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A Pharmacological Evaluation of A Herbal Cocktail

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Abstract: An herbal cocktail comprising of seeds, stem and leaves of seven African plants extensively used in South-Western Nigeria for the management and treatment of inflammation and tumor of the breast was investigated for analgesic and anti-inflammation activities. The analgesic properties of the ethanol extract was investigated using three *in vivo* mice test models (mice constriction, hot-plate and formalin-induced pain test) while anti-inflammatory activities of the same were evaluated using the Carageenan and egg albumin-induced oedema test systems *in vivo*. Present findings indicated that the cocktail at a concentration of 400-1600 mg kg^{-1} produced significant inhibition ($p<0.05$) response in both phases of the formalin pain model. The acetic acid-induced abdominal constriction also showed a dose dependent pain inhibition pattern directly related to the amount of extract administered. Instructively, the extract exhibited higher analgesic activity than acetylsalicylic acid but lower than morphine (2 mg kg^{-1}). The cocktail (400-1600 mg kg^{-1}) exhibited anti-inflammatory activity but inhibition observed at 1600 mg kg^{-1} in the 5 and 6 h was very significant. It compared favourably with the reference drug (Indomethacin 10 mg kg^{-1}). Consequently, it is our suggestion that the cocktail may possess analgesic and anti-inflammatory properties.

Key words: Analgesic, anti-inflammatory, herbal cocktail, decoction

INTRODUCTION

Medicinal plants are widely used for medications in Africa for the treatment and management of a myriad of diseases and according to da Silva Frutuoso *et al.* (2007) they have continued to play key roles in health care management around the world. The secondary metabolites of these plants have provided man with a variety of useful substances necessary for sustaining life on earth. Most countries in the tropics are richly endowed with these natural products and the indigenous people have over time, mastered and maximized the use of these substances from plants for the treatment of various diseases confronting man. The chemical diversities derivable from the plants make them important resource for the isolation of bioactive organic compounds (Basso *et al*., 2005). Thus, *Desmodium gangeticum* widely used in India and reported to contain flavone and isoflavonoid glycosides, is the main ingredient in many Ayurvedic formulations used for diabetes disease management (Govindarajan *et al*., 2007). Some other plants which have demonstrated analgesic and anti-inflammatory activities include *Rheedia longifolia* Planch and Triana (da Silva Frutuoso *et al*., 2007), *Sida cordifolia* Linn. (Sutradhar *et al*., 2007), *Mezoneuron benthamianum* Baill (Mbagwu *et al*., 2007), *Hippocratea africana* (Okokon *et al*., 2008).

Therefore, the present study was done to investigate the local claim that the cocktail known as JOLOO has analgesic and anti-inflammatory activities.

MATERIALS AND METHODS

Plant materials and extract preparation: The seven plant materials used for this study, *Butyrospermum paradoxum* seed (PCGH 437), *Securidata longepunculata* bark (PCGH 439), *Tetrapleura tetraptera* stem (PCGH 382), *Hoslurdia opposita* (PCGH 322) leaves, *Xylopia aethiopica* seed (PCGH 441), *Olax subscorpioidea* stem (PCGH 438) and *Allium ascalonicum* (PCGH 440) were sourced in August 2007 from traditional medicine practitioners in Totoro village, Abeokuta Ogun State of Nigeria. They were identified and authenticated by the Department of Pharmacognosy, School of Pharmacy, College of Medicine, University of Lagos. Herbarium specimens were deposited at the Department of Pharmacognosy. The

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cocktail was prepared according to the ratio of 5:1: 3: 2: 4:1:3, respectively to produce the desired pharmacological action. Samples were air dried, powdered and allowed to stand in 500 mL 95% cold ethanol for 72 h. They were thereafter decanted and filtered using a muslin cloth. The extract was further evaporated to dryness in an oven at 40°C. Finally, the dried extract weighed 19.2 g. The cocktail used in this study was obtained when reconstituted to a concentration of 100 mg mL^{-1}.

