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Antinociceptive and Anti-inflammatory properties of a polyherbal extract of *Plumbago zeylinica* and *Capsicum frutescens* in rodents

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ABSTRACT

The aim of the present study is to evaluate the anti-nociceptive and anti-inflammatory properties of an extract of a polyherbal mixture containing the leaves and roots of *Plumbago zeylinica* and fruits of *Capsicum frutescens*. Acute toxicity effect of WAP was evaluated in mice. The antinociceptive and anti-inflammatory activities of WAP (100–400 mg/kg) were investigated in hot plate, acetic acid-induced writhing, carrageenan-induced paw oedema and air pouch models. No death was recorded when WAP was administered at a dose of 2000 mg/kg. Oral treatment with WAP elicited inhibitory activity in hot plate test. Abdominal writhing was significantly reduced by WAP at 100, 200 and 400 mg/kg (54.5 ± 1.6 , 20.0 ± 7.4 , 38.4 ± 4.8) as compared with the control group (71.0 ± 2.6). Carrageenan-induced oedema formation was significantly ($p < 0.05$) reduced at WAP (100, 200, and 400 mg/kg) by 23.5, 27.3 and 31.4%, respectively, and by reference to indomethacin (10 mg/kg, 31.2%). WAP (200 and 400 mg/kg) significantly reduced exudate volume (37.2 and 44.1%), protein (42.0 and 54.1%), total leucocytes (59.1 and 60.2) and neutrophils count (57.3 and 58.0%) in the carrageenan-induced air pouch in rats. Similarly, WAP treated animals showed reduced nitrites and malondialdehyde levels and increased glutathione levels. Furthermore, WAP (400 mg/kg) neither affected locomotory activity nor induced gastric lesion in mice. The results of this study revealed that extract of a polyherbal mixture containing the leaves and roots of *Plumbago zeylinica* and fruits of *Capsicum frutescens* possesses anti-nociceptive and anti-inflammatory activity.

Keywords: Polyherbal, *Plumbago zeylinica*, *Capsicum frutescens*, inflammation, rats

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INTRODUCTION

The rich biodiversity of medicinal plants constitutes the greatest asset to human health. The dependence of human beings on plants dates back to the origin of the human race. Medicinal plants served as the major agents for primary health care for several years before the advent of conventional medicine (Sen and Semantha, 2015). Multi-agent medicines is a developing theme in modern drug therapy, which is the combination of drugs with synergistic effects to improve efficacy and reduce toxicity (Risberg *et al.*, 2011). However, this represents nothing new for phytotherapy since many medicinal plants are combined in a herbal concoction. This arrangement of herbal ingredients into formula have shown potential interactive effects including mutual enhancement,

mutual assistance, mutual restraint and mutual antagonism (Aslam *et al.*, 2016).

There is an increasing popularity in the development and assessment of herbal formulations for various beneficial health and functional effects (Capasso *et al.*, 2000). In the Nigerian herbal market space, there are many unregistered products being sold in the market, because scientific evidence is still lacking, it may put the health of their users at risk of toxicity (Oreagba *et al.*, 2011). There is therefore need to evaluate the pharmacological effect and toxicity of some of these polyherbal formulations. A Polyherbal formulation containing the leaves and roots of *Plumbago zeylenica* and *Capsicum frutescens* fruits herein referred to as WAP, is sold as herbal remedy for the management of inflammatory conditions and pain in South West, Nigeria. *Plumbago*

zeylanica leaves and roots and dried fruits of *Capsicum frutescens*, have been reportedly used as an anti-inflammatory, anti-nociceptive, antidiabetic and anticancer agents (Gbadamosi and Erinoso, 2016).

Plumbago zeylanica is distributed as a weed plant in many parts of the tropical and subtropical countries. Its medicinal values has been mentioned in traditional African medicine (TAM) and Ayurveda ethnomedicine (Burkill, 1985, Ngarivhume *et al.*, 2015; Chaudhari and Chaudhari, 2015). The leaves is said be useful in the treatment of infections, ulcers, diarrhoea, sore and swelling (Ganesan and Gani, 2013). A paste of the leaves helps to relieve painful rheumatic areas and chronic itchy skin problems (Ngarivhume *et al.*, 2015). The roots has been reported to have antifertility, diuretic, antimicrobial, antiulcer and antidiarrhoeal activities (Mandavkar and Jalalpure, 2011). Plumbagin is said to be the main phytochemical constituents in both the leaves and root extracts (Lin *et al.*, 2003; Hsieh *et al.*, 2005).

Capsicum species are known for their therapeutic potentials in the treatment of arthritis, rheumatism, stomach aches, skin rashes and wounds (Francis, 2005). Capsaicin is the major component of *Capsicum* species, constituting about 70% of the total pungent acid amides. Other analogue includes capsaicinoid, dihydrocapsaicin (Calixto *et al.*, 2000). The therapeutic effects of *Capsicum* species have been linked to the presence of capsaicinoid, phenolic compounds and carotenoid contents (Hernandez-Ortega *et al.*, 2012). Capsaicin in found as an ingredient in several commercially available formulations for muscle pain (Calixto *et al.*, 2000). *Capsicum frutescens* fruit extract and capsaicin have been reported to possess anti-nociceptive and anti-inflammatory properties (Jolayemi and Ojewole 2012, 2014).

