

## The Effect of Aqueous Extract of Leaf of *Ficus capensis* Thunb (Moraceae) on *in Vivo* Leukocyte Mobilization in Wistar Rats

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**Abstract:** Immune system (the body's defense system which protects the body from diseases), is subject to modification by substances to either enhance or suppress its ability to resist invasion by pathogen. *Ficus capensis* Thunb. (Moraceae), a wild fig tree, is believed by the Igala people of Kogi State in Nigeria to possess an immune boosting property, hence, forming part of most of their traditional remedies for several ailments. This study was aimed at investigating, so as to ascertain this claim. Twenty wistar strain albino rats divided into four groups of five animals each were used. One hour prior to introduction of an inflammatory stimulus, each rat in groups (Group 2-4) received oral administration of 100, 150 and 250 mg/kg, respectively of aqueous extract of leaf of *Ficus capensis* Thunb (Moraceae). The control group (Group 1) received distilled water. After four hours, the animals were sacrificed and both Total and Differential Leukocyte Counts were performed on the peritoneal fluid obtained from these animals. Evaluation of the data obtained from this study indicated a significant ( $p < 0.05$ ) dose-dependent increase in leukocyte mobilization, with doses 150 and 250 mg/kg giving total leukocyte count of  $4.44 \pm 0.39 \times 10^9$  and  $6.10 \pm 0.86 \times 10^9/L$ , respectively, the most mobilized being Neutrophils. The results obtained from this study suggest that the extract might have a pharmacologically active substance which may be responsible for the above effect and its applications in traditional medicines as an immune boosting agent.

**Keywords:** *Ficus capensis* thunb, immunity, inflammation, leukocytes mobilization, peritoneum

### INTRODUCTION

The stress and pressure of modern society take a toll on immune system. Those with weakened immunity are more susceptible to infection and disease. The need to maintain or rebuild a healthy defense has led researchers to minerals, plants and fungi in search of natural substances with health-supporting properties (Nworu *et al.*, 2007). Plants have been used safely and effectively to enhance wellness from time immemorial and, in olden days, herbal preparations from roots, leaves, flowers and barks were the only remedies available to our ancestors (Sofowora, 2003). Herbal preparations have been known to the people over a long period of time and so, they have more faith in their therapeutic capacities than they do for the conventional medicines which are new to them (Yusuf *et al.*, 2003). Traditional medicinal uses of plants indicate the presence of biological active constituents in plants; these bioactive constituents have provided inspiration in the development of an impressive number of synthetic drugs, serving as a lead for the discovery of modern medicines (Alves and Rosa, 2005). Compounds that are capable of interacting with the immune system to up

regulate or down regulate specific aspects of the host response are classified as *Immunomodulators* (Nworu *et al.*, 2007). Those compounds which appear to stimulate the human immune response are being sought for the treatment of cancer, immunodeficiency diseases, or for generalised immune-suppression following drug treatment; for combination therapy with antibiotics and as adjuncts for vaccines (Jong *et al.*, 1983; Jong and Birmingham, 1992). Those compounds that suppress immune reactions are potentially useful in the remedy of autoimmune (an abnormal immune response against self-antigens) or certain gastro-intestinal tract diseases (e.g., Crohns) (Badger, 1983). Leukocytes, or white blood cells, are responsible for the defense of the organism, hence, forming the immune system (Daniela and Giorgio, 1997). The human immune system is highly complex and consists of two categories of defense mechanisms - the innate (non-specific) and the adaptive (specific) systems (Atal *et al.*, 1986; Guyton and Hall, 2006). These two mechanisms could be modified by substances to either enhance or suppress their ability to resist invasion by pathogens (William, 2001). The research work is therefore aimed at investigating the effect of aqueous extract of the

plant *Ficus capensis* Thunb. (Moraceae) on *in vivo* leukocyte mobilization in albino rats treated with inflammatory stimulus.

## MATERIALS AND METHODS

**Animals:** Adult Wistar strain albino rats (130-200 g) of both sexes, purchased from the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria were used for the study. The animals were housed in wire mesh cages under environmental conditions of 28±2°C and 12 h light/dark cycles. They were fed with standard pellets and had unrestricted access to clean drinking water.

**Plant material:** The fresh leaves of *Ficus capensis* Thunb. (Moraceae) were collected in May 2011 from a tree within Samaru campus of the Ahmadu Bello University, Zaria. The plant specimen was authenticated by taxonomist U.S Gallah at the herbarium of the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria. The voucher number of the plant is 6803. The leaves were air-dried and then grounded into powder.

