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CRUDE AQUEOUS LEAVE EXTRACT OF *CARICA PAPAYA LINN* (PAWPAW) REDUCED ANXIETY AND FEAR RELATED BEHAVIOUR IN CD1 MICE

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ABSTRACT

Among many other medicinal uses of the leaves of *Carica papya*, they are dried and smoked either to relieve asthma or as tobacco substitute. The effect of aqueous leave extract of *Carica papya* on anxiety and fear was therefore studied in 20 male CD1 mice (20-30g body wt). Mice were either treated with 0mg/kg (Control, n=10) or 10mg/kg body weight of aqueous leaf-extract of *C. papaya* (p.o.; n=10) for 14 days before testing in the elevated plus maze (EPM), light/dark transition (LD) box and open field (OF). The results in the elevated plus maze showed higher risk taking behaviour such as frequency of entries and percentage time in the open arm for the *C. papaya*-treated group of mice compared to control ($p< 0.01$), whereas the risk assessment behaviour such as frequency of stretch attend postures (SAP), and the displacement reactions such as frequency and duration of grooming were lower in the test groups ($p<0.05$) compared to control. In the light/dark box test, the results followed a similar trend; with decreased SAPs in both light and dark chambers for mice treated with the aqueous leaf-extract of C. papaya, but increase time spent in the light chamber ($p< 0.01$) for the mice treated with *C. papaya* extract. The test group of mice also spent more time in the light region of the box when compared to their control ($p< 0.01$). The open field test showed increased centre square activity ($p< 0.01$) but decreased SAP ($p<0.001$) and grooming ($p< 0.05$). These behaviour in both the EPM, LD box and OF tests indicate that the mice treated with aqueous leaf-extract of *C. papaya* were less anxious and more willing to take risks. Therefore, the aqueous leaf-extract of *Carica papaya* reduced anxiety and fear in the mice.

KEYWORDS: *Carica papaya*, anxiety, mice.

1. INTRODUCTION

Carica papaya commonly known as Pawpaw belongs to the family of *Caricacaea*. It contains many biologically active compounds among which are Papain and Chymopapain (Chinoy,1995). These compounds aid digestion and their levels vary in fruits, latex, leaves and roots. These compounds' quantities also vary with the age and the sex of the plant, with the female and hermaphrodite trees yielding more crude papain. Other compounds found in *Carica papaya* are Alkaloids, Carpaine, Dehydro carpaines, Flavonols, Benzy/ glucosinolate, Tannins (mostly in the leaves), Linalool, Cis- and trans linalool oxide, X – lina lenic acid, Nicotine, Methylbutanoate, X- phellandrene, Butanoic acid, X- terpinene, Gamma- terpinene, 4-terpineol, Terpinolene (in the fruits), Methyl thiocyanate and benzyl isothiocyanate, Papain, Chymopapain 'a' and 'b' (in the latex and exudates) (Franco *et al*, 1993).

Pawpaw leaves have a wide range of uses. In Nigeria, due to the digestive property of papain, papaw leaves are wrapped round meats to tenderize it during cooking (Eckey, 1954). Its fresh fruits are also used for production of concoctions, jam etc. In the West Indies, the leaves are cooked and eaten like spinach, and in Asia, used as tea (Brucher, 1989). The leaves are boiled and drank for eradicating cancer traces, used in treating diarrhoea, asthma, malaria, worms and sores and for preparing soaps and oils (Rector-Page, 1992). The leaves are oestrogenic – promote milk production, facilitate child birth when taken in the third trimester and increase female libido and may enhances menstruation. However, when taken during early pregnancy, it induces abortion, this is due to papain, which dissolves a protein responsible for adhering the newly fertilized egg to the wall of the uterus (Rector-Page, 1992).

