Anti-inflammatory Effect of Aqueous Extract of Coffee plant leaves (Coffea canephora) in Rats.

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Abstract
The anti-inflammatory and analgesic activities of aqueous extract of Coffea canephora leaves was investigated in rats. Egg albumin- induced hind paw oedema was used to assess the anti- inflammatory activity of the extract. The results of the anti-inflammatory study revealed that 60, 120 and 180 mg/kg of the extract reduced the formalin-induced oedema significantly (P<0.05) at the beginning of 2 hours when compared to the control group. The present study showed that the aqueous extract of Coffea canephora has anti-inflammatory activity that could be mediated via modulators of pain and inflammation.

Keywords: coffea canephora, anti-inflammatory.

Introduction
Inflammation is a response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, cell migration, tissue breakdown. Inflammation is a protective response of tissues to injury, intended to eliminate the initial cause of cell injury as well as necrotic cells and tissue resulting from the original insult (Mitchell et al, 2002).Inflammation may also be described as complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissues would compromise the survival of the organism. Although it is a defence mechanism, the complex events and mediators involved in inflammatory reaction can be induced, maintained and aggravated by many diseases (Malaya et al., 2003).

Coffea canephora also known as Robusta coffee is a variety of coffee, which has its origins in central and western sub-Saharan Africa. It is a species of flowering plant in the Rubiaceae family. Though widely known as Coffea robusta, the plant is scientifically identified as Coffea canephora, which has two main varieties - Robusta and Nganda (Dagoon, 2005).
The plant has a shallow root system and grows as a robust tree or shrub to about 10 metres. It flowers irregularly, taking about 10–11 months for cherries to ripen, producing oval-shaped beans. The robusta plant has a greater crop yield than that of C. arabica, and contains more caffeine - 2.7% compared to arabica's 1.5% (Nesbitt 2005). It is also said to be less susceptible to pests and disease, therefore it needs less herbicide and pesticide than Arabica (Benoit and Stefano, 2005). Approximately 20% of the coffee produced in the world is robusta (coffeeresearch.org, 2007).

In a Dutch study of dietary which was carried out on over 40,000 participants with a mean follow up of over ten years it was found that taking over three cups of coffee a day reduces the risk of type 2 diabetes mellitus(van Dieren et al 2009). This finding was confirmed in a randomised control study published in 2011. They found no changes in glucose metabolism following coffee consumption, but saw significant improvements in fat metabolism and liver function over a 10 week period, comparing coffee consumption with non-coffee beverages. This study confirmed the “beneficial metabolic effects of long-term coffee consumption” (Wedick et al 2011). They also commented on the possible anti-inflammatory effects of coffee, and its hepato-protective property. In this work we investigated the anti-inflammatory effect of the aqueous extract of leaves of Coffea canephora in rats.

MATERIALS AND METHODS:
Plant Materials
Coffea canephora was purchased from a local supplier in Jos, Plateau State, Nigeria on December 2012
Preparation of Extract
The fresh spiny leaves of *Coffea canephora* were dried under a shade and reduced to a coarse powder using a mortar and 50g of the powder was soxhlet extracted with 250 ml of distilled water at 100°C for 72 hours. The extract was slowly evaporated to dryness using a rotary evaporation at 40°C to yield 6.18% W/V of dry weight of residue which was stored at –4°C until use.

Animals
Wistar rats of either sex (weighing 100-140g) were obtained from the animal house unit of University of Jos, Jos Nigeria. The animals were housed under standard environmental conditions and fed and water provided *ad libitum*.