Animals: Swiss mice weighing 20-32 g and Albino rats (100-180 g) of either sex, bred in the animal house of the University of Lagos were used for this study. Approval was obtained from the University of Lagos Ethical Committee on the use of animals for research purposes. The animals were maintained under standard environmental conditions as described by the method of Bishayee and Chanterjee (1994) as reported by Mbagwu *et al.* (2007). They had access to water and standard feed *ad libitum.*

Analgesic activity

Acetic acid induced-abdominal constriction assay: The method adopted was as described by Mohamad *et al.* (2005). Fifty Swiss albino mice of either sex were divided into five groups (Control-1; Reference-2 and Test 3-5) of ten animals each. The control animals were given 10 mL kg^{-1} of normal saline only while the reference groups were injected with 100 mg kg^{-1} acetylsalicylic acid subcutaneously. The test animals were treated intraperitoneally (i.p.) with varying doses of 400, 800 and 1600 mg kg^{-1} of the ethanol extract respectively. These doses were determined from preliminary studies in our laboratory and the LD_{50} was found to be 2000 mg kg^{-1}.

Forty minutes after treatment, a dose of 10 mL kg^{-1} of 0.6% v/v acetic acid solution in normal saline was administered i.p. to all the groups to induce pain. Thereafter, the number of abdominal constrictions occurring within 5 and 15 min were counted. The fewer number of constrictions observed among the test groups compared with the constrictions recorded for the control group was considered as evidence of the presence of analgesia and was expressed as a percentage inhibition of constrictions (Amir and Kumar, 2005). Data calculation was according to the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of constrictions (control)} - \text{Mean No. of constrictions (treated)}}{\text{Mean No. of constrictions (control)}} \times 100$$

Formalin induced-pain test: The method of Shibata *et al.* (1979) as reported by Mbagwu *et al.* (2007) was used. Fifty Swiss mice of either sex were divided into two-control and three test treatment groups of 10 mice each. Each of the test groups were given the cocktail at a dose of 400, 800 and 1600 mg kg^{-1}, respectively while the two control groups received 10 mL kg^{-1} normal saline orally and 100 mg kg^{-1} of acetylsalicylic acid (ASA) subcutaneously. Thirty min after, 20 mL of 1% formalin was injected subcutaneously into the right hind paw of pretreated mice to induce pain. Responses were measured five min after formalin injection, for the first phase and the second phase were taken 15-30 min later. The licking of the injected paw and the duration was indicative of pain.

Hot plate induced-pain test: The hot plate method was done as described by Gupta *et al.* (2007) with minor modifications. Mice were divided into five groups of ten animals each. A dose of 400, 800 and 1600 mg kg^{-1} of the cocktail was administered orally to the three test groups while 10 mL kg^{-1} of distilled water was given to the control and 2 mg kg^{-1} morphine administered subcutaneously to the reference group. Thirty min after, the animals were dropped gently on the hot-plate set at 55±1°C. The reaction time was determined as the interval between placement of the animals on the hot plate and the moment the animal either licks its fore-paws or jumps out of the plate.

Anti-inflammatory activity

Carageenan-induced paw oedema: Induced pedal inflammation in rat hind paw by the subplantar injection of the phlogistic agent described by the method of Winter *et al.* (1962) as reported by Owoyele *et al.* (2004) with minor modifications.

The Albino rats of either sex used for this study were fasted for 12 h but allowed access to water only. The ethanol extract 400 and 600 mg kg^{-1} was administered orally to the test groups of rat while indomethacin 10 mg kg^{-1} was given subcutaneously to the reference group. The control group received only normal saline orally. To induce paw oedema, 0.1 mL of 1% Carageenan diluted in distilled water was injected into the sub-plantar region of the right hind paw 1 h after treatment. Oedema was assessed immediately after Carageenan injection at intervals of 0, 1, 2, 3, 4, 5 and 6 h using the cotton thread method described by Bamgbose and Naomesi (1981). The increase in paw swelling was measured and percentage inhibition calculated.

