was based on the method of Akah et al., [32] using a pregnant and a non-pregnant Swiss albino rat. The non-pregnant Swiss albino rat was injected with Stilbesterol (0.1mg/kg) intramuscularly 24 hours before the experiment. Both rats were killed. The two uterine horns of the animals were cleaned free from fatty and connective tissues and trimmed. Tubular segments of approximately equal length (2-3 cm) were removed from the uterine horns by cutting of both ends and then transferred to a petri dish containing Tyrode solution. The tissue gotten from the pregnant rat was threaded at the top and the bottom; the bottom thread was attached to the tissue- holder, while the thread is attached to the recording device.

The preparation was subjected to a resting tension of 1.0 g and allowed to equilibrate for 30 – 45 minutes before it was challenged with CPMLE. Graded doses of acetylcholine and oxytocin was administered; 2, 4 and 8 ug for acetylcholine; 1, 2, 4 and 8 ug for oxytocin, to establish their effect. Subsequently, Doses of CPMLE were added to the bath fluid sequentially, and washed out 3-4 times after the maximum responses of the tissues were attained. Distilled water (i.e. the vehicle) in which CPMLE was dissolved was used as control fluid for MLE. Concentrations of bath applied CPMLE were repeated where appropriate and/or regular intervals of 3 – 20 minutes after the last washing. The CPMLE induced responses of the uterine muscle preparations were recorded isometrically by means of the force-displacement transducer. This similar procedure was repeated for the pregnant rat.

2.7. Evaluation of haematological parameters

The determination of the blood sample collection, differential leucocyte count, packed cell volume, haemoglobin concentration, red blood cell count, and white blood cell count was done according to Odoh et al., [33]. Blood collection was through the optical plexus of the rats using a heparinized (plain) haematocrit capillary. The blood sample was placed in an EDTA bottle for storage and collection for experiment.

2.7.1. Differential leucocyte count

A thick blood film was made on a grease free microscope slide and was allowed to dry. The dry blood film was stained with Leishman stain and was washed off after ten minutes and was allowed to air-dry. The prepared slide was viewed in the microscope, while the neutrophils and the lymphocytes were counted and their percentage composition calculated.

2.7.2. Packed cell volume (PCV)

Haematocrit capillary tube was filled with blood by placing one of the open ends of the tube in the blood bottle and tilting it at an angle about 30°. One end of the filled capillary tube was sealed with a plastercine and the tube centrifuged

for 20 minutes at 300 rpm in a haematocrit centrifuge. A haematocrit reader was used to read off the length of the packed cell in percentage

2.7.3. Haemoglobin (Hb) concentration

Sahli haemoglobinometre was used for the determination of haemoglobin concentration. Up to the ten (10) mark of the Sahli tube was 0.1 N HCl placed. With the Sahli blood pipette, 20 μ l of blood was placed into the Sahli tube and was sucked up and down. The mixture was allowed to stand for 5 minutes for the formation of acid haematin. The dark mixture formed was diluted gradually with distilled water till the colour when compared with that in the haemoglobinometre is slightly darker than the standard. The dilution continued till the colour turns exactly and slightly paler than the standard. The volumes of the noted colour change were taken and the average of the of the slightly darker and paler were compared and was ensure that the variation is not more than ± 5 for the average value to be adopted. The average value was applied in the formula below:

$X(Y)/100$ where X is the 14 g Hb in 100ml of blood, Y is the average value of dilutions.

2.7.4. Red blood cell (RBC) count

The following equipment were used for the experiment; the microscope, haemocytometre (counting chamber), red cell pipette, ringer solution and cover slip. Using the dilution pipette with RED mixer from haemocytometer kit, blood was drawn up to the 0.5 mark. Continuing to hold the pipette as horizontal as possible, Ringer's solution diluent was drawn up to the 101 mark (Dilution of 1 to 200). The tip of the pipette was sealed with the finger and shaken well to mix.

