

## Anti-Inflammatory, Anti-Pyretic and Anti-Diarrhoeal Properties of an Anti-Haemorrhoid Tri-Herbal Pill.

\*Joy Okpuzor<sup>1,\*</sup>, Adeola Michael Oloyede.

1. Department of Cell Biology and Genetics, Faculty of science, University of Lagos, Akoka, Lagos, Nigeria

\*Corresponding Author: email- [joyokpuzor@yahoo.com](mailto:joyokpuzor@yahoo.com) ; Phone No: +234 8023065999

### Abstract

A Nigerian tri-herbal decoction branded “Jedi-Jedi Pill” made from *Croton penduliflorus*, *Cassia podocarpa* and *Manihot esculenta*, is widely regarded as effective for treating haemorrhoids and associated diseases. The anti-inflammatory, anti-pyretic, anti-diarrhoeal activities and the overall drug safety of an anti haemorrhoid preparation known as “Jedi-Jedi pill” was investigated using carrageenan induced oedema, 2, 4-Dinitrophenol-induced pyrexia, Castor oil-induced diarrhoea models in rats. Doses of the decoction (300 - 1500mg<sup>-1</sup>kg body weight (b. wt.)), were orally administered to Albino rats (150 – 200g) and Mice (15-30g) of either sex to evaluate side effects and mortality within 72h and to assess drug safety. Paw inflammation was induced in rats hind paw by the subplanter injection of Carrageenan while Oedema was assessed immediately after at intervals of 0 - 6h using the cotton thread method. Pyrexia was induced in the rats by the administration of 10mg<sup>-1</sup>kg b. wt., of 2, 4-Dinitrophenol intraperitoneally while measurement was by inserting a clinical thermometer into their anal cavities for about 2min. Castor oil method was used to induce diarrhoea while adsorbent paper examination was done to determine the presence of wet stool every hour for 4h. Acute toxicity studies produced no mortality but weakness and bloody eyes especially at the highest dose of 1,500 mg<sup>-1</sup>kg b. wt was observed. Anti-inflammatory activity studies showed a dose dependent trend in percentage inhibition which peaked at 900 mg<sup>-1</sup>kg b. wt. This activity was comparable with the reference drug Indomethacin. Activity against hyperthermia was insignificant. The preparation significantly reduced (p<0.05) diarrhoeal frequency in rats at 900 mg<sup>-1</sup>kg b. wt., but this activity was significantly lower than the reference drug. This investigation reports that Jedi-jedi pill possesses anti-inflammatory and anti-diarrhoeal capabilities but lacks anti-pyretic action. Though non toxic, it has some side effects. [Nature and Science 2009;7(8):89-94] (ISSN 1545-0740).

**Keywords:** *Croton penduliflorus*, *Cassia podocarpa*, *Manihot esculenta*, Anti-inflammatory, Anti-pyretic, Tri-herbal and Anti-diarrheal.

### 1. Introduction

Medicinal plants have been of great importance to the healthcare needs of individuals and their communities. The use of herbal preparations made from medicinal plants is widespread in developing countries. In these local communities where medicare is not so easily accessible due in part to lack of healthcare facilities and the high cost of orthodox treatment (Zhu et al., 2002; Okochi et al., 2003), recourse to traditional medicine offers the only hope of staying healthy and alive. The most important bioactive constituents of medicinal plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga et al 2005). Crude drugs obtained from medicinal plants have been used to treat all manner of ailments in most traditional societies.

In African traditional medicine, a mixture of herbs and plants are cooked, macerated or made into tincture to treat different diseases. The tri-herbal

preparation popularly known as “Ogun jedi-jedi” likewise is prepared from *Manihot esculenta*, *Cassia podocarpa* and *Croton penduliflorus*, mucilage and potash is believed to be effective for the treatment of haemorrhoids. The plants from which this preparation was formulated have been used in ethno medicine to treat different ailments. Cassava (*Manihot esculenta* Crantz) serves as a food and an important tuber in the provision of energy for the teeming millions of people from tropical African countries and has been ranked as an important human calorie source, behind rice, sugar cane and maize (FAO, 1995; Siqueira et al., 2007). Adeyemi et al., (2008) reported that cassava tuber is effective against diarrhoea, fever, headache, aches and pains among other medicinal uses. *Cassia podocarpa* and *Croton penduliflorus* have been reported to have laxative properties (Asuzu et al., 1988, Elujoba et al., 1989). Mucilage from cassava was found to be a very good binding agent (Uhumwangho, 2006), and has antioxidant properties

(Fu et al, 2004). Iijima and Ohzeki (2008) had shown from clinical studies that replacement therapy with NaCl and KCl in Congenital chloride diarrheal patient normalized serum electrolytes and only KCl was administered in adolescence.