Egg albumin-induced inflammation: Hind paw inflammation in rats was induced by injecting Egg albumin (0.1 mL 1% w/v in normal saline) into the sub-plantar tissue of the right hind paw. Control animals were administered 10 mL kg^{-1} distilled water orally. The cocktail at a dose 400-1600 mg kg^{-1} and chloropheniramine

(100 mg kg^{-1}) were administered to the test and reference groups 90 min before injecting egg albumin to induce inflammation. The linear paw circumference was assessed at 15 min interval over 180 min using the cotton thread method (Bamgbose and Naomesi, 1981).

Statistical analysis: Results are expressed as mean±SEM. Statistical analysis was done using students t-test and results were considered significant when $p<0.05$.

RESULTS

Analgesic studies

Mice abdominal constriction assay: The animals injected with 10 mL kg^{-1} of 0.6% acetic acid intraperitoneally presented 54.3±1.69 constrictions (n = 10) in 15 min. The group treated with the 400-1600 mg kg^{-1} cocktail exhibited dose dependent and significant, reductions in number of constrictions by 24.9, 53.6 and 74.0%, respectively (Table 1). The dosage ranges of 800 and 1600 mg kg^{-1} of the cocktail, produced significant inhibition ($p<0.05$) greater than 100 mg kg^{-1} acetylsalicylic acid which inhibited abdominal constriction by 47.1%.

Formalin test: Four hundred to sixteen hundred milligram per kilogram of the cocktail demonstrated dose-related inhibitions against both phases of formalin-induced pain (Table 2). An inhibition of 38.9% was recorded at the concentration of 400 mg kg^{-1} while 800 mg kg^{-1} and 1600 mg kg^{-1} doses, produced inhibition that is (56.6 and 65.3%) higher than ASA in the first phase. Significant pain inhibition was recorded, at all the doses of the cocktail and acetylsalicylic acid (100 mg kg^{-1}) in the second phase. However, the duration of paw licking was longer among rats treated with 400 mg kg^{-1} of the extract than those in the 800 and 1600 mg kg^{-1} group.

Hot plate test: Table 3 shows the reaction time of mice to hot plate-induced pain. The reaction time in mice treated with the cocktail showed a dose relationship with the dosage administered. Although the cocktail inhibited better that the control at all doses, morphine returned a higher pain tolerance time of 7.2 sec.

Anti-inflammatory studies

Carrageenan-induced paw oedema in rats: The cocktail administered one hour before carrageenan showed a fluctuating rhythm with recourse to time in a biphasic pattern (Table 4a). Although the various doses of the cocktail (400-1600 mg kg^{-1}) inhibited inflammation, inhibition at 1600 mg kg^{-1} was very significant at the 5 and 6 h. The cocktail compared favourably with the reference drug (Indomethacin 10 mg kg^{-1}) which also produced a very significant inhibition.

Egg albumin-induced oedema: Egg albumin produced rapid swelling that peaked in 90 min (2.61±0.02). The swelling fluctuated over the period of the experiment (Table 4b). Oedema inhibition was significant in the rats treated with cocktail dose of 800 and 1600 mg kg^{-1} from 120 to 180 min. Rats treated with 800 and 1600 mg kg^{-1} of

Table 1: Effect of herbal cocktail on acetic acid-induced constriction in mice

Groups	Dose (mg kg^{-1})	No. of constrictions	Inhibitions (%)
Control	-	54.3±1.69	-
Cocktail	400	40.8±1.55	24.9
	800	25.2±1.33	53.6
	1600	14.1±1.72	74.0
Acetylsalicylic acid	100	28.7±1.69	47.1

Values expressed as mean±SEM; $p<0.05$ significantly different from control (Students t-test)

Table 2: Effect of cocktail on formalin induced pain

Groups	Dose (mg kg^{-1})	0-5 min	Inhibition (%)	15-30 min	Inhibition (%)
Control		73.2±3.89	-	64.0±1.77	-
Cocktail	400	44.7±1.93	38.9	18.6±0.82	70.9
	800	31.7±2.00	56.6	4.7±1.170	77.0
	1600	25.4±3.37	65.3	6.9±1.690	89.2
Acetylsalicylic	100	40.5±4.37	44.7	19.0±2.49	70.3