Half of the content of the pipette was emptied into a waste container and a small amount of the diluted blood was placed into one chamber of the haemacytometer to just fill the chamber of the haemacytometer. The preparation was allowed to sit for a minute (for cells to settle). The center of the grid was focused with 100x objective and was counted with 400x objective. The count of each five fields (each with 16 smallest squares) with a clicker (fields: top right and left, bottom right and left, center) was noted. Include in the count all cells touching left and bottom sides, ignore cells touching top and right sides. The RBCs per cm^3 was calculated by adding the cells in the 5 groups and multiplying by 10,000 (i.e., add four zeros).

2.7.5. Total white blood cell (WBC) count

Same as in RBC except that the diluting fluid is 1.5 % acetic acid tinted with methyl violet. The pipette is similar but with different graduation. Unlike the RBC, the leukocytes cells in the entire 9 big grid was counted and applied in the formula $(n \times 200/9)$.

2.8. Statistical analysis

Data obtained were analysed using one-way ANOVA using graph pad prism 5.04. Results were presented as mean \pm SEM. Differences between means of treated and control groups were accepted significant at $p < 0.05$, Dunnett's post-hoc LSD test [34].

3. Results

3.1. Percentage yield of the extract

The extraction process yielded 33% of *Carica papaya* methanol leaf extract (CPMLE).

3.2. Phytochemical Constituents of Extract

The phytochemical analysis showed that CPMLE tested positive to saponins, carbohydrate, alkaloids, glycosides, proteins, terpenoids, tannins, resins, oils and steroids. The results were shown in Table 1.

Table 1 Phytochemical analysis of *Carica papaya* leaf extract

Constituents	Relative abundance
Alkaloids	+
Carbohydrate	+
Flavonoids	-
Glycosides	+
Oils	+
Proteins	+
Reducing sugar	-
Resins	+
Saponins	+
Steroids	+
Terpenoids	+
Tannins	+

KEY: - Absent; + Present

3.3. Acute toxicity

The extract caused no deaths in the first 24 hours among the three groups of mice that received 10, 100, 1000 mg/kg. Also no deaths occurred at the end of the next 24 hours with the second set of three groups that received 1600, 2900, 5000 mg/kg. This shows that the extract is relatively safe and has a wide range of ED₅₀. The results were shown in Table 6.

Table 2 Acute toxicity of *Carica papaya* leaf extract

Stage of test	Extract	Dose (mg/kg)	Mortality
1	MLE	10	0/3
		100	0/3
		1000	0/3
2	MLE	1600	0/1
		2900	0/1
		5000	0/1
Control			0/1

3.4. Oxytocic studies

3.4.1. Pregnant models

In the pregnant model, after the viability test, 1, 2, 4, 8, 16 and 32 µg of Oxytocin (an exogenous uterine stimulant) on the pregnant rat uterus tissue gave several dose-related contractions on the kymograph. 1, 2, 4, 8, 16, 32, 64 µg of acetylcholine (a known oxytocic agent) also showed contractions on the kymograph. A 20, 40, 80, 160 and 320 mg of CPMLE showed a spontaneous rhythmic contraction almost immediately as compared with the oxytocic effect of Acetylcholine and Oxytocin. Figures 1-5 illustrates a typical trace obtained.

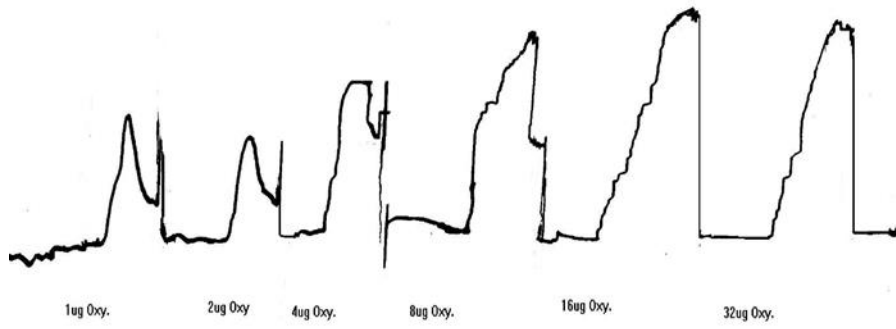


Figure 1 Effect of Oxytocin on rat uterus (pregnant model)



Figure 2 Effect of acetylcholine on rat uterus (pregnant model)