The use of combined plants parts to alleviate pain is a common practice today. Plant derived herbal preparations have been used for hundreds and even thousands of years to obtain effective pain relief. Evaluation of herbal extracts for antinociceptive activity is widely undertaken by researchers. The process used to prepare herbal formulations are not well standardized. Some herbal formulations carries claims that have not be verified, and the possible side effects and potential drug interactions not known. Therefore such medicinal preparations are not without risk. The aim of the present study was to investigate the toxicity, antinociceptive and anti-inflammatory propeties of WAP (a polyherbal extract containing leaves and roots of *Plumbago zeylanica* and dried fruits of *Capsicum frutescens*).

MATERIALS AND METHODS

Test Substance: WAP is finely ground polyherbal extract containing leaves and roots of *Plumbago zeylanica* and dried fruits of *Capsicum frutescens*, obtained from a local herbal vendor in Abeokuta, Ogun State, Nigeria. The powder material was soaked in distilled water for 24 hours, filtered and evaporated *in vacuo* (40 °C). The resulting extract was administered to animals by gavage using oral canula.

Experimental animals: Wistar rats (150 - 200g) or swiss mice (18 - 25g) of either sex were obtained from the Central

animal house, University of Ibadan. The animals were housed in cages maintained under standard conditions of light and humidity and fed with pelletized feed (Vital Feeds Ltd, Jos Nigeria) and received water *ad libitum*. The animals were acclimatized for two weeks in the Pharmacology laboratory at the University of Ibadan prior to carrying out the experiments. All experimental procedures were carried out with strict compliance to The "Principle of Laboratory Animal Care" (NIH Publication No. 85-23) and ethical guidelines for investigation of experimental pain in conscious animals (Zimmerman, 1985).

Evaluation of acute toxicity effects of WAP in mice:

Healthy male and female swiss mice weighing 18 g were used for the acute oral toxicity study. All mice of both sexes were fasted overnight without food but allowed water before treatment. A single high dose as recommended by the Organization for Economic Co-operation and Development guideline 432 was followed. WAP (2000 mg/kg) dissolved in distilled water was administered by gavage to three male and three female mice, and distilled water (0.1 mL/10 g body weight) was given to three male and three female mice as control groups. Following single administration, signs of possible toxicity were observed every hour for the first four hours and daily for 7 days. The visual observations included changes in the skin and fur, locomotor activity, piloerection, tail elevation, traction, motor incoordination, respiration, lacrimation, salivation, diarrhea, vocalization and death. After the 7th day observation, the animals were sacrificed to measure organ/body weight indices. The organs (liver, kidney, heart, stomach) were removed, weighed and evaluated for macroscopic abnormalities.

The relative organ weights were calculated as follows;

$$\text{Relative organ weights} = \frac{\text{Absolute organ weight (g)} \times 100}{\text{Body weight of mice on sacrifice day}}$$

Evaluation of antinociceptive activity of WAP in acetic acid-induced nociception in mice: In the acetic acid-induced nociception in mice as described by Koster *et al.*, (1959), writhing was induced by intraperitoneal injection of 0.6% acetic acid (0.1 mL/ 10g body weight).

Five groups of mice (n=5) were used in this study comprising the vehicle (0.1 mL/10g distilled water), indomethacin (10mg/kg), or WAP (100, 200, 400mg/kg). Each mouse was placed inside the plexiglass observation chamber immediately after treatment according to the time schedule of treatment. Immediately after injection of acetic acid (0.1 mL/10 g b.w.), the latency time to the beginning of the first contraction of the abdominal muscle was measured and the frequency of writhing occurring between 5 and 20 minutes counted. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs.

Evaluation of antinociceptive activity of WAP in hot plate test:

Thermal noxious stimulus was produced in mice by placing them on the hot plate (UgoBasile hot/cold plate 35100, Italy) according to a method described by Hole and Hunskaar (1972). Five groups of five mice each were selected for this

study, group 1 served as control and received the vehicle (distilled water, 0.1 mL/10g b.w.). WAP at the concentration of 100, 200 and 400 mg/kg b.w. was administered orally to groups 2, 3 and 4, respectively and group 5 received Pentozocine (5 mg/kg b.w.). The mice were placed singly on the hot plate which was maintained at $55 \pm 1^{\circ}$ C. Reaction time was recorded when the animals licked their fore-and hind paws or jumped; before (0) and at 60, 90, 120 and 150 min after administration of test drugs. The mice which reacted within 20 s were selected for the study.

The mean percentage maximum possible effect (% MPE) was calculated as:

$$\% \text{ MPE} = \frac{\text{Post-drug latency} - \text{Pre-drug latency}}{\text{Cut-off time} - \text{Post drug latency}} \times 100$$

Assessment of effect of WAP on locomotory activity in

Open-field test: To assess the possible nonspecific muscle relaxants or the sedative effects of WAP, the motor performances of the mice were evaluated in the open-field apparatus (Archer, 1973). Groups of mice ($n = 5$) were treated with vehicle (0.1 mL/10g, p.o.), WAP (400mg/kg, p.o.) 1 h before the performance of the test. The mice were placed in the center of the UgoBasile activity cage apparatus and allowed to have free ambulation for 5 min of observation of the locomotion frequency (horizontal activity and vertical activity).