Values expressed as mean±SEM; $p<0.05$ significantly different from control (Students t-test)

Table 3: Effect of cocktail on hot plate test

Groups	Dose (mg kg^{-1})	Reaction time (sec)	Inhibition (%)
Control	-	2.2±0.1	-
Cocktail	400	2.5±0.2	13.63
	800	3.5±0.2	59.09
	1600	5.4±0.4	145.45
Morphine	2	7.2±0.5	227.30

Values expressed as mean±SEM; $p<0.05$ significantly different from control (Students t-test)

Table 4a: Effect of the cocktail on carrageenan-induced rat paw oedema

Dose (mg kg^{-1})	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Control	2.33±0.02	2.51±0.01	2.45±0.03	2.49±0.03	2.43±0.02	2.38±0.03	2.41±0.04
400	2.41±0.01	2.53±0.02	2.26±0.1c	2.37±0.02^{a}	2.27±0.02^{c}	2.25±0.01^{b}	2.23±0.02^{b}
800	2.62±0.02	2.65±0.01^{c}	2.35±0.04^{a}	2.52±0.02	2.30±0.02^{c}	2.20±0.02^{c}	2.21±0.03^{c}
1600	2.38±0.03	2.54±0.02	2.28±0.02^{c}	2.44±0.03	2.52±0.01^{c}	2.18±0.01^{c}	2.13±0.02^{c}
Indometh	2.15±0.11	2.08±0.12^{b}	2.07±0.11^{b}	2.15±0.12^{a}	2.10±0.11^{a}	2.02±0.12^{a}	1.91±0.11^{c}

Values are Mean±SEM; (N = 10); $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$

Table 4b: Effect of cocktail on egg albumin-induced rat paw oedema

Treatment (mg kg^{-1})	15 min	30 min	45 min	60 min	75 min	90 min
Control	2.65±0.01	2.40±0.02	2.57±0.03	2.55±0.01	2.61±0.02	2.61±0.02
400	2.52±0.02^{b}	2.47±0.02^{a}	2.54±0.02	2.29±0.02^{c}	2.32±0.02^{c}	2.40±0.03^{c}
800	2.48±0.03	2.37±0.02	2.51±0.27	2.31±0.03^{c}	2.33±0.03^{c}	2.38±0.02^{c}
1600	2.41±0.02^{b}	2.39±0.02	2.32±0.02	2.29±0.02^{c}	2.32±0.03^{c}	2.42±0.01^{c}
Chlorphen	2.64±0.01	2.35±0.01^{c}	2.58±0.02^{c}	2.30±0.01	2.34±0.01^{c}	2.41±0.01^{c}
Treatment (mg kg^{-1})	105 min	120 min	135 min	150 min	165 min	180 min
Control	2.60±0.02	2.58±0.03	2.55±0.03	2.52±0.02	2.52±0.02	2.50±0.03
400	2.38±0.03	2.36±0.03^{c}	2.36±0.01^{c}	2.33±0.01^{c}	2.30±0.02^{c}	2.28±0.02^{c}
800	2.37±0.02^{c}	2.30±0.02^{c}	2.28±0.02^{c}	2.19±0.01^{c}	2.14±0.01^{c}	2.10±0.02^{c}
1600	2.41±0.02^{c}	2.34±0.02	2.32±0.02^{c}	2.22±0.02^{c}	2.22±0.01^{c}	2.10±0.02^{c}
Chlorphen	2.41±0.02^{c}	2.32±0.03	2.30±0.01^{c}	2.21±0.02^{c}	2.20±0.02^{c}	2.14±0.02^{c}

Values are mean±SEM; $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ significantly different from control (student's t-test)

cocktail produced significant inflammation inhibition from 120-180 min as shown in Table 4b. This compared with the activity of the chlorpheniramine over the same period significantly ($p<0.05$).