Papain is a proteolytic enzyme, having a sulphydric group as an active centre, classified among tiolproteatis which helps digestion, acting locally in a gastrointestinal tract. *C. papaya* extracts are thus also used in problems of dyspeptic type like flatulence and swollen intestine (Leipner, 2000). The fruit juice of *C. papaya* contains anti-hypertensive agents which exhibit mainly alpha-adrenoceptor activity (Eno, *et al,* 2000). Also, *Carica papaya* stimulates the stomach to increase secretions, release Histamine from the body tissue, depresses the Central Nervous System (CNS), and kills some intestinal parasites (Heinemann, 1995) and filarial worms (Ghosh, *et al*, 1998). *C. papaya* leaves are dried and smoked to relieve asthma or used as tobacco substitutes (Rector-Page, 1992).

Daily oral doses of benzene and alcohol extracts (20 mg/kg; 30 days) did not affect body weight, reproductive organ weights, liver weight or kidney function of rats. However, aqueous extracts (1mg/kg; 7-15 days) given orally to female rats caused infertility and irregular oestrous cycle (Chinoy, 1995). Male rats given ethanol seed extracts orally (10 to 50 mg per day for 30, 60 or 90 days) or intramuscularly (0.1 or 1.0 mg per day for 15 or 30 days) had decreased sperm motility (Chinoy, 1997a). Oral doses also decreased testis mass and sperm count in the rat (Chinoy, 1997b). Allergy to the latex could cause Anaphylactic Shock as well as Dermatitis since its latex is irritant (Gupta *et al*, 1990).

In spite of the many research works done on this plant extract, not some much has been done on its effect on neurobehaviour. In some local communities in Cross River State, Nigeria, the leaves are dried and smoked as tobacco substitute, as the young men of these local communities say that it provide kicks. In some other communities in the state however, it is used by herbalist in the management of asthma as smoke from its leaves it is believed to free the airways. This research was aimed at investigating the effect of crude leave extracts of *Carica papaya* on anxiety in mice using models such as the Elevated plus maze, the Light/ Dark transition box and open field test.

2. MATERIALS AND METHODS

2.1 Preparation of extract

Middle aged fresh *C. papaya* leaves were sun dried to crispy form and ground to fine powder. Then, 450g of powder was soaked in 3 litres of distilled water for about 12 hours. The mixture was filtered to using Whatman No. 1 filter paper. The filtrate was evaporated using an electric rotary evaporator at room temperature of about 26^{o}C to a paste, which was refrigerated until required for use. The percentage yield obtained from this process of extraction was 10.24%.

2.2 Animals care and drug administration

Twenty male albino mice (20-30g body weight; 90-120 days old) were obtained from the animal house of the Department of Physiology, University of Calabar, Calabar. The mice were fed normal animal feed (Vital feeds) for growers and exposed to an average 12 hour light/dark cycle. All animals had free access to feed and water. The animals were divided into two groups of 10 animals each; control and test. The control mice received distilled water 0.1ml/10g body weight orally (p.o.) while the test group of mice received 10mg/kg of the *C. papaya* leaf-extract (p.o.). The extract was administered for 14 days before subjecting the animals to behavioural tests in the Elevated plus maze, Light/dark transition box and open field. The dose was chosen based on some earlier studies which showed the leaf-extract of C. papaya to be safe even up to doses of 2g/kg body weight (Ismail *et al*, 2014).

2.3 Behavioural tests protocol

The **Elevated plus maze** used by Bisong et al, (2010) was employed to assess anxiety in the mice. It has a pair of closed arms and open arms, each arm measuring 31cm in length and 5.5cm in width, and a centre square measuring 5cmx5cm. It is elevated from the floor by about 45cm. Each mouse was picked using a plastic holding container to avoid handling stress and placed in the centre square between the open and closed arms, and allowed to explore the maze for 5 minutes. The following behaviour were score: frequency of open arm entries, duration in the open arm, percentage of time spent in the open arms, frequency of stretch attend postures (forward elongation of the head and shoulders followed by retraction to original position), as well as frequency and duration of grooming. The maze was cleaned up with 70% alcohol to reduce olfactory influence (Lister, 1987; Trulas and Skolnick, 1993).