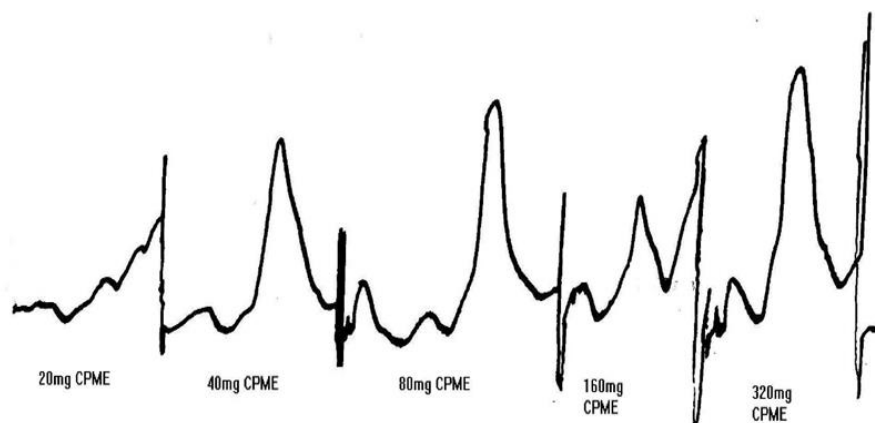


Figure 3 Effect of CPME on rat uterus (pregnant model)

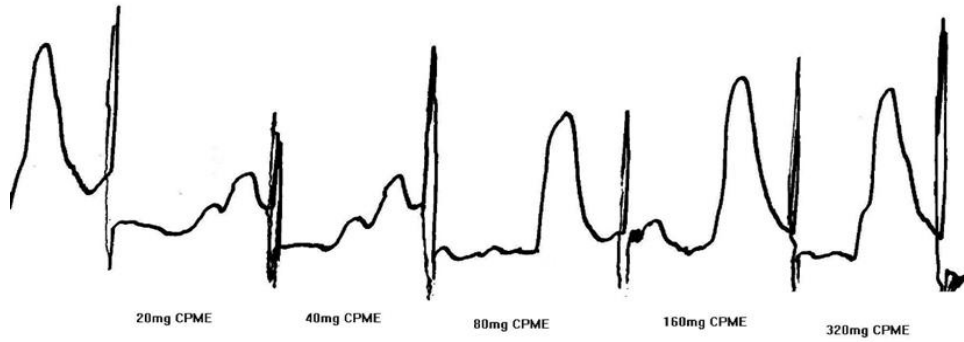


Figure 4 Effect of CPME on rat uterus (pregnant model)

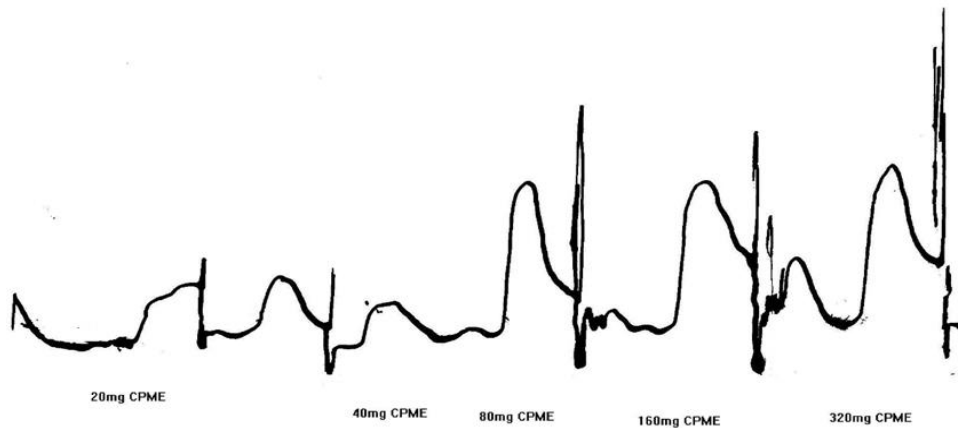


Figure 5 Effect of CPME on rat uterus (pregnant model)

3.4.2. Non-pregnant model

After the viability test, 1, 2, 4 and 8 μ g of Oxytocin and 1, 2, 4 and 8 μ g Acetylcholine gave contractions respectively when applied on the non-pregnant rat uterus tissue. A 20, 40, 80, 160 mg of CPME contracted the uterus tissue. Then a co-administration of 20 mg of CPME and 8 μ g of oxytocin as well as a 20 mg of CPME and 4 μ g of acetylcholine gave a dose-related increase in the basal tone of uterine contractions signifying potentiation. Figures 6-11 illustrates a typical trace obtained.

