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Evaluation of the Influence of Methanol Leaf Extract of *Ocimum Gratissimum* on Some Immunologic Indices in Albino Rats

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Abstract: *Immunomodulatory activity of methanol leaf extract of Ocimum gratissimum was investigated in immune-suppressed wistar albino rats using immunologic/haematologic indices. The animals were divided into four equal groups. Group 1and 2 received 100 and 300 mg/kg body weight of methanol leaf extract of Ocimum gratissimum respectively. Group 3, the negative control received only 2% pyrogallol while group 4 received only normal feed for 21days after which blood was drawn from ocular puncture for assays. The results showed a significant ($p<0.05$) increase in the total white blood cell count in group 2 compared to the negative control group. Also, there was a non-significant ($p>0.05$) increase, when compared to the negative control in the packed cell volume and haemoglobin levels. There was a significant ($p<0.05$) increase in primary and secondary antibody titre of rats given both low and high doses of the extract compared to the negative control. Equally the delayed type hypersensitivity values showed no significant ($p>0.05$) difference in the test groups compared to the negative control. Therefore, the results of the study showed that immunomodulatory effect of Ocimum gratissimum leaf extract is via stimulation of antibody production and enhanced inflammatory response.*

Key words: *immunomodulatory activity, haematologic indices, Ocimum gratissimum, pyrogallol.*

I. Introduction

The immune system is the body's defence against infectious organisms and other invaders and is made up of a network of cells, tissues and organs that work together to protect the body (Basil *et al.*,1996). The immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated in the form of an immune memory, and allows the adaptive immune system to mount faster and stronger response (Mayer *et al.,* 2006). Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Prolonged inflammation, known as chronic inflammation leads to progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process (Liszewski *et al.*,1996). Inflammation is an important non-specific defense reaction of tissue injury, such as that caused by a pathogen or wound (Prescott *et al.* 2005). Inflammation is one of the first responses of the immune system to infection (Kawai and Akira 2006). Immunomodulators are substances that are capable of interacting with the immune system to upregulate or down-regulate specific aspect of the host response (Stanilove *et al*., 2005; Utoh-Nedosa *et al*., 2009). It is also known as biologic response modifier or immunoregulator which is function as drug leading predominantly to a non-specific stimulation of immunological defense mechanisms (Tzianabos, 2000). These may include some bacterial product, lymphokines and plant derived substances.

Ocimum gratissimum is a rich source of minerals such as iron and copper, and contains eugenol which serves protective role against diseases, (Odemena and Onyeneke, 1998). This vegetable is used as herbal remedy for malaria, as anti-convulsant and against cough, fever and conjunctivitis. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh (Onajobi, 1986). The plant contains iron, magnesium, calcium and vitamins (Salmien *et al.,* 2008).The present study investigated the immunomodulatory activity of methanol leaf extracts of *Ocimum gratissimum* in immune system of wistar albino Rats.

II. Materials And Methods

Plant Sample Collection, Preparation and Extraction

Fresh leaves of *Ocimum gratissimum* were collected from Ogwumabiri market, Abia State, Nigeria and were identified by Dr.Omosun Garuba of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike. The leaves were dried at room temperature and ground to powder using an electric blender (Christy and Norris – 47262, England). A quantity 150g of the powdered leaves was macerated in 600 ml 60% methanol. This was filtered after 48hours and then evaporated to dryness. The residue was kept at 4-8°C used as stock.

EXPERIMENTAL DESIGN

Wistar albino rats were purchased from Empire Farms, Nsukka, Enugu State and weighing