DISCUSSION

Plants are useful sources of crude drugs and they are believed to possess minimal side effects. They may be used singly or as a decoction or cocktail to treat diseases. Consequently, there has been increased interest in typing medical plants for various pharmacological actions. We have presented pharmacological results favouring the potential use of JOLOO cocktail as an analgesic and anti-inflammatory agent. It effectively inhibited pain and inflammation without remarkable changes in animal behaviour and no mortality was recorded. The evidence of effective pain inhibitive analgesic properties was confirmed from the abdominal constriction test, formalin and the hot-plate tests. The hot-plate method is one of the most common tests for evaluating the analgesic efficacy of drugs/compounds in rodent. It is also considered selective for opioid-like receptor (Somchit *et al*., 2004). Present results showed that the cocktail exerts dose-dependent analgesic effect both peripherally and centrally. Although the morphine effect was more pronounced by 82% as compared to the highest cocktail concentration (1600 mg kg^{-1}), nonetheless the effect of the cocktail was significant. The cocktail however inhibited both phases of formalin induced pain which is a model considered very useful in elucidating the mechanism of pain and analgesia (Okpo *et al*., 2001), in constriction and hot-plate tests. Drugs which act mainly centrally such as narcotics, inhibit both phases of formalin induced pain also while peripherally acting drugs like aspirin only inhibit later phase pains (Santos *et al*., 2000) thus buttressing the fact that our cocktail may be acting both peripherally and centrally.

Considering that acetic acid induced constriction test is normally used to evaluate the peripheral analgesic effect of drugs, the response is thought to be mediated by peritoneal mast cells (Ribeiro *et al*., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Sutradhar *et al*., 2007). This result is an indication of a probable inhibition of prostaglandin released during inflammation lending credence to the fact that the cocktail possesses also anti-inflammatory properties. In peripheral tissues, prostaglandins and kinines have been implicated in the pain process (Hajare *et al*., 2000) and abdominal constrictions induced by injection of the cocktail i.p. is said to be the consequence of sensitisation of the chemosensitive nociceptors by prostaglandins as reported by Maria *et al*. (1997) and quoted by Ndebia *et al*. (2007). It is therefore indicative that the pain killing effect of our cocktail may be by the inhibition of prostaglandins synthesis and confirms the peripheral action of Acetylsalicylic acid as reported by Rang *et al*. (1995) and quoted by Ndebia *et al*. (2007).

Carrageenan-induced hind paw oedema is the acclaimed standard experimental models for acute inflammation studies. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility (Chakraborty *et al*., 2004). The cocktail exhibited convincing anti-inflammatory activity

using the carrageenan and egg-albumen induced rat paw oedema models respectively. We observed from our investigations that the cocktail reduced paw swelling as effectively as indomethacin and chlorpheniramine in a dose-dependent relationship. Present cocktail may therefore, be inhibiting mediators of acute inflammation as stated by Mossa *et al.* (1995) and reported by Olaleye *et al.* (2004). Many compounds have been proposed as inflammatory mediators released locally at the site of inflammation and having biological properties that cause the signs and symptoms of inflammation (Galti *et al.*, 2001).

The data from present results showed that although, the cocktail was slightly effective in reducing inflammation in the early phase, it markedly reduced mediators of late response in the injured tissues. The decrease in carrageenan-induced paw oedema was most pronounced between the third and fifth hour of inflammatory response, which corresponds to the phase of prostaglandin release (Dannhardt and Kiefer, 2001) as shown from our studies. Sutradhar *et al.* (2007) had isolated a compound which inhibited carrageenan induced paw oedema in rats and suggested inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. There has however been reports that acute inflammatory response is characterized by the release of histamine and 5-HT as immediate responses of inflammation (early phase) whereas the kinnins and prostaglandins mediate the more prolonged delayed-onset responses (late phase) (Di Rosa *et al.*, 1971; Goetzel, 1980).

Consequent upon the results obtained from our investigations, the cocktail possess analgesic and anti-inflammatory properties and further affirms its use by the traditional healers.

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