The **light/dark transition box** test adapted by Bisong *et al*, (2017) was also used as one of the tests to assess anxiety. The apparatus is a 45 x 27 x 27 cm box made of plywood and consists of two chambers connected by an opening (7.5 x 7.5 cm) located at floor level in the center of the dividing wall. The small chamber measures 18 x 27 cm and is painted black. The larger chamber measures 27 x 27 cm and is painted white, with bright illumination was provided by a 60 watt table lamp. The floor of both chambers are marked by grid lines which divide it into 9 x 9 cm squares and is covered with transparent glass. Mice were carried into the test room in their home cages and placed in the center of the white chamber facing the opening and were allowed to explore the apparatus for 5 minutes. An observer sitting quietly about 1 m from the apparatus recorded the behaviour of the animals in the box. These included: Transitions (Number of times the mouse crossed between the light and dark chambers), duration in the light chamber, duration in the dark chamber, frequency of rearing, stretch attend postures, grooming. After 5 minutes, mice were removed from the box by the base of their tails and returned to their home cage. The maze was then cleaned with a solution of 70% ethyl alcohol and permitted to dry between tests (Hascoët and Bourini, 1998).

The **open field test** was also used to assess anxiety. It simultaneously measures locomotor behaviour, exploratory and anxiety-like behavioiur (Walsh & Cummins, 1976). The open field apparatus is made of plywood (72 cm×72 cm floor; 36 cm) walls, one of which is transparent for observing the animals. Blue lines drawn on the floor divide the floor into sixteen 18 cm×18 cm squares, and central square (18 cm×18 cm) in the middle clearly marked. Mice were individually introduced to the open field using a small bucket and allowed to explore for 5 minutes for behaviour such as frequency of lines crossed, grooming, centre square activity and stretch attends postures to be scored (Brown eta al, 1999; Bisong et al, 2010).

2.4 Statistical Analysis
Data obtained from the study were analysed using the Student's t- test. Data were presented as means ± standard error of mean. Probability level of P< 0.05 was accepted as significant.

2.5 Ethical approval
Appropriate ethical approval for this research was obtained from the Animal Ethics Committee of the Faculty of Basic Medical Sciences University of Calabar, Calabar Nigeria, with protocol number 014PY20416 dated 14th April, 2016.

3. RESULTS
3.1 Comparison of activities in the Elevated plus maze between mice administered aqueous leave extract of Carica papaya and control mice
The frequency of entry into the open arm was significantly higher in the test group of mice administered *Carica papaya* leave extract compared to control, p < 0.001. The duration in the open arm and the percentage time in the open arm were also, significantly higher in the test group compared to control (p < 0.01). The frequency of stretch attend postures was significantly lower in the test when compared to control (p< 0.05). The frequency and duration of grooming in the closed arm of the maze were lower in the test group compared to control (P<0.001). The summary of these comparisons is shown in Table 1 below.

Table 1: Comparison of activities in the Elevated plus maze test between mice administered aqueous leave extract of *Carica papaya* and control mice.

Behaviour scored	Control group	*C. papaya* group	Level of significance
Frequency of open arm entry (/5minutes)	4.4 ± 0.36	11.7 ± 0.47	0.001
Duration in open arm (seconds)	54.2 ± 7.61	93.7 ± 11.17	0.01
Percentage time in the open arm (%)	18.07 ± 2.54	31.23 ± 3.72	0.01
Stretch attend postures (/5 minutes)	11.5 ± 1.91	7.3 ± 1.02	0.05
Frequency of grooming in the closed arm (/5minutes)	6.2 ± 0.80	1.7 ± 0.39	0.001
Duration of grooming in the closed arm (Seconds)	91.5 ± 16.95	27.06 ± 8.06	0.001

3.2 Comparison of activities in the Light/Dark transition box between mice administered aqueous leave extract of Carica papaya and control mice
Table 2 shows the summary of activities (behaviour scored) in the light/dark transition box test following treatment with the aqueous leaf-extract of C. papaya. The frequency of transitions, frequency of line crosses in the light and dark chambers were significantly lower in the test group of mice when compared to control (p< 0.01). Although the frequency of rearing in the light chamber did not differ, the frequency of rearing for the test group was significantly lower in the dark chamber (p< 0.001). The frequency of stretch attend postures (SAP) was lower for the test group when compared to control both in the light and darks chambers of the apparatus (p< 0.01). The mice treated with the aqueous leaf-extract of C. papaya spent more time in the light chamber of the apparatus when compared to their control counterparts (p< 0.01).