The present study was undertaken to evaluate the pharmacological properties of ogun jedi-jedi widely sold in motor parks, markets and other public places to a cross section of our untutored population by medicine hawkers. The tri-herbal preparation was made in a crude pill –like form for aesthetic purposes. The efficacy and safety of such herbal preparation is a source of concern to us as scientists working in this field.

## 2. Materials and Methods

### 2.1 Plant materials and Sample preparations:

The tri-herbal formulation locally known as “Jedi-Jedi Pill” was purchased from medicine hawkers at a motor park in Lagos metropolis. They are presented in brownish pill form and weighs approximately 500mg per pill. The formulation consists of 20% *Croton penduliflorus*, 25% *Cassia podocarpa*, 15% *Manihot esculenta*, 20% Potash and 10% Starch mucilage. The pills were dissolved in water and administered to the animals according to their body weights (b. wt.).

### 2.2. Animals

Albino rats (150 – 200g) and Mice (15-30g) of either sex obtained from Biochemistry Department of Nigerian Institute of Medical Research (NIMR) Yaba, Lagos were used for the study. Approval was obtained from the University of Lagos Ethical Committee on the use of animals for research purposes. The rats and mice were fed standard laboratory diet and water *ad libitum*. They were maintained under standard environmental conditions as described by Bishayee and Chanterjee, (1994).

### 2.3. Acute toxicity (LD<sub>50</sub>) study

Acute toxicity study was carried out as reported by Aniagu et al., (2005). This was done orally (p.o) using mice (n=25) and Rats (n=25). The mice and rats were randomly divided into five groups of five of animals respectively. The dose levels used ranged from 300 to 1500mg<sup>-1</sup> kg b. wt. The mice and rats were observed for signs of adverse side-effects and death within 72h after treating them with the preparation. The acute toxicity LD<sub>50</sub> was calculated as the geometric mean of the dose that resulted in 50% mortality.

### 2.4. Anti-inflammatory activity

Paw inflammation was induced in rats hind paw by the subplanter injection of phlogistic agent (Carrageenan) as described by Oloyede et al., (2008) with minor modifications.

The albino rats used for this study were fasted for 12h but allowed access to water. The aqueous preparation dose of 300 to 900mg<sup>-1</sup>kg b. wt., was administered orally to the test group of rats, while indomethacin 25mg<sup>-1</sup>kg b. wt., dosage was administered orally to the reference group (positive control). Distilled water 1 mg<sup>-1</sup>kg b. wt., was orally given to the control group (negative group).

To induce paw oedema, 0.1ml carrageenan diluted in distilled water was injected into the sub-planter region of the right hind paw 1hr after the treatment. Oedema was assessed immediately after carrageenan injection at intervals of 0, 1, 2, 3, 4, 5 and 6h using the cotton thread method described by Bamgbose and Neomesi, (1981). The increase in paw swelling was measured and percentage inhibition was calculated.

### 2.5. Anti-pyretic activity

Anti-pyretic activity of the preparation was carried out using the methods of Berken et al (1991). Rats were weighed and randomized into five groups of five rats per group. The baseline body temperatures of the rats were taken by inserting a clinical thermometer into their anal cavities for 2min. The steady temperature readings obtained were recorded as the pre-treatment temperatures. Pyrexia was induced in the rats by the administration of 10mg<sup>-1</sup>kg b. wt., of 2, 4-Dinitrophenol (DNP) intraperitoneally. Hyperthermia developed 30min later after DNP administration. Different doses of the preparation (ranging between 300 - 900 mg<sup>-1</sup>kg) were given orally, aspirin (100 mg<sup>-1</sup>kg i.p) and distilled water (10 mg<sup>-1</sup>kg b. wt.,) were administered orally to the treatment and control groups of animals. Rectal temperatures were obtained at 1hr interval for 5hr.

### 2.6. Anti-diarrhoeal activity

Anti-diarrhoeal activity of the preparation was evaluated using the castor oil-induced diarrhoeal model in rats (Awouters et al., 1978). Five groups of five rats per group were used for the study. The rats were fasted for 24h prior to the experiment. Distilled water 10 mg<sup>-1</sup>kg b. wt., was given to group I (control group) orally. Group II received 100mg aspirin/kg orally while the other three groups were treated with 300, 600 and 900 mg<sup>-1</sup>kg b. wt., respectively. One hour after the treatment, rats in all the groups were given 1 ml castor oil/100 g<sup>-1</sup> body weight orally. The rats were separated into individual cages having adsorbent paper beneath and examined for the

presence and frequency of wet stool every hour for 4h. Absence or delay in production of watery stool was regarded as protective or positive.