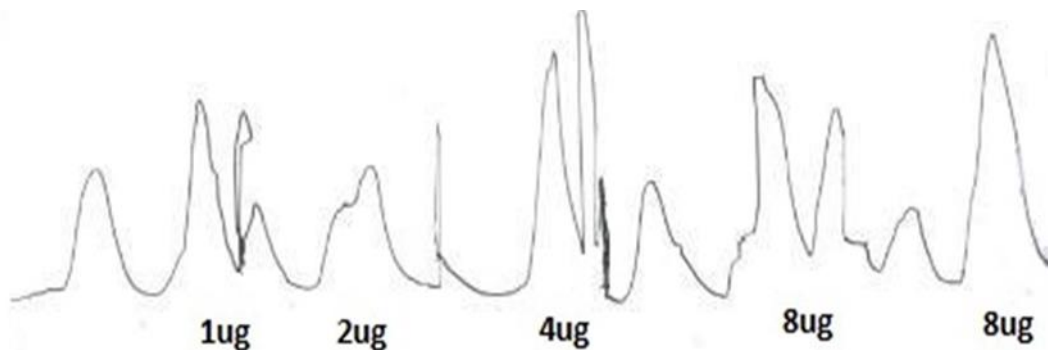


Figure 6 Effect of Oxytocin on rat uterus (non-pregnant model)

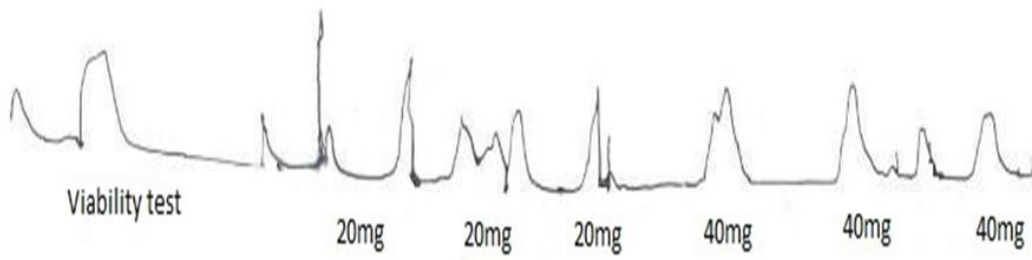


Figure 7 Effect of CPMLE on rat uterus (non-pregnant model)

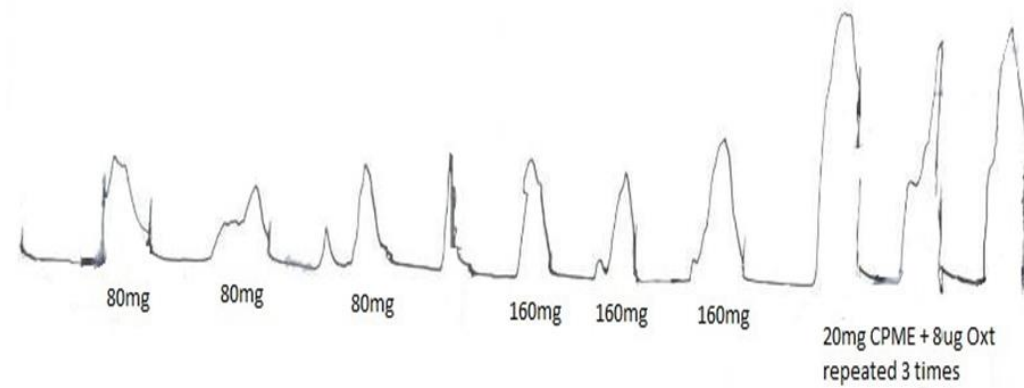


Figure 8 Effect of CPMLE on rat uterus showing potentiation with oxytocin (non-pregnant model)