Table 2: Comparison of activities in the Light/dark transition box test between mice administered aqueous leave extract of *Carica papaya* and control mice.

Behaviour scored	Control group	*C. papaya* group	Level of significance
Frequency of transitions (/5minutes)	16.20 ± 1.67	9.80 ± 2.20	P< 0.01
Frequency of line crosses in the light chamber (/5 minutes)	53.20 ± 5.02	28.90 ± 2.42	P< 0.001
Frequency of line crosses in the dark chamber (/5 minutes)	55.60 ± 10.02	23.30 ± 4.61	P< 0.01
Frequency of rearing in the light chamber (/5 minutes)	16.90 ± 2.93	14.3 ± 2.21	NS
Frequency of rearing in the dark chamber (/5 minutes)	25.10 ± 1.88	14.10 ± 2.40	P< 0.001
Frequency of stretch attend postures in the light chamber (SAP, /5minutes)	11.75 ± 1.38	7.30 ± 1.01	P< 0.01
Frequency of stretch attend postures in the dark chamber (SAP, /5minutes)	10.60 ± 1.56	3.80 ± 0.88	P< 0.001
Time spent in light chamber of the LD box (seconds)	132.2 ± 6.37	190.60 ±18.18	P< 0.01

NS – not significant vs control

3.3 Comparison of behaviour in open field test between mice administered aqueous leave extract of Carica papaya and control mice

Fig. 1 showed that the frequency of line crosses did not differ between control (113.7 ± 14.67/5minutes) and the *C. papaya* group (97.9 ±11.52/5minutes). The frequency of stretch attend postures (Fig. 2) was lower in the test group of mice administered *Carica papaya* leave extract (5.3 ±1.71/5min) when compared to control (16.1 ± 2.32/5min; $p < 0.001$). The frequency and duration of grooming (Fig. 3 and Fig. 4) were also lower in the *C. papaya* leaf-extract group when compared to control ($p< 0.05$, $p< 0.01$ respectively). Frequencies of grooming were 7.5 ±1.06 and 4.1 ±0.73 in the control and C. papaya groups respectively. The durations of grooming were 19.3 ±0.17s and 7.2±1.11s respectively for control and the C. papaya group. The frequency of centre square entries (Fig 5: control = 2.1±0.17/5min and C. *papaya* = 5.4±1.01/5min) and duration in the centre square (Fig. 6: control= 3.2±1.01s and *C. papaya* = 8.8±1.24s) were higher in the *C. papaya* group compared to control ($p < 0.01$).

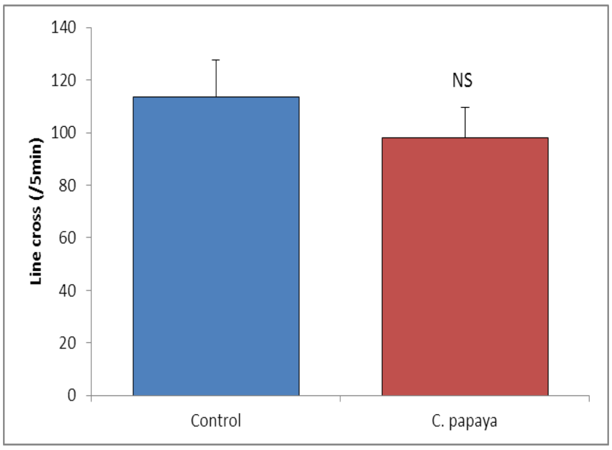

Fig 1: Comparison of frequency of line crosses in open field test between mice administered aqueous leave extract of *Carica papaya* and control. *NS- Not significant versus control*