### 2.7. Statistical analysis

Results were expressed as the mean  $\pm$  standard error of mean (S.E.M). Statistical analysis of data was carried out using Student's *t*-test. Differences in mean were considered to be significant when  $p \leq 0.05$ .

## 3. Results

### 3.1. Acute toxicity studies

General weakness, sluggishness and bloody eyes were the major behavioural changes observed in the rats and mice at 1200 and 1500 mg<sup>-1</sup>kg b. wt., doses. No death was recorded at any of the doses administered. Oral LD<sub>50</sub> was therefore not determined because mortality was not observed.

### 3.2. Effect on carrageenan induced inflammation

The effect of the tri-herbal preparation on carrageenan induced rat paw oedema is shown in Table 1. The control animals progressively exhibited increasing paw volume in response to carrageenan injection during the study. The anti inflammatory activity become noticeable after the third hour at doses of 300 and mg<sup>-1</sup>kg b. wt., whereas the same activity became evident at the second hour in that of 900 mg<sup>-1</sup>kg b. wt., The oral administration of 300mg dose did not produce any significant effect but doses of 600 and 900mg of the preparation produced a significant ( $p < 0.05$ ) inhibition of the rat paw oedema. At the sixth hour the sample exhibited a significant anti inflammatory activity at a dose of 900mg when compared to control. The maximum paw oedema percentage inhibition of 73.3% and 93.3% respectively (Table 2) was observed at doses of 600 and 900 mg<sup>-1</sup>kg b. wt., when compared to the control group, but lower than that of Indomethacin.

**Table 1a: Effect of "Jedi-Jedi Pill" on Carrageenan-induced Rat Paw Oedema**

Treatment	Dose	0hr	1hr	2hr	3hr
Control	10ml/10kg	2.4 $\pm$ 0.02	2.54 $\pm$ 0.04	2.62 $\pm$ 0.04	2.78 $\pm$ 0.02
Crude	300mg	2.50 $\pm$ 0.03	2.66 $\pm$ 0.06	2.74 $\pm$ 0.05	2.76 $\pm$ 0.04
	600mg	2.54 $\pm$ 0.02**	2.72 $\pm$ 0.04***	2.82 $\pm$ 0.04**	2.86 $\pm$ 0.02
	900mg	2.56 $\pm$ 0.02**	2.74 $\pm$ 0.05	2.70 $\pm$ 0.04*	2.68 $\pm$ 0.04
Indomethacin	25mg	2.40 $\pm$ 0.03	2.52 $\pm$ 0.04	2.56 $\pm$ 0.02	2.70 $\pm$ 0.03

**Table 1b: Effect of "Jedi-Jedi Pill" on Carrageenan-induced Rat Paw Oedema**

Treatment	Dose	4hr	5hr	6hr
Control	10ml/10kg	2.66 $\pm$ 0.02	2.68 $\pm$ 0.04	2.70 $\pm$ 0.03
Crude	300mg	2.70 $\pm$ 0.05	2.66 $\pm$ 0.04	2.64 $\pm$ 0.05
	600mg	2.76 $\pm$ 0.04	2.68 $\pm$ 0.04	2.62 $\pm$ 0.02
	900mg	2.66 $\pm$ 0.04	2.60 $\pm$ 0.04	2.58 $\pm$ 0.04*
Indomethacin	25mg	2.54 $\pm$ 0.02***	2.48 $\pm$ 0.02***	2.44 $\pm$ 0.04***

Values: Mean  $\pm$  SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  significantly different from control (student's *t*-test)

**Table 2: Percentage Inhibition of "Jedi-Jedi Pill" on Carrageenan-induced Rat Paw Oedema**

Treatment	Dose (mg)	Percentage inhibition						
		1hr	2hr	3hr	4hr	5hr	6hr	
Crude	300	0	0	31.6	23.1	42.9	53.3	
		600	0	0	15.8	15.4	50.0	73.3
		900	0	36.4	68.4	61.5	85.7	93.3
Indomethacin	25	14.3	27.3	21.1	46.2	71.4	86.7	

### 3.3. Anti-pyretic studies

The tri-herbal preparation did not exhibit any anti-pyretic activity at 300-900mg<sup>-1</sup> kg dose. The basal rectal temperature of the control was 37.88  $\pm$  0.45<sup>c</sup> (n=5).Thirty minutes after DNP administration, the mean rectal

temperature of the rats was  $38 \pm 0.21^\circ \text{C}$ . The rectal temperature remained hyperthermic in all the groups throughout the 4hr period. Aspirin (100mg/kg) produced significant ( $p < 0.05$ ) reduction of hyperthermia throughout the study (Table 3).