**Preparation of plant extract:** The powdered material (230 g) of *Ficus capensis* Thunb. (Moraceae) was macerated with distilled water (1.2 L) and left for 72 h. The mixture was stirred at intervals using a sterile glass rod. The extract was filtered and the filtrate was evaporated to dryness at 40°C with the aid of a rotary evaporator attached to a vacuum pump. The concentrated extract was stored in air tight containers prior to use.

**Chemicals:** These included phosphate buffered saline (ph 7.4 containing; 8 g/L NaCl, 0.20g/L KCl, 1.44g/L Na<sub>2</sub>HPO<sub>4</sub> • 2H<sub>2</sub>O, 0.24 g/L KH<sub>2</sub>PO<sub>4</sub>), Normal saline (containing 0.9% NaCl), chloroform, Turks solution (White blood cell fluid), Leishman's stain, buffered distilled water (ph 6.8).

**Experimental design:** Twenty adult wistar strain albino rats of both sexes (weighing between 130 and 200 g) were divided into four groups of five animals each as follows:

- Group 1:** Received 1ml of distilled water
- Group 2:** Were administered with 100 mg/kg b w of aqueous extract of *Ficus capensis*
- Group 3:** Were treated with 150 mg/kg b w of aqueous extract of *Ficus capensis*
- Group 4:** Received 250 mg/kg b w *Ficus capensis*, respectively via oral route.

**Evaluation of *In vivo* leucocytes mobilization:** The effect of the aqueous extract of leaves of *Ficus capensis* Thunb. (Moraceae) on *in vivo* leucocytes migration induced by inflammatory stimulus was investigated using the methods of Ribeiro *et al.* (1991), as modified by Nworu *et al.* (2007). One hour after oral administration of graded doses 100, 150 and 250 mg/kg body weight of the aqueous leaves extract of *Ficus capensis* Thunb. (Moraceae) to each albino rat in the groups (Group 2-Group 4), including those in (Group 1) being the control group received intraperitoneal injections of 1mL of 3% (w/v) agar suspension in normal saline. Four hours later, all the animals from all groups were sacrificed and the peritoneum washed with 5 mL of a 5% solution of Phosphate Buffered Saline. The peritoneal fluid was collected into EDTA bottles and total and differential leucocytes counts were performed on the perfusates.

**Determination of total and differential leukocyte count:** Total and differential leukocyte count were carried out by method described by Dacie and Lewis (1991). Briefly, 20 µL of the perfusate was drawn up into the Pasteur pipette after which 380 µL of the Turk's solution (White blood cells diluting fluid) was sucked up to mix with the above volume. The counting chamber was cleaned and the cover slip mounted on it. The diluent (i.e. the diluted sample containing Turk's solution and perfusate) was mixed and later introduced into the counting chamber and then allowed to stand for 2 min. The setup was then mounted on a light microscope and the cells counted in four large squares of the counting chamber using × 10 magnification. To determine differential leukocyte count, Leishman staining technique was used. A drop of the fluid (20 µL) was placed on one end of a grease-free glass slide using an applicator. Another glass slide (spreader) was used to make a smear of the fluid on the glass slide using the push wedge technique, (i.e., the spreader was placed at an acute angle -45° in front of the fluid until the fluid ran along the edge of the spreader, then, the spreader was moved forward with an even movement and a fine film of the fluid was obtained). The film was allowed to dry after which Leishman's stain was used to cover the film and allowed to stand for 2 min. Thereafter, buffered distilled water of PH 6.8, (two times the quantity of Leishman's stain used) was used to flood the film. The setup was allowed to stand for 8 min after which the stain was rinsed with more buffered distilled water of PH 6.8. The slide was left to dry and then examined on the microscope using the oil immersion objective lens of ×100 magnification. The cells were counted and differentiated on morphological basis using tally counter and results expressed in percentage.