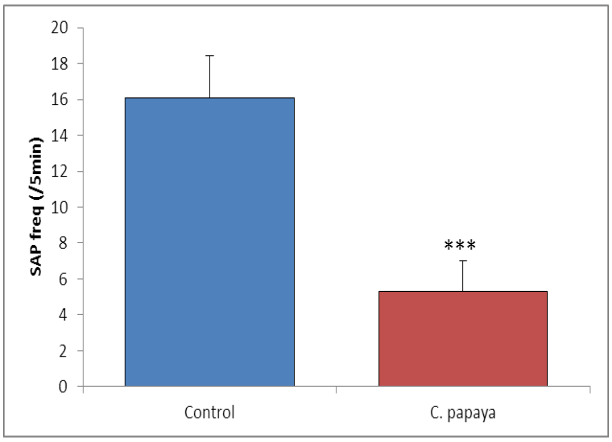

Fig 2: Comparison of frequency of stretch attend postures (SAP) in open field test between mice administered aqueous leave extract of *Carica papaya* and control. *-** *Significant at p< 0.001 versus control*

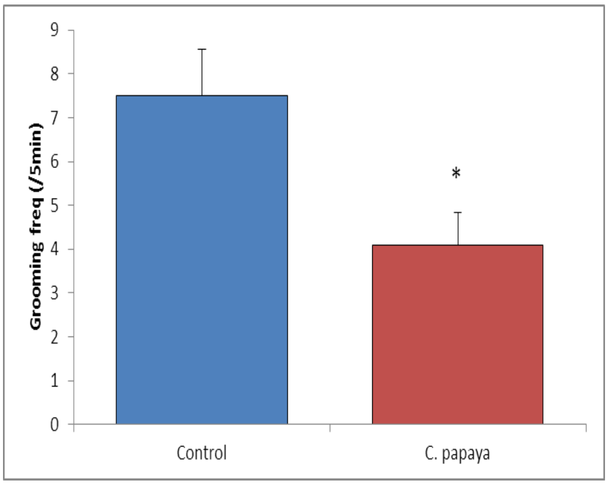

Fig 3: Comparison of frequency of grooming in open field test between mice administered aqueous leave extract of *Carica papaya* and control. *- *Significant at p< 0.05 versus control*

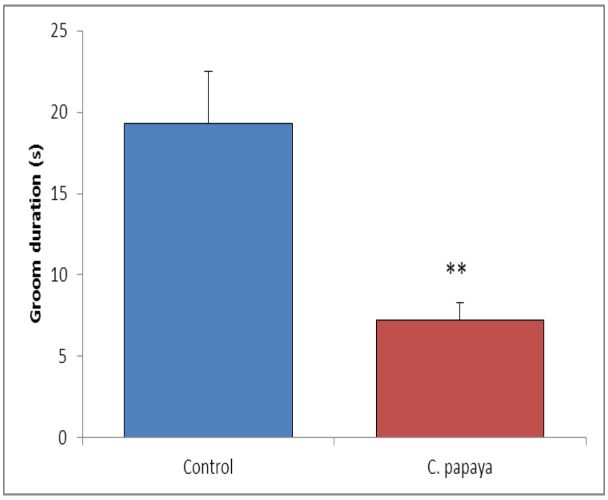

Fig 4: Comparison of duration of grooming in open field test between mice administered aqueous leave extract of *Carica papaya* and control. **- *Significant at p< 0.01 versus control*