Anti-inflammatory screening
The anti-inflammatory activity of the leaf extract of *Coffea canephora* was investigated using egg albumin induced rat paw oedema test. 25 wistar rats were randomized into five groups of five. Animals were deprived of food overnight and water only during the experiment to keep them in a fasting state. The grouping was based on the paw volume measured at 0 minute. Group 2, 3 and 4 were pre-treated with 60 mg/kg, 120 mg/kg and 180 mg/kg body weight respectively. Group 5 was given the standard drug (300 mg/kg Aspirin) and group 1 the negative control received normal saline 10 ml/kg orally, 30 minutes before administration of 0.1 ml of egg albumin into the right hind paw of each rat in the sub plantar region to induce inflammation; Oedema was assessed in terms of the linear diameter at the injected hind paw using veneer calliper, which was used to estimate the degree of inflammation and inhibition of oedema at 0 hr and was measured subsequently at an interval of 20 min for the total duration of 120 minutes.

Statistical Analysis
Data are expressed as mean ± standard error of mean (SEM) and analysed using the ANOVA. *P* < 0.05 was accepted as significant.

RESULTS
The effects of aqueous extract of the leaves of *Coffea canephora* on egg albumin-induced hind paw oedema in rats are shown as a graphical representation (figure 1). The experiment revealed significant difference between rat groups treated with the extract and that of the control.

Graphical representation of the effect of the aqueous extract of *Coffea canephora* leaves on egg albumin induced rat paw oedema.

Discussion:
The genetic elimination of A2A adenosine receptors has been shown to disengage the critical immune-suppressive mechanism and cause the dramatic exacerbation of acute inflammatory tissue damage by T cells and myeloid cells.

Caffeine consumption is typically chronic in humans it was therefore interesting to examine whether acute consumption of *coffe canephora* leaves affects the outcome of inflammation. In contrast to an acute administration, chronic consumption of caffeinated water alone (without an acute dose) failed to augment the tissue damage.
The anti-inflammatory screening of *Coffea canephora* leaves was carried out using the egg albumin induced oedema test, which is a widely accepted model for the evaluation of anti-inflammatory effects of drugs (Abbah *et al.*, 2010).

Egg albumin is used to screen agents with anti-inflammatory effect especially if inflammation was not intended to be sustained for long. (Osadepe *et al.*, 2006). Inflammation is elicited through prostaglandin synthesis the most frequently encountered mechanism of action among anti-inflammatory drug. (Vane, 1971) Inflammation is always accompanied by pain. Inflammation and pain are linked by cyclooxygenase enzymes, most especially cox2 which help in the synthesis of prostaglandins most especially PGE2 AND PGF2α which occurs in high concentration at the inflamed site. Prostaglandin itself does not cause pain but acts indirectly either by stimulating or by sensitizing the nociceptors to the pain producing substances released from the damaged tissue of the inflamed area.

Results from the study showed that the aqueous extract exerts a time dependent decrease in rat paw oedema. The significant oedema reduction started at 20 minutes after administration at 120 mg/kg. There was an appreciable reduction in the paw size over a period of 2hours and percentage inhibition rose from 20% at 20 minutes to as high as 50% at 120 minutes, with 120 mg/kg showing a more time dependent decrease in paw volume. Compared to the negative control, 60 mg/kg showed the least inhibitory effect at an inconsistent reduction in paw volume. The extract produces a significant dose dependent decrease in paw volume over time. This could be due to a quick onset of action followed by a slow elimination process. At time interval of 2hrs, the extract produced as significant inhibition (p<0.05) of the egg albumin induced edema at all doses used including Aspirin.

The effect of the extract would most likely be as a result of caffeine present in the leave of *Coffea canephora*. Caffeine is a methyl xanthine, and methyl xanthine is known to have anti-inflammatory properties. This is due to the similarity in molecular structure to the nucleotide adenosine. Caffeine blocks the action of adenosine by action at a competitive inhibitor for the A1 and A2a adenosine receptor. It is also possible that one of the mechanism of anti-inflammatory activity of the extract maybe due to inhibition of the Cyclooxygenase enzyme and reduction of prostaglandins synthesis.

**Conclusion**

The study showed that the extract of *Coffea canephora* leaves possessed anti-inflammatory activities. Its anti-inflammatory activity may be attributed to the blockade of adenosine as a competitive inhibitor for the A1 and A2a adenosine receptor; inhibition of Cyclooxygenase enzyme and reduction in prostaglandins synthesis.

**References**