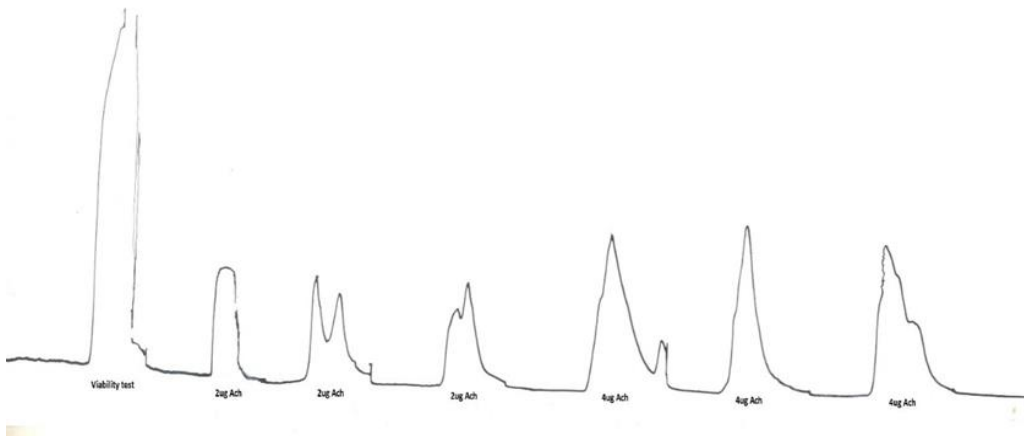


Figure 9 Effect of acetylcholine on rat uterus (Non-pregnant model)

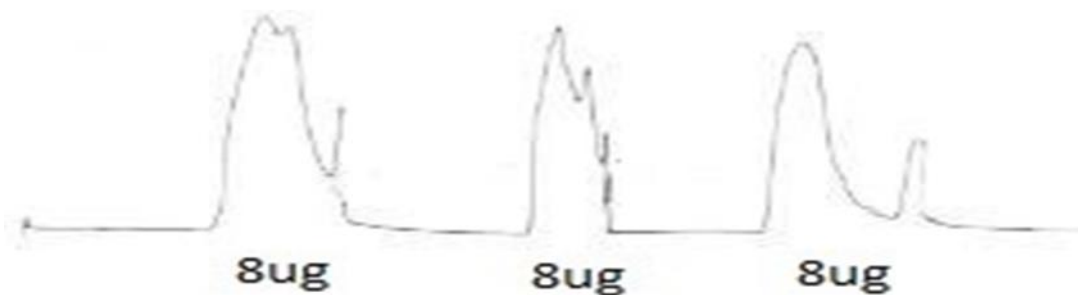


Figure 10 Effect of acetylcholine on rat uterus (Non-pregnant model)

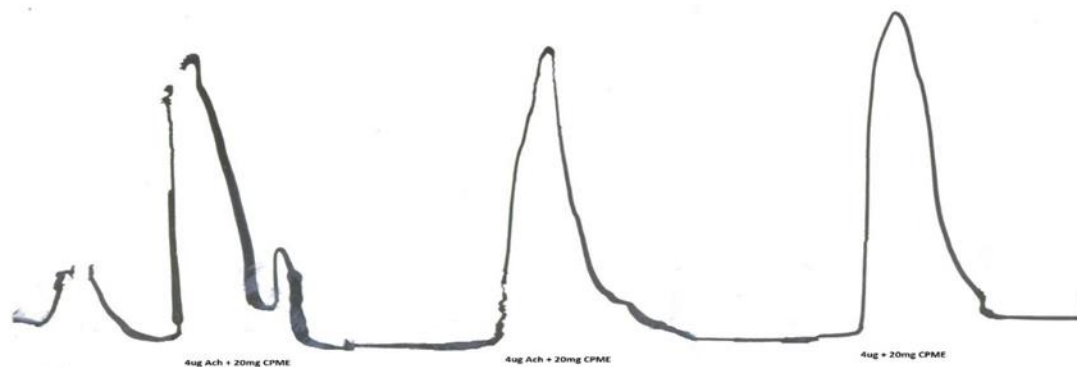


Figure 11 Effect of acetylcholine and CPMLE on rat rterus showing potentiation

3.5. Haematological Analysis

3.5.1. The effect of CPMLE on Haemoglobin Concentration

The results showed that 400 mg/kg of CPMLE increased the haemoglobin concentration significantly on day 7. The dose of 100 and 200 mg/kg maintained a steady increase on day 14; 200 and 400 mg/kg increased the haemoglobin concentration on day 21 (Table 3).