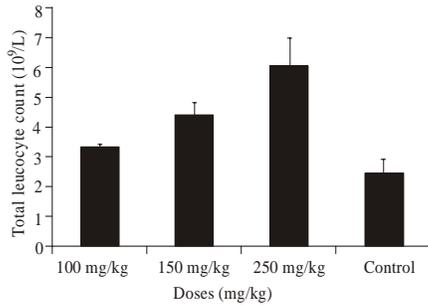


Fig. 1: Effect of aqueous extract of leaf of *Ficus capensis* thunb. (Moraceae) on mobilization of total Leukocytes. Values are presented as mean±SEM (Bars represent mean ± SEM) for five animals in each group; Values are statistically significant compared to control group at <sup>a</sup>: p<0.05; ns not significant

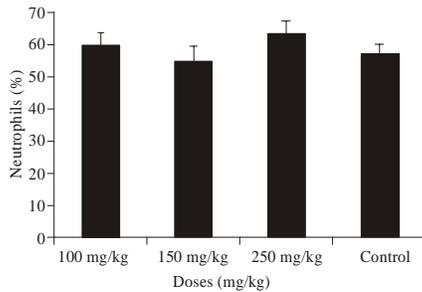


Fig. 2: Effect of aqueous extract of *Ficus capensis* thunb. (Moraceae) on mobilization of neutrophils. Values are presented as mean±SEM (Bars represent mean±SEM) for five animals in each group; Values are statistically significant compared to control group at <sup>a</sup>: p<0.05; ns: not significant

**Statistical analysis:** Data were expressed as mean±SEM. Statistical significance (considered at p<0.05) between the various groups was determined by One-way analysis of variance (ANOVA) using Post Hoc Tests.

## RESULTS

In this study an acute model of agar induced peritonitis was used to examine the effect of Aqueous extract of leaf of *Ficus capensis* Thunb. (Moraceae) on leukocyte mobilization from bone marrow and their recruitment to the site of inflammation. The plant extract was observed to increase leukocyte mobilization in all the treated groups. A significant leucocyte mobilization (p<0.05) of the extract was observed in the groups treated with 150 and 250 mg/kg b w doses of the plant extract, each yielding total leukocyte count of 4.44×10<sup>9</sup> and 6.10 ×10<sup>9</sup>/L, respectively when compared to control (Fig. 1). However, the most mobilized being neutrophils at dose of 250 mg/kg b w. Although the effect of the plant extract at doses 100 and 150 mg/kg b w showed no

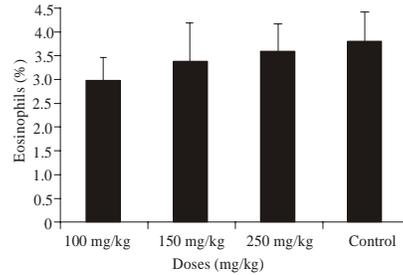


Fig. 3: Effect of aqueous extract of leaf of *Ficus capensis* thunb. (Moraceae) on mobilization of eosinophils. Values are presented as mean±SEM (Bars represent mean±SEM) for five animals in each group; Values are statistically significant compared to control group at <sup>a</sup>: p<0.05; ns not significant

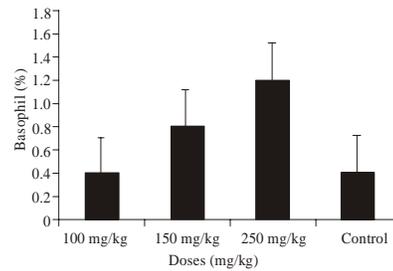


Fig. 4: The effect of aqueous extract of leaf of *Ficus capensis* thunb. (Moraceae) on mobilization of basophils. Values are presented as mean ± SEM (Bars represent mean±SEM) for five animals in each group; Values are statistically significant compared to control group at <sup>a</sup>: p<0.05; ns not significant

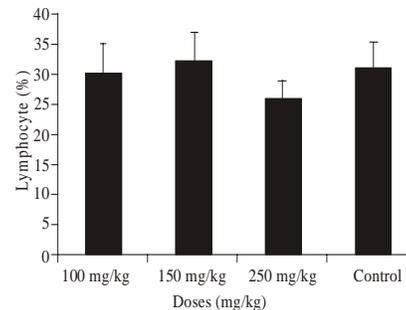


Fig. 5: The effect of aqueous extract of leaf of *Ficus capensis* thunb. (Moraceae) on mobilization of lymphocytes. Values are presented as mean±SEM (Bars represent mean±SEM) for five animals in each group; Values are statistically significant compared to control group at <sup>a</sup>: p<0.05; ns not significant

statistically significant difference (p>0.05) when compared to control group (Fig. 2). In addition, there was no statistically significant (p>0.05) differential leukocytes count in all groups administered with various doses of the plant extract 100, 150 and 250 mg/kg b w when compared to control group (Fig. 3-6).