Assessment of gastro-ulcerogenic potential of WAP in

mice: In order to assess the gastrointestinal tolerability effect of WAP after acute administration, mice were divided into three groups ($n=3$) and fasted over night for 18 hours. Group I received Vehicle (0.1 mL/10 g distilled water), group II received WAP (400 mg/kg) and group 3 received high dose of indomethacin (40 mg/kg). The animals were observed for 4 hours and then sacrificed by deep ether anaesthesia. Then the abdomen of each mouse was opened through the greater curvature and examined under hand lens for lesions or bleedings.

Evaluation of anti-inflammatory effect of WAP in

carrageenan-induced paw oedema in rats: Inflammatory oedema was induced in the right hind paw of female rats according to the method described by Winter *et al.*, (1962). The animals were divided into five groups ($n=5$); group 1 was negative control group that was orally pre-treated with vehicle (10 mL/kg distilled water), groups 2-4 were orally pre-treated with WAP (100, 200 and 400 mg/kg) and group 5 received indomethacin (10 mg/kg). One hour after oral pre-treatment, 0.1mL of 1% carrageenan was injected into the right hind paw of each rat under the subplantar aponeurosis. Measurement of paw volumes was done before and at 1, 2, 3, 4, and 5 h after injection of carrageenan using the Ugo Basile (7134) digital plethysmometer. The increase in paw volume was calculated as percentage and plotted against time (hour). The percentage of inhibition of oedema formation was calculated with the formula below

$$\% \text{ inhibition} = \frac{[(\text{Mean volume})_{\text{control}} - (\text{Mean volume})_{\text{treatment}}]}{(\text{Mean volume})_{\text{control}}} \times 100$$

Evaluation of anti-inflammatory effect of WAP in

carrageenan-induced air pouch model: Female Wistar rats weighing 150-200g were anaesthetized with Ketamine (100 mg/mL), and 20 ml of sterile air was injected subcutaneously into the shaved area of the dorsal region (Martins *et al.*, 1994). Three days after, the pouch was re-inflated with 10 mL of air. On the 6th day following the first air injection, the animals were divided into five groups ($n=5$); saline (1% tween 80; 10 mL/kg), carrageenan (1% tween 80; 10 mL/kg), WAP 200 mg/kg, 400 mg/kg and Indomethacin (10 mg/kg). After 1 h of pretreatment, 2 mL sterile saline or carrageenan (1% in sterile saline) was injected directly into the pouches. Twenty four hours after the carrageenan injection, the animals were anesthetized with deep ether. The pouches were washed with 2 mL of sterile saline, and the exudates recovered. The volumes of recovered exudates were measured and divided into aliquots for leucocytes counts, protein, nitrites, reduced glutathione (GSH), and malondialdehyde (MDA) assays.

Estimation of leucocytes and protein concentration:

Total number of leucocytes in the recovered exudates was determined by using a Neubauer chamber with Turks stain. Differential cell counts were performed on smears stained with May Grünwald-Giemsa. Exudates protein level as a measure of increase in vascular permeability was assessed using the Biuret method (Gornall *et al.*, 1949). Briefly, 0.2 mL of cell free exudates was mixed with 2.8 mL of Biuret reagent and allowed to incubate in the dark for 30 min. The absorbance was read at 540 nm in UV-Vis spectrophotometer (752N, INESA).

Estimation of nitrites, malondialdehyde and reduced glutathione levels

Cell free exudates was used to measure the production of nitric oxide in the air pouch. Samples were incubated with Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid), and absorbance at 550 nm was determined by using UV-Vis spectrophotometer (752N, INESA). Estimation of MDA level as a marker of lipid peroxidation in the exudates was determined using the thiobarbituric acid reactive substance (TBARS) method of Nagababu (2010). The method as described by Sin *et al.*, (2001) was adopted in the determination of reduced glutathione concentration in the recovered exudates from the air pouch.

RESULTS

Acute toxicity result: Administration of 2000 mg/kg of WAP in mice showed no signs of morbidity or mortality immediately or during the observation period. Alterations in behavioural changes were absent in both the male and female mice. The changes in body weight in the female mice was not significantly ($p > 0.05$) different, however, WAP (2000 mg/kg) significantly ($p < 0.05$) reduced body weight gain in male mice when compared to the control group (Fig 1A). No gross pathological findings were noted when the mice were sacrificed at the termination of the study. No statistically significant difference ($p > 0.05$) in relative organ weights in both sexes when compared to the control (Fig 1B).