Table 3 Result of the effect of CPMLE on haemoglobin (Hb) concentration (g/dl)

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
CPMLE	100	10.67 ± 0.37	11.00 ± 0.25	15.00 ± 0.20	14.50 ± 0.41
CPMLE	200	10.98 ± 0.49	11.54 ± 0.35	16.52 ± 0.32*	16.02 ± 0.40*
CPMLE	400	10.86 ± 0.51	12.98 ± 0.61*	15.76 ± 0.35*	15.84 ± 0.33*
Distilled water (5 ml/kg)	-	10.74 ± 0.47	10.50 ± 0.45	14.58 ± 0.27	13.96 ± 0.20

Values represented as Mean ± SEM of the results from triplicate analysis, *p<0.05 significant. Comparison with control group; CPMLE = Carica papaya Methanol leaf extract

3.5.2. The effect of CPMLE on PCV

CPMLE at a dose of 400 mg/kg increased the PCV was increased on day 7 while 200 and 400 mg/kg also increased PCV on days 14 and 21 with respect to the negative control (Table 4).

Table 4 Result of the effect of CPMLE on PCV (%)

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
CPMLE	100	31.00 ± 1.00	32.20 ± 0.73	50.75 ± 2.81	42.50 ± 1.26
CPMLE	200	32.20 ± 1.16	33.80 ± 1.02	67.20 ± 3.47*	48.60 ± 1.47*
CPMLE	400	32.00 ± 1.38	38.80 ± 2.44*	59.20 ± 3.68*	48.40 ± 1.29*
Distilled water (5 ml/kg)		31.60 ± 1.29	31.50 ± 0.87	47.75 ± 1.03	41.20 ± 0.97

Values represented as Mean ± SEM of the results from triplicate analysis, *p<0.05 significant. Comparison with control group; CPMLE = Carica papaya Methanol leaf extract

3.5.3. The effect of CPMLE on RBC

CPMLE at a dose of 400 mg/kg increased the RBC count significantly on day 7 while 200 and 400 mg/kg also caused an increase on days 14 and 21 (Table 5).

Table 5 Result of the effect of CPMLE on RBC (*10⁶ cells/mm³)

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
CPMLE	100	3.00 ± 0.34	3.36 ± 0.09	4.58 ± 0.19	4.61 ± 0.24
CPMLE	200	3.21 ± 0.15	3.44 ± 0.17	5.58 ± 0.34*	5.00 ± 0.18*
CPMLE	400	3.16 ± 0.13	3.98 ± 0.27*	5.30 ± 0.20*	5.01 ± 0.15*
Distilled water (5 ml/kg)		3.10 ± 0.15	3.10 ± 0.12	4.39 ± 0.11	4.13 ± 0.06

3.5.4. The effect of CPMLE on WBC

The results showed that there was an increase in the WBC count with the dose of 400mg/kg of CPMLE on day 7. Also there was a significant reduction in WBC count with the dose of 200mg/kg on day 14 before returning to normal on day 21 (Table 6).

Table 6 Result of the effect of CPMLE on WBC (*10⁶ cells/mm³)

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
CPMLE	100	5878.4 ± 220.85	6708.0 ± 177.49	5572.5 ± 340.69	5745.0 ± 314.89
CPMLE	200	6392.0 ± 424.13	6664.0 ± 192.91	4810.0 ± 145.95*	5136.8 ± 309.68
CPMLE	400	6444.4 ± 335.65	5882.0 ± 158.70*	4956.0 ± 378.12	4836.0 ± 382.02
Distilled water (5 ml/kg)		6688.4 ± 198.44	7195.5 ± 226.69	7308.8 ± 358.85	5644.0 ± 322.89

Values represented as Mean ± SEM of the results from triplicate analysis, *p<0.05 significant. Comparison with control group; CPMLE = Carica papaya Methanol leaf extract

3.5.5. The effect of CPMLE on neutrophils

The results showed an increase in neutrophil count on day 7 with the dose of 100 and 200 mg/kg of CPMLE. It also showed a decrease with the dose of 200 and 400 mg/kg on days 14 and 21 (Table 7).