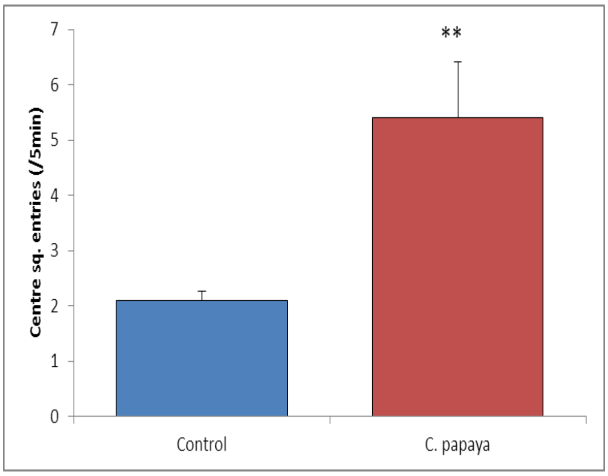

Fig 5: Comparison of frequency of centre square entries in open field test between mice administered aqueous leave extract of *Carica papaya* and control. **- *Significant at p< 0.01 versus control*

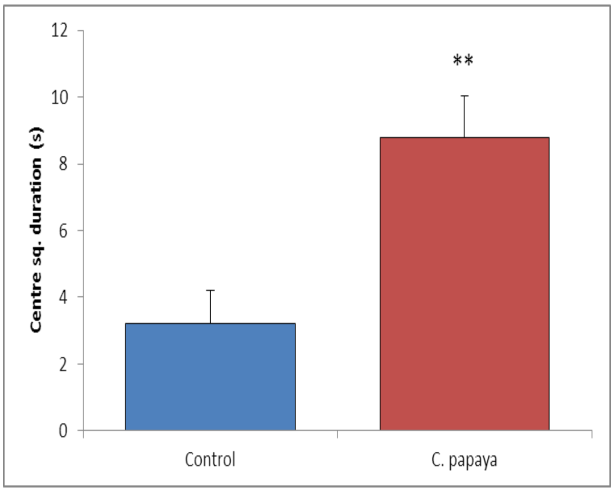

Fig 6: Comparison of duration of centre square entries in open field test between mice administered aqueous leave extract of *Carica papaya* and control. ****-** *Significant at p< 0.01 versus control*

4. DISCUSSION

The elevated plus maze and the light-dark transition box are models used to assess anxiety and fear (Brown et al, 1999). These models are based on the physiological principle that rodents have a natural aversion of for open space and heights. Therefore, when exposed to the elevated plus maze, fearful mice (animals) will avoid the open arm and spend more time in the closed arm of the elevated plus maze and the dark region of the light/dark box (Lister, 1987; Rodgers *et al*, 1993a, Rodgers *et al*, 1993b).

In the Elevated plus maze experiment, the major behaviours used to assess anxiety include risk assessment behaviours like open arm activity, Stretch attend postures and grooming (Lister, 1990). Avoidance of the open arms and increased stretch attends and grooming means increased fear and anxiety. The animals administered aqueous crude extract of *Carica papaya* spent more time in the open arms of the elevated plus maze. This was shown in a higher percentage time in the open arm. These *C. papaya* treated animals also showed lower frequency and duration of grooming and even fewer stretch attend postures. Fewer risk assessment behaviour in these mice indicate decreased anxiety in the mice.

In the Light and Dark box, the risk assessment behaviour, stretch attend postures, in both light and dark chambers were decreased in the mice treated with *C. papaya* extract, ndicating decreased anxiety and fear. Following treatment with *C. papaya* extract, the mice spent more time exploring the illuminated (light) chamber of the box, against the normal observation for mice which is avoidance of open and or well lithe spaces, thus indicating decreased anxiety and fear in this group of mice.

The behaviour which show general activity were however decreased in the *C. papaya* extract group. The transition between the light and dark chambers of the box, frequency of line crossing and rearing in both chamber of the maze were decreased. This indicate a sedative behaviour. However, this sedative activity of the extract did not mask the anti-anxiety effect. This is however likely due to the sedative effect of the extract.

In conclusion, the aqueous leaf-extract of *Carica papaya* decreased anxiety/fear-related behaviour in the elevated plus maze and light/dark transition box tests, therefore it has anxiolytic effect. As expected for drugs that would relieve anxiety, it had a sedative effect.

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