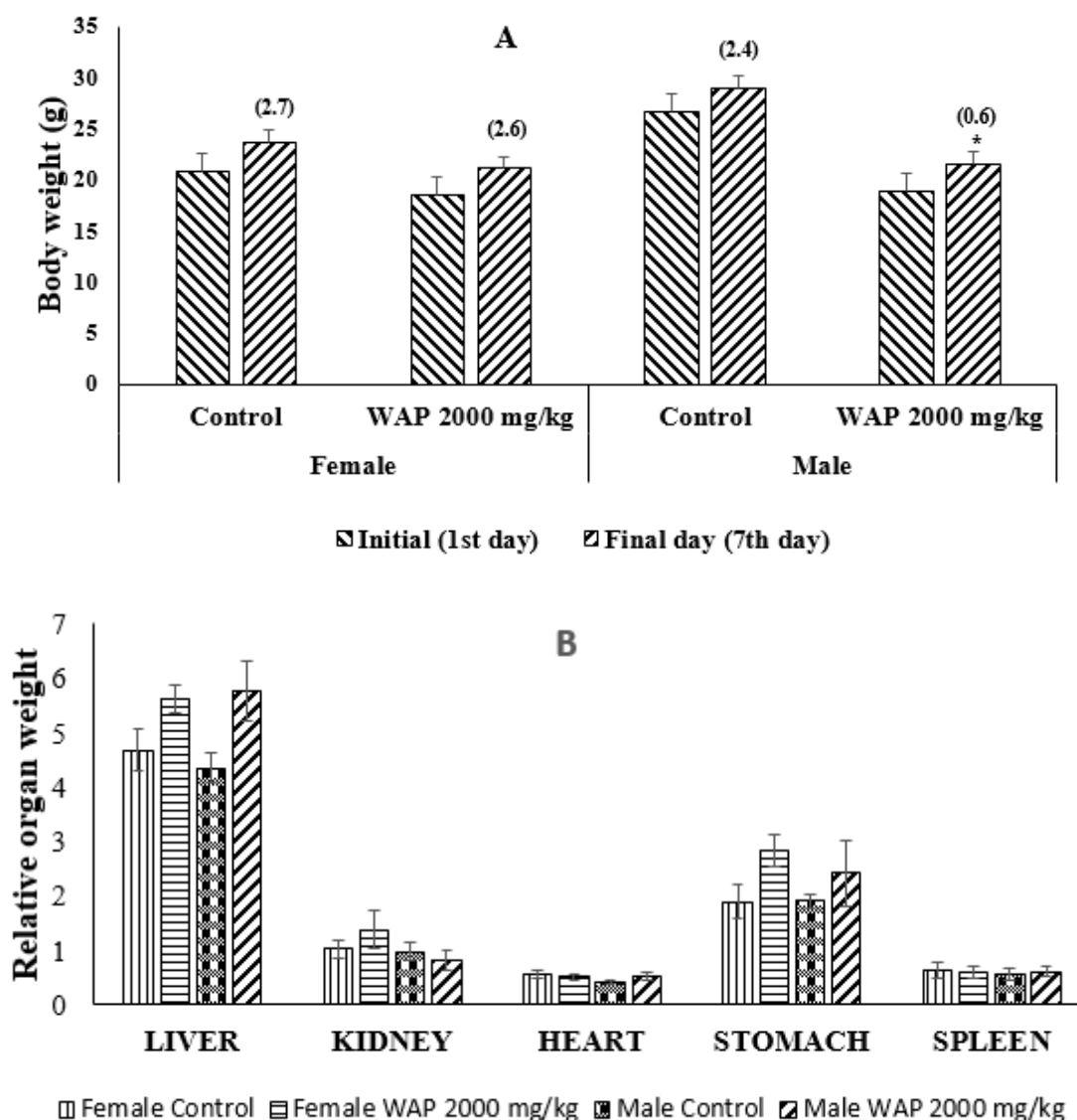


Figure 1:

Effect of acute oral administration of WAP on (A) weight gain in mice, and (B) relative organ weights. Data represent Mean \pm SEM of three mice, values in parenthesis are weight gains. * Denotes $p < 0.05$ using student's t-test.

Antinociceptive effect of WAP in acetic-acid writhing test in mice:

The results of latency time to the beginning of the first writhe (onset of writhing) and the frequency of writhes after intraperitoneal injection of acetic acid is presented in Fig 2. WAP (100, 200, 400 mg/kg) did not significantly ($p > 0.05$) affect the latency of writhing when compared to control. The frequency of writhes was significantly ($p < 0.05$) decreased by WAP at the doses of 100, 200 and 400 mg/kg (54.5 ± 1.6 , 20.0 ± 7.4 , 38.4 ± 4.8) compared to vehicle (71.0 ± 2.6). The positive control Indomethacin (10 mg/kg) significantly ($p < 0.05$) inhibited the frequency of writhing (10.3 ± 3.0).

Antinociceptive effect of WAP in hot plate test in mice:

The WAP at doses of 200 and 400 mg/kg but not 100 mg/kg did significantly ($p < 0.05$) showed anti-nociceptive effect in the hot plate test (Fig 3A). The area under the curve for prolonged latency at 60 -150 min is as shown in Fig 3B. WAP at 200 and

400 mg/kg significantly increased maximal possible effect by 44.1% and 56.2%, respectively. Pentazocine (5 mg/kg) showed a significant ($p < 0.05$) maximal possible effects (50.0%) when compared to control.

Effect of WAP (400 mg/kg) on locomotor activity in Open field test:

A group of mice (n=5) was used to examine the effect of WAP (400 mg/kg) on locomotor activity. Locomotor activity was recorded for a duration of 5 min after 2 hour post drug administration. The changes in exploratory and rearing activity after acute administration of WAP is presented in Fig 4. Mice treated with WAP 400 mg/kg did not cause any significant ($p > 0.05$) reduction in the ambulatory locomotor activity (total horizontal activity count) or rearing (total vertical count) during the five minutes test period when compared to the control group in the open-field test.