**Table 3: Effect of “Jedi-Jedi Pill” on D-amphetamine-induced Pyrexia in Rats**

Treatment	Dose (mg/kg)	Baseline Temp	Post-DNP Temp				
			1hr	2hr	3hr	4hr	
Control	10ml/kg	37.9±0.5	38.8±0.2	37.6±0.4	27.8±0.3	37.8±0.3	37.7±0.3
Extract 1	300mg	38.1±0.3	38.4±0.2	38.2±0.3	38.3±0.2	38.3±0.1	38.5±0.1
Extract 2	600	37.3±0.4	38.4±0.2	38.5±0.3	37.3±0.4	38.3±0.2	37.9±0.2
Extract 3	900	38.1±0.2	38.7±0.3	37.7±0.4	37.6±0.3	37.5±0.3	37.4±0.3
Aspirin	100	38.2±0.4	38.3±0.3	39.4±0.2**	39.3±0.2**	39.2±0.2**	38.8±0.2*

### 3.4. Anti-diarrhoeal activity

The 900 mg<sup>-1</sup>kg b. wt., dose of the decoction significantly ( $p < 0.05$ ) protected the rats against castor oil-induced diarrhoea at 1hr and 2hr. Table 4 shows that the percentage inhibition of the Jedi-Jedi pill on castor oil-induced diarrhoea at doses 300-900 mg<sup>-1</sup>kg b. wt., increased dose-dependently throughout the period of study. Aspirin at 100 mg<sup>-1</sup>kg b. wt., was also observed to significantly protect the rats against castor oil-induced diarrhoea in 1 and 2hr of the study. It also had a pronounced percentage inhibition especially at 1 and 3hr respectively (Table 5).

**Table 4: Effect of “Jedi-Jedi Pill” on Castor oil-induced Diarrhoea in Rats**

Treatment	Dose (mg/kg)	1hr	2hr	3hr	4hr
Control	10ml/kg	4.40 ± 1.03	5.40 ± 1.80	3.40 ± 1.5	2.00 ± 0.84
	300mg	1.80 ± 0.86	2.40 ± 1.36	3.80 ± 1.72	2.40 ± 1.12
	600mg	2.80 ± 1.24	0.40 ± 0.24	1.00 ± 0.89	1.00 ± 0.32
	900mg	0.20 ± 0.20**	0.40 ± 0.24*	1.00 ± 0.89	1.00 ± 0.32
Aspirin	100mg	0.40 ± 0.24**	0.60 ± 0.04*	0.06 ± 0.40	1.40 ± 0.87

Values are mean ±SEM \*P<0.05 significantly different from control (Student's t-test).

**Table 5: Percentage inhibition of “Jedi-Jedi” on Castor oil-induced diarrhoea in rats**

Treatment	Dose	Percentage inhibition in parenthesis			
		1hr	2hr	3hr	4hr
Control	10ml/kg	16	16	17	6
	300mg	9 {43.0}	13 {18.7}	4 {76.5}	16 {-166}
	600mg	9 {77.7}	10 {37.5}	10 {41.2}	1 {83.3}
	900mg	4 {75.0}	4 {75.0}	3 {82.4}	1 {83.3}
Aspirin	100mg	0 {100}	7 {56.3}	5 {70.5}	3 {50.0}

Values are expressed as percentage inhibition, N = 5.

## 4. Discussion

Jedi-Jedi Pill is a Nigerian local tri-herbal formula prepared with three plants *Croton penduliflorus*, *Cassia podocarpa*, *Manihot esculenta*, mucilage and potash. It is one of several herbal preparations sold openly in motor parks and market places in Lagos metropolis Nigeria, by medicine hawkers and it is widely used and acclaimed to be effective for the treatment of haemorrhoids. Although

acute toxicity studies did not indicate any mortality, adverse side effects like general weakness, sluggishness and bloody eyes were observed.