Table 7 Result of the Effect of CPMLE on neutrophils (%)

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
CPMLE	100	72.80 ± 1.02	72.40 ± 1.50*	64.25 ± 1.31	66.50 ± 1.71
CPMLE	200	73.40 ± 0.98	68.60 ± 1.54*	57.60 ± 2.73*	58.80 ± 1.02*
CPMLE	400	71.80 ± 1.50	42.60 ± 7.08	60.80 ± 1.62*	56.40 ± 1.03*
Distilled water (5 ml/kg)		72.00 ± 1.41	28.25 ± 1.65	70.00 ± 2.00	70.00 ± 0.71

Values represented as Mean ± SEM of the results from triplicate analysis, *p<0.05 significant. Comparison with control group; CPMLE = Carica papaya Methanol leaf extract

3.5.6. Effect of CPMLE on lymphocytes

The results showed a decrease in lymphocyte count on day 7 with the dose of 100 and 200 mg/kg. It also showed an increase with the dose of 200 and 400 mg/kg on days 14 and 21 (Table 8).

Table 8 Result of the effect of CPMLE on lymphocytes (%)

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
CPMLE	100	27.20 ± 1.02	27.60 ± 1.50*	35.75 ± 1.31	33.50 ± 1.71
CPMLE	200	26.60 ± 0.98	31.40 ± 1.54*	42.40 ± 2.73*	41.20 ± 1.02*
CPMLE	400	28.20 ± 1.50	57.40 ± 7.08	39.20 ± 1.62*	43.60 ± 1.03*
Distilled water (5 ml/kg)		28.00 ± 1.41	71.75 ± 1.65	30.00 ± 2.00	30.00 ± 0.71

Values represented as Mean ± SEM of the results from triplicate analysis, *p<0.05 significant. Comparison with control group; CPMLE = Carica papaya Methanol leaf extract

3.6. Effect on the body weight

The body weight of the treatment and control rats were as shown in Table 9. There were gradual increases in body weight of treatment and control rats weekly. The body weight of the treatment rats were significantly different as compared to the control rat. There was a significant increase in body weight with the dose of 100, 200 and 400 mg/kg of CPMLE on day 14 and 21.

Table 9 Result of the effect of CPMLE on body weight

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
CPMLE	100	122.58 ± 4.17	152.43 ± 3.99	157.99 ± 10.55*	162.40 ± 3.86*
CPMLE	200	127.79 ± 5.04	153.22 ± 5.34	153.42 ± 4.63*	150.82 ± 6.84*
CPMLE	400	134.50 ± 5.21	154.00 ± 6.30	158.76 ± 4.95*	161.50 ± 5.30*
Distilled water (5 ml/kg)	-	134.11 ± 2.66	147.10 ± 2.92	133.56 ± 8.35	141.22 ± 2.94

Values represented as Mean ± SEM of the results from triplicate analysis, *p<0.05 significant. Comparison with control group; CPMLE = Carica papaya Methanol leaf extract

4. Discussion

The therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to its phytochemical constituents [35]. The phytochemical analysis showed that CPMLE tested positive to saponins, carbohydrates, alkaloids, glycosides, proteins, terpenoids, tannins, resins, oils and steroids. Due to the

presence of saponins in CPMLE it was found that “Saponins has relationship with sex hormone involved in controlling the onset of labor in women and the subsequent release of milk” [36]. With this fact we can attribute its oxytocic effect to the presence of saponins in the methanol leaf extract.