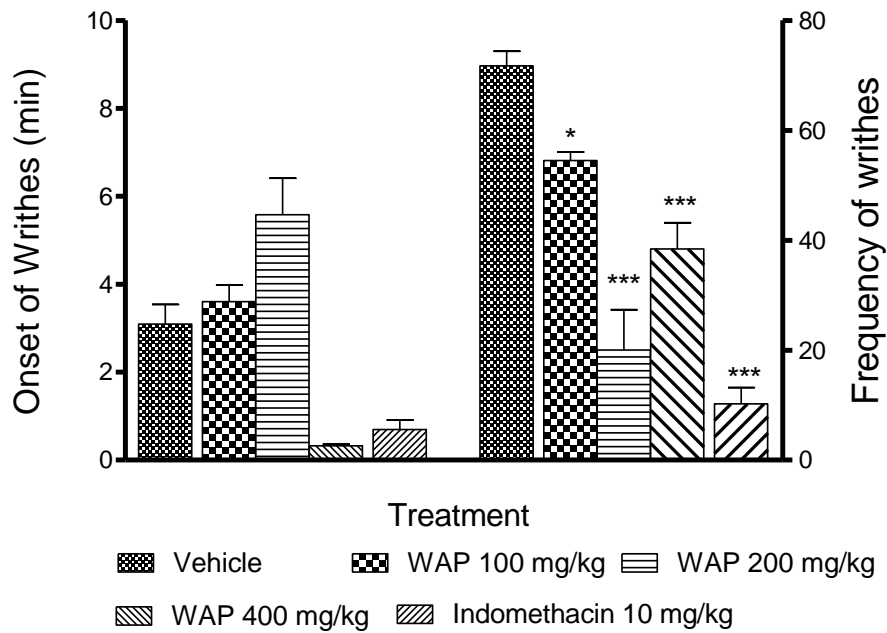


Figure 2: Antinociceptive effect of WAP (100, 200, 400 mg/kg) in acetic acid-induced nociception in mice. Data represent Mean \pm SEM of five mice, values in parenthesis are percentages of inhibition.*** $p < 0.001$, by 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group.

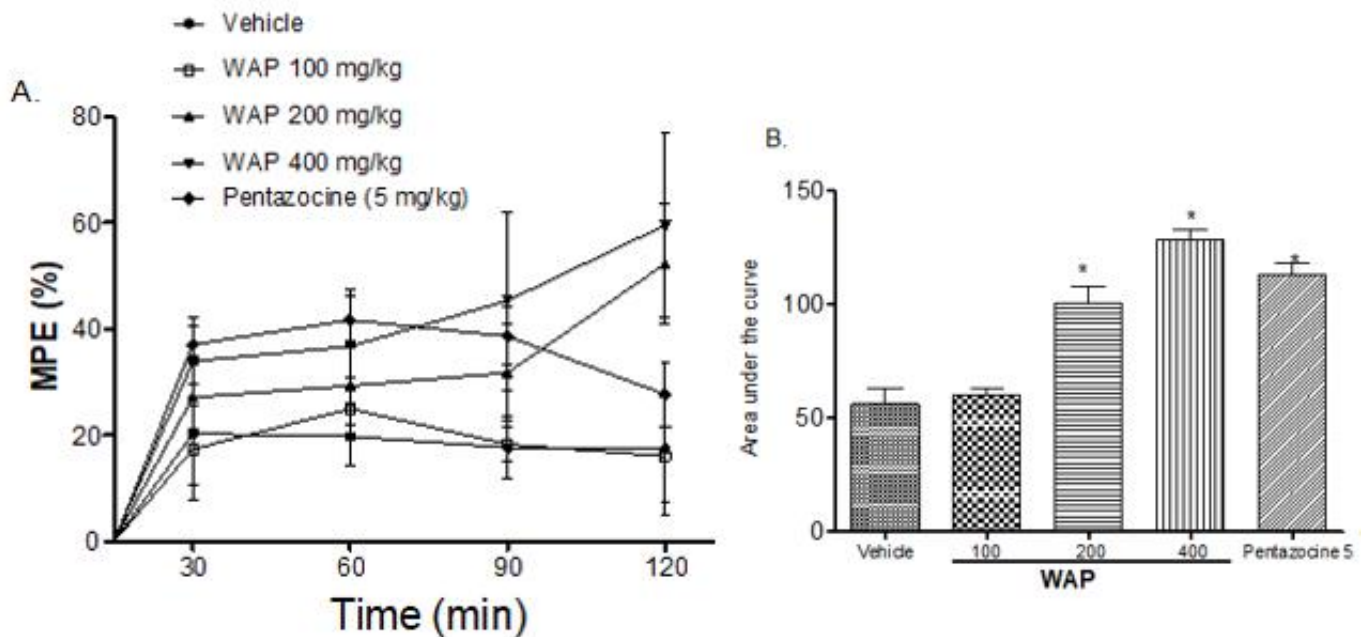


Figure 3: Antinociceptive effect of WAP in hot plate (A) Percent maximal possible effect (B) Area under the curve. Data represent Mean \pm SEM of five mice, values in parenthesis are percentages of inhibition.** $p < 0.01$, by 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group.

WAP is not ulcerogenic to mice stomach: Assessment of the ulcerogenicity of WAP (400 mg/kg) in mice showed that the

extract did not cause damage to the gastric mucosa. Indomethacin (40 mg/kg) did showed gross macroscopic lesions and bleeding in the stomach (data not shown).

Antioedema effect of WAP in carrageenan-induced rat paw oedema: Intraplantar injection of carrageenan to rats paw resulted in time dependent increase in paw volume for a period of 1-5 h post carrageenan injection (Fig A). Pretreatment of rats with WAP in doses of 100, 200 and 400 mg/kg significantly inhibited the carrageenan-induced increase in the oedema volume of the paws in a dose dependent manner. The percentage of inhibition showed that WAP (100, 200, and 400 mg/kg) inhibited increase in paw volume dose dependently by 23.5, 27.3 and 31.4%, respectively (Fig 5B). Similarly, indomethacin-treated group showed significant anti-oedema effect (31.2%).