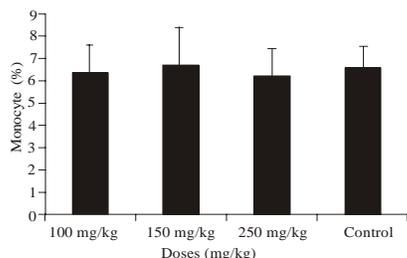


Fig. 6: The effect of aqueous extract of leaf of *Ficus capensis* thunb. (Moraceae) on mobilization of monocytes. Values are presented as mean±SEM (Bars represent mean±SEM) for five animals in each group; Values are statistically significant compared to control group at <sup>a</sup>: p<0.05; ns not significant

## DISCUSSION

Immunity or resistance to infection is derived from the activity and intact functioning of two tightly interrelated systems, *the innate immune system* and the *adaptive immune system*. Elements of the innate or natural immune system include exterior defenses, such as skin and mucous membranes; phagocytic leukocytes; and serum proteins, which act nonspecifically and quickly against microbial invaders. Microbes that escape the onslaught of cells and molecules of the innate immune system face destruction by T and B cells of the adaptive or acquired immune system. Activation of the adaptive immune system results in the generation of antibodies and cells that specifically target the inducing organism or foreign molecule. Unlike the innate system, adaptive or acquired immune responses develop gradually but exhibit memory. Therefore, repeat exposure to the same infectious agent results in improved resistance mediated by the specific aspects of the adaptive immune system (Rhoades and Tanner, 2004). Interestingly, the innate and adaptive mechanisms of the immune system could be modified by substances to either enhance or suppress their ability to resist invasion by pathogens (Nworu *et al.*, 2007). Leukocytes are rapidly mobilised from the bone marrow into the blood during infections or inflammatory reactions. A blood neutrophilia is a characteristic feature of infections and inflammatory disorders, due initially to the rapid mobilisation of neutrophils (being the body's first line of defence) from the bone marrow reserve and their subsequent migration into the tissues (Antje *et al.*, 2008). This is so because, neutrophil granules serve as reservoirs for digestive and hydrolytic enzymes prior to delivery into the phagosome (David *et al.*, 2008), as studies have indicated that azurophilic granule contents possess microbicidal activity and may play an important role in the tissue destruction observed during inflammatory reactions (Spitznagel, 1990; Elsbach and

Weiss, 1998; Ganz *et al.*, 1985). Similar effects have been reported with the methanolic extract of seed of *Garcinia Kola* (Nworu *et al.*, 2007) and the co-ordinated action of G-CSF and ELR+CXC chemokines (Antje *et al.*, 2008), where the later furthered their research work on the factors responsible for such effect and determined that the rapid mobilisation of neutrophils from the bone marrow is driven by the coordinated actions of the CXC chemokines, KC and MIP-2 and the cytokine G-CSF acting via distinct mechanisms. Both G-CSF and GM-CSF have a multitude of pharmacological effects, including increasing the proliferative activity of progenitor cells, shortening the time for neutrophil production and maturation in the marrow, accelerating the release of maturing cells from the marrow to the blood, augmenting the production of neutrophil granule proteins and stimulating the release of proteases and perhaps other constituents from the cells to their surroundings (David *et al.*, 2008). Moreover, Previous studies have documented significant increases in blood levels of chemokines, Granulocyte Colony Stimulating Factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF) in animal models of acute inflammation and in patients with infections, (Metcalf *et al.*, 1996; Shabbazian *et al.*, 2004; Cataisson *et al.*, 2006; Noursadeghi *et al.*, 2002; Sugimoto *et al.*, 2005; Call *et al.*, 2001) suggesting that these chemokines and cytokines may potentially stimulate mobilization of leukocytes, especially neutrophils from the bone marrow during inflammatory reactions. G-CSF was also demonstrated to play a critical role in the leukocytosis caused by bacterial infection (Gregory *et al.*, 2007).

Therefore, it could be deduced from these previous studies that the aqueous extract of the plant (*Ficus capensis* Thunb.) used for this present study has a stimulatory effect on these cytokines and chemokines and probably, regulatory effect on their receptors, which may be responsible for its effect on increase in leukocytes mobilization.

## CONCLUSION

It could be concluded from this study that oral administration of aqueous extract of leaf of *Ficus capensis* Thunb. (Moraceae) to wistar rats led to a dose-dependent increase in leukocyte mobilization when an agar-induced acute inflammatory stimulus was introduced into the animals, suggesting that the extract might have a pharmacologically active substance capable of causing the above effect, therefore, justifying the claim of some ethnic groups in Nigeria, as an immune-boosting agent. Although the data provided in the present work appears to justify the folkloric use of the plant as an immune-boosting agent, the validity of its usefulness needs to be further ascertained.

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