After the acute toxicity study, the extract caused no deaths in the first 24 hours among the three groups of mice that received 10, 100 and 1000 mg/kg. Also no deaths occurred at the end of the next 24 hours with the second set of three groups that received 1600, 2900, 5000 mg/kg. This shows that the extract is relatively safe and has a wide range of ED50. This is in line with the findings of Halim et al [37]; who reported no mortalities with the aqueous extract. However, there were behavioral changes such as depression, reduced motor activity and ataxia, this could be due to the presence of cyanogenic glycosides in *C. papaya* [38; 39]. These glycosides have been known to have deleterious effects on the brain due to cytotoxic hypoxia, leading to various degrees of nervous signs [40]. This may be related to the folkloric use of the plant as a muscle relaxant and sedative [41], which has been validated [42].

There was a gradual increase in body weight of treatment and control rats weekly. The increase in body weight of the treatment rats was not significantly different from the control rat. The food and water consumption of the treatment rats were also not significantly different as compared to the control rats measured throughout the study. The increase in body weight of all groups weekly were considered normal and gradually as observed in rats of similar age group in a published reference [43]. The increases in body weight were in line with the increase in food and water consumed by the rats.

After the viability test, 1, 2, 4, 8, 16 and 32 µg of Oxytocin (an exogenous uterine stimulant used as the standard drug) was applied to the pregnant rat uterus tissue which gave several dose-related contractions on the kymograph. A 1, 2, 4, 8, 16, 32, 64 µg of acetylcholine (a known oxytocic agent) also showed contractions on the kymograph. A 20, 40, 80, 160 and 320 mg of CPMLE when added showed a spontaneous rhythmic contraction almost immediately as compared with the oxytocic effect of Acetylcholine and Oxytocin.

A 100, 200 and 400 mg/kg dose of CPMLE cumulatively applied to the bath-fluid caused dose related, significant increase in the baseline tone and induced spontaneous, rhythmic, myogenic contractions of the uterine muscle strips taken from stilbesterol-pretreated, non-pregnant female rats. A 200 and 400 mg/kg dose of CPMLE when sequentially added to the bath-fluid provoked contractions of the uterine muscle preparations. Sequentially applied acetylcholine (1, 2, 4 and 8 µg) and oxytocin (1, 2, 4 and 8 µg) also induced dose-dependent, significant contractions of the uterine muscle preparations taken from the non-pregnant rats. A 200–400 mg/kg dose of CPMLE potentiated Acetylcholine and Oxytocin-induced contractions of the isolated uterine muscle strips in a dose-dependent manner. These similar findings justified the use of *Spondias mombin* by traditional birth attendants in labor induction, augmentation, and as postpartum astringent [11].

The observations made during the study of the haematological parameters were quite interesting. There was a significant ($p < 0.05$) increase in mean haemoglobin concentration, red blood cell count and packed cell volume as compared to control receiving distilled water. There was a significant ($p < 0.05$) decrease in the white blood cell count recorded for rats in the test groups relative to that observed in the control group. These results are in agreement with previous observations of increase in platelet count after treatment with leaf extract of *Carica papaya*. *Carica papaya* leaf extract has been found to accelerate the increase in platelet, haemoglobin, PCV and RBC count and shortened the hospitalization period [44].

5. Conclusion

In conclusion, it is clear that the methanol extract of *Carica papaya* leaves induced spontaneous, rhythmic, myogenic contractions of the uterine muscle strips taken from stilbesterol-pretreated, non-pregnant female rats as well as from pregnant rats. It also significantly increased mean haemoglobin concentration, packed cell volume and red blood cell count as compared to the negative control. There was a significant ($p < 0.05$) decrease in the WBC recorded for rats in the test groups relative to that observed in the control group without causing any acute/subacute toxicity. Therefore, we suggest that *Carica papaya* leaf extract can be used as a medicine in labor induction and augmentation. However, this is a preliminary study and more work is needed to isolate and to identify the biologically active ingredients of *Carica papaya* leaves that are responsible for these effects.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors whose names are listed above certify that they have NO affiliations with or involvement in any organization or entity with any financial interest.

Statement of ethical approval

Approval for the use of animal subjects was secured from the Animal Research Ethics Committee, University of Nigeria, Nsukka. The animals were handled according to International protocol for handling laboratory animals as documented in the European Community guideline, revised Council Directive, 2010/63/EU.

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