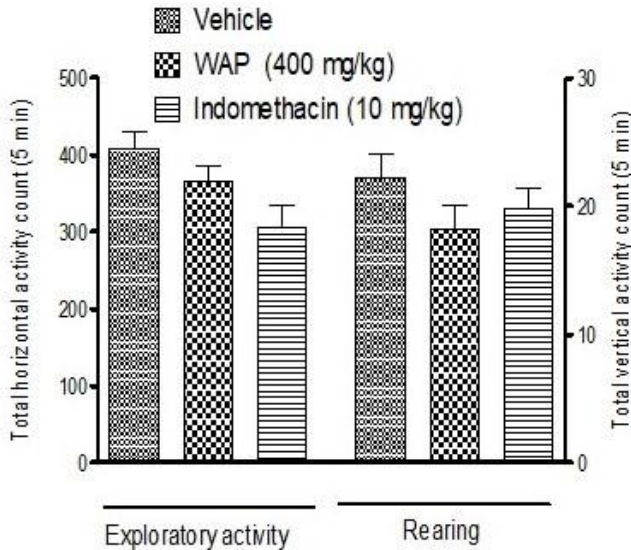


Figure 4: Open field activity: Total horizontal activity count during the five-minute test period.

Anti-inflammatory effect of WAP on exudate volume, protein concentration and leucocyte migration in inflammation induced by carrageenan in rat air pouch

A significant increase in exudates volume and protein concentration was observed in the animals bearing air pouches injected with carrageenan, as compared to the air pouch injected with normal saline (Table 1). Administration of WAP in doses of 200 and 400 mg/kg resulted in a significant decrease in the exudates volume (37.2 and 44.1%) and protein concentration (42.0 and 54.1%), respectively. In response to treatment with indomethacin, a significant decrease in the exudates volume (48.9%) and protein concentration (81.4%) was observed.

Injection of carrageenan into the air pouch caused a significant increase of 95.9% in the number of leucocytes that migrated into the pouch. Pre-treatment with 200 and 400 mg/kg of WAP resulted in the inhibition of leucocytes migration by 59.1% and 60.2%, respectively (Table 1). WAP further caused a significant reduction in the neutrophil and monocytes count when compared with carrageenan control. WAP at doses of 200 and 400 mg/kg pre-treatment resulted in a significant reduction in neutrophils by (57.3 and 58%) and

monocytes by (60.6 and 69.7%), and lymphocytes (64.6, 66.0%), respectively.

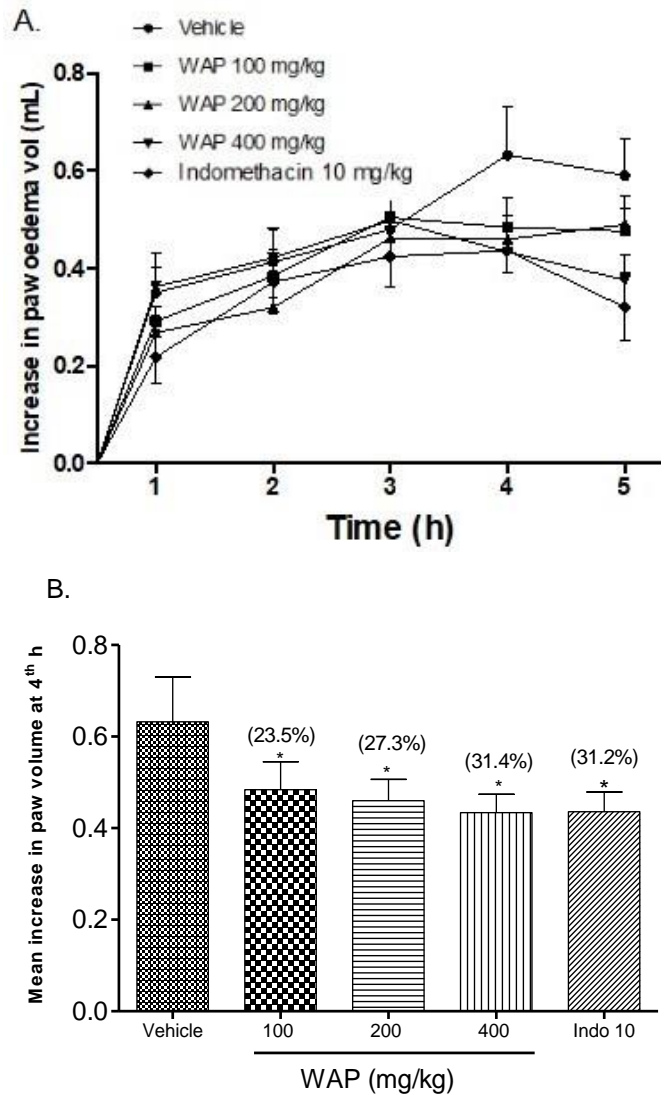


Figure 5: Anti-oedema effect of WAP (100, 200, 400 mg/kg) carrageenan-induced rat paw oedema. (A) Increase in rat paw oedema (B) Percent inhibition at 4th hour. Data represent Mean \pm SEM of five rats, values in parenthesis are percentages of inhibition.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, by 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group

Effects of WAP on nitrite level, index of lipid peroxidation and reduced glutathione in carrageenan-induced air pouch.: Injection of carrageenan into the air pouch caused about 100-fold increase in nitrite (an indirect measure of NO) production compared to the saline injected animals (group 1). Pretreatment with WAP in doses of 200 and 400 mg/kg showed significant reduction of nitrite level by 42.0 % and 42.2% respectively compared to carrageenan group (Table 2).