Carrageenan-induced rat paw oedema is a suitable test for evaluating anti-inflammatory drugs. Its oedema formation in rat paw is a biphasic event which involves various inflammatory mediators (Ahamed et al 2005). Chemical mediators such as histamine and serotonin are released in the first phase

(the first 2hrs after carrageenan administration) while in the second phase (3-5hrs after), Kinins, prostaglandins and other slow reacting substances become active (Hernandez-perez and Gallazo 2002). This pill exhibited inhibitory effect on carrageenan induced rat paw oedema at all doses used in this study. The 600 mg<sup>-1</sup>kg b. wt., dose inhibited oedema significantly ( $p < 0.05$ ) throughout the first phase, whereas the 900 mg<sup>-1</sup>kg b. wt., dose oedema inhibition occurred in both phases. The reference drug (Indomethacin) exhibited inhibition throughout the second phase and the level of its inhibition pattern is comparable with the tri-herbal preparation. The highest percentage inhibitions observed in the carrageenan induced oedema at 600 and 900 mg<sup>-1</sup>kg b. wt., showed a dose-dependent trend. It is most probable that the inhibition of different types of chemical mediators of inflammation may be involved in the biphasic inhibition pattern observed. Therefore the ability of "Jedi-Jedi Pill" to inhibit the biphasic events of carrageenan induced rat paw oedema by suppressing inflammation confirms its anti-inflammatory properties.

Antipyretics are known to prevent rise in body temperature generally in response to endogenous pyrogens as excessive rise in body temperature may cause irreversible tissue damage and possibly death (Tijani *et al.*, 2008). Cyclooxygenase (COX) which is the enzyme that converts arachidonic acid to prostaglandin (PG) is activated by pyrogens. The pill which did not produce any anti-pyretic effect at 300-900 mg<sup>-1</sup>kg b. wt., shows that there is continued synthesis of prostaglandins and this supports the biphasic pattern of carrageenan induced inflammation. It is therefore not surprising that COX activity which was activated by exogenous DNP administration was not affected by the preparation since synthesis of prostaglandins continued. Therefore, it is suggested that the pill does not compete with arachidonic acid at the active site of COX; hence it does not have antipyretic activity. Antipyretics have been reported to compete with arachidonic acid at the active site of cyclooxygenase (Insel, 1996). Most of the currently available antipyretics inhibit both cyclooxygenase 1 and cyclooxygenase 2 (COX-1 and COX-2, respectively), inhibiting the synthesis of prostaglandin and thromboxane (Insel, 1996). Inhibition of COX-2 is thought to mediate, at least in part, the anti-pyretic action of aspirin and related antipyretic drugs while inhibition of COX-1 results in the unwanted side effects associated with this drug.

The crude pill inhibited castor oil-induced diarrhoea in rats in a dose-dependent manner producing maximal inhibition at 600 and 900 mg<sup>-1</sup>kg

b. wt., respectively. Inhibition of experimental diarrhoea and reduction in faecal output by a substance are the basis of the pharmacological evaluation of a potential anti-diarrhoeal agent (Akah *et al.*, 1999). It was able to protect against castor oil-induced diarrhoea, and the reduction in faecal output in this study. The mode of action may be through the inhibitory action on the transmembrane fluxes of Ca<sup>2+</sup> as suggested by Seung *et al.* (2004), therefore it may be suppressing diarrhoea by direct inhibition of myolysis via calcium blockade, and possibly by its antimicrobial potential against *Escherichia coli* and other micro-organisms causing diarrhoea.

The mucilage and potash added in the tri-herbal preparation may have re-enforced the crude pill's efficacy. Mucilage from cassava was found to be a very good binding agent (Uhumwangho, 2006), and has antioxidant properties (Fu *et al.*, 2004). Iijima and Ohzeki (2008) had shown from clinical studies that replacement therapy with NaCl and KCl in Congenital chloride diarrhoeal patient normalized serum electrolytes and only KCl was administered in adolescence. A combined effect of the *Manihot esculentus*, starch mucilage and potash may probably have suppressed the laxative and purgative effect of *croton penduliflorus* seed (Asuzu *et al.*, 1988) and *cassia podocarpa* (Elujoba *et al.*, 1989) to produce the observed anti-diarrhoeal effect in the rats.

In summary, this study reports that a crude preparation, "Jedi-jedi" pill possesses anti-inflammatory and anti-diarrhoeal capabilities but lacks anti-pyretic action. It therefore, partially supports the claim by traditional medicine practitioners and hawkers that this tri-herbal preparation is effective for managing haemorrhoids. The study also reports its non toxicity but with side effects. Further research is therefore recommended to investigate the full implications of observed side effects.

#### Correspondence to:

Joy Okpuzor, Department of Cell Biology & Genetics, University of Lagos, Akoka – Lagos, Nigeria.

Cellphone: +234 802 306 5999

Email: [joyokpuzor@yahoo.com](mailto:joyokpuzor@yahoo.com)

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