Table 1:

Effect of WAP on exudate volume, protein concentration and leucocytes migration in the inflammation induced by carrageenan in the rat air pouch

Parameters	Saline	Carrageenan	WAP (200 mg/kg)	WAP (400 mg/kg)	Indomethacin (10 mg/kg)
Exudates volume (mL)	0.20 ± 0.05	3.62 ± 0.18 [#]	2.28 ± 0.25 ^{***}	2.03 ± 0.14 ^{***}	1.85 ± 0.24 ^{***}
Protein concentration (mg/mL exudates)	0.37 ± 0.19	177.5 ± 7.34 [#]	102.9 ± 4.85 ^{***}	81.5 ± 15.40 ^{***}	33.03 ± 4.21 ^{***}
Total leucocytes (x ¹⁰ /mL exudates)	4.13 ± 0.24	100.9 ± 6.98 [#]	41.3 ± 1.94 ^{***}	40.25 ± 3.00 ^{***}	54.27 ± 2.21 ^{***}
Neutrophils (x ¹⁰ /mL exudates)	2.91 ± 0.15	67.92 ± 6.63 [#]	28.95 ± 2.28 ^{***}	28.49 ± 3.53 ^{***}	37.29 ± 1.47 ^{**}
Monocytes (x ¹⁰ /mL exudates)	0.13 ± 0.01	4.36 ± 0.60 [#]	1.72 ± 0.10 ^{***}	1.32 ± 0.15 ^{***}	1.74 ± 0.04 ^{***}
Lymphocytes (x ¹⁰ /mL exudates)	1.05 ± 0.10	27.92 ± 0.65 [#]	9.86 ± 0.90 ^{***}	9.46 ± 0.53 ^{***}	14.32 ± 1.04 ^{***}

p < 0.001 vs saline. ** *p* < 0.01, ****p* < 0.001 vs carrageenan control, by 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group.

Table 2:

Antioxidants effects of WAP in the carrageenan-induced air pouch in rats

Treatment	Nitrites (µM/ mL exudates)	MDA (ηM of MDA/ mL exudates)	GSH (µM GSH/ mL exudates)
Saline	0.03 ± 0.01	0.28 ± 0.05	0.28 ± 0.05
Carrageenan	3.81 ± 0.28 [#]	2.96 ± 0.13 [#]	0.22 ± 0.02
WAP (200 mg/kg)	2.21 ± 0.27 ^{***}	1.31 ± 0.17 ^{***}	0.37 ± 0.04 [*]
WAP (400 mg/kg)	2.20 ± 0.22 ^{***}	1.46 ± 0.12 ^{***}	0.49 ± 0.02 ^{***}
Indo (10 mg/kg)	1.08 ± 0.19 ^{***}	0.92 ± 0.05 ^{***}	0.59 ± 0.04 ^{***}

p < 0.001 vs saline. ** *p* < 0.01, ****p* < 0.001 vs carrageenan control, by 1-way ANOVA followed by Newman-Keuls Multiple comparison post hoc test compared to vehicle group

Indomethacin (10 mg/kg) treatment significantly lowered nitrite level by 71.7%. LPO was significantly elevated in carrageenan-injected groups compared to saline injected control. However, WAP (200 and 400 mg/kg) significantly reduced lipid peroxidation and prevented carrageenan-induced depletion of GSH. Indomethacin (10 mg/kg) performed better than WAP at 200 and 400 mg/kg.

DISCUSSION

The results of the present study revealed that WAP possesses antinociceptive and anti-inflammatory activities in models of nociception and inflammation in rodents.

Result of the acute toxicity test of WAP showed that there was no mortality or any significant change in the behavior of the mice recorded at the dose of 2000 mg/kg. Although there was no death recorded during the period of observation, but we observed a significant reduction in body weight gained in WAP-treated male mice compared to control. Based on the results of the preliminary toxicity testing, we can safely say the LD₅₀ is larger than 2000 mg/kg. There has been no other toxicity studies report on this polyherbal formulation containing roots and leaves of *Plumbago zeylanica* and fruits of *Capsicum frutesens*. Acute toxicity tests provide

preliminary information on the toxic nature of a material for which no other toxicological information is available.

WAP showed significant antinociceptive effect in the acetic acid-induced abdominal writhing test. This is a model of visceral pain associated with the release of biogenic amines (e.g., histamine and serotonin), bradykinin, prostaglandin E2 and PGF_{2α} which caused activation of visceral nociceptors (Duarte *et al.*, 1988). Non-narcotic analgesics such the non-steroidal anti-inflammatory drugs (NSAIDs) showed potent antinociceptive effects in this model (Rossato *et al.*, 2015). The effect in acetic acid-induced writhing revealed that WAP antinociceptive effect involves peripheral, spinal and supraspinal inhibition of pain. Similarly in the hot plate test, WAP demonstrated significant antinociceptive effect by increasing the latency time. The hot plate test is considered to be selective for centrally acting antinociceptive compounds, like morphine, while peripheral acting analgesics are known to be inactive on this kind of thermal stimulus. There are reports of anti-nociceptive effect of plumbagin from *Plumbago zeylanica* (Sheeja *et al.*, 2010) and capsaicin from *Capsicum frutesens* fruits extract (Mousseau *et al.*, 1994; Jolayemi and Ojewole, 2014). Reduction in the number of writhing and the increase maximal possible effect to thermal pain suggests that the polyherbal formulations might be acting peripherally and centrally in alleviating pain.

There has been reports of effects of the roots and leaves of *P. zeylanica* on the central nervous system (Sharma and

Kaushik, 2014). Therefore in order to rule out the chances of false positive effect of WAP (400 mg/kg), we evaluated its effect on spontaneous locomotory function in the activity meter cage. WAP insignificantly reduced locomotory activity but no effect was observed on rearing activity. The results revealed that the observed antinociceptive effect of WAP was not as a result of sedation or impairment of motor activity in mice.

Fruits of *capsicum frutescens* is suspected to have ulcerogenic potential, but there has been no confirmation to these claims. On the other hand, there is scientific claim on the antiulcer properties of roots of *P. zeylanica* (Agbaje and Adeniran, 2008). We evaluated the ulcerogenic potential of WAP in mice. Unlike indomethacin, acute dose of WAP (400 mg/kg) did not caused gastric mucosal damage after oral administration. These results suggest that acute dosage of WAP is safe on the gastrointestinal tissue when compared with indomethacin. NSAIDs are highly associated with gastrointestinal distress causing peptic ulceration and bleeding (Al-Sayed and El-Naga, 2015).

The present study revealed that WAP possesses significant anti-inflammatory potential in carrageenan-induced rat paw oedema. This model was taken as a prototype of the exudative phase of inflammation and routinely used for evaluating the anti-inflammatory activity of natural products (Della Loggia *et al.*, 1986). Development of oedema is biphasic, the first phase is due to release of histamine and serotonin for 1 h, after which increase vascular permeability is maintained by the release of kinins up to 2.30 h. The second phase from 2.30 h to 6 h appeared to be due to release of prostaglandins, this phase is closely associated with the migration of leucocytes in the inflamed site (Di Rosa *et al.*, 1971).

WAP produced statistically significant reduction of carrageenan-induced paw oedema with comparable activity to indomethacin (10 mg/kg). The initial phase of oedema, which is not inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin or aspirin, has been attributed to the release of histamine, 5-hydroxytryptamine (5-HT) and bradykinin. The second accelerating phase of swelling has not only been correlated with the elevated production of prostaglandins, but also has been attributed to the induction of inducible cyclooxygenase (COX-2) in the hind paw (Posadas, 2004). The ability of WAP to reduce increase in paw oedema in the later phase showed that it might be suppressing the release of inflammatory mediators like prostaglandin E₂. Sheeja *et al.*, (2010) made similar observation on anti-inflammatory effect of crude extract and fraction of *P. zeylanica* in carrageenan-induced rat paw oedema.

In another study, anti-inflammatory properties of WAP was evaluated in carrageenan-induced air pouch in rats. The air pouch model of inflammation serves as an important model that can be used to assess the inhibition of increase vascular permeability, the suppression of leucocytes migration and activation, the inhibition of release of inflammatory mediators (NO, TNF- α , IL-1 β , PGE₂), as well the in vivo antioxidant markers from the inflammatory exudates (Martins *et al.*, 1994).

In this study, WAP (200 and 400 mg/kg) reduced exudates volume, protein and nitrite accumulation in the

exudates. Carrageenan is known to evoked increase in vascular permeability, with consequent increase in protein leakage and the accumulation of nitric oxide. Nitric oxide is produced from L-arginine by nitric oxide synthase and involved in various pathophysiological processes including inflammation and septic shock (Raetz, 1993). A drug capable of preventing in vivo release of pro-inflammatory mediators like NO could potentially possess anti-inflammatory activities. In this study, we found that WAP at 200 and 400 mg/kg dose dependently inhibited the production of NO in carrageenan-induced air pouch.

Local neutrophil infiltration and activation contributes to the carrageenan inflammatory response by producing, among other mediators, oxygen- derived free radicals such as superoxide anion (O₂⁻) and hydroxyl radicals (Salvemini *et al.* 1996). WAP suppressed leucocytic cells recruitment from the vessels into the area of inflammation. It significantly reduced the total leucocyte, neutrophil and monocytes numbers in the exudates. Some other studies have shown inhibition neutrophil migration and activation as a key step in modulating the progression of acute of inflammation (Rosa *et al.*, 2015).

More evidences now abounds suggesting the involvement of free radicals in the pathogenesis of inflammation (Gutteridge, 1995). Free radicals-induced cell damage involves lipid peroxidation, which is implicated in the pathogenesis of the inflammatory process. In view of this we tested the possibility of the WAP in reducing carrageenan-induced lipid peroxidation in vivo. The result revealed that WAP reduced the level of lipid peroxidation and as well prevented the depletion of reduced glutathione. This indicates that the extract prevents formation of hydrogen peroxide and superoxide anion possibly by its free radical scavenging properties.

In conclusion, the overall results demonstrated extracts containing leaves and roots of *Plumbago zeylanica* and dried fruits of *Capsicum frutescens*, possess both central and peripheral antinociceptive activities, and is safe on the gastrointestinal tissues and exhibited significant anti-inflammatory and antioxidant activities in acute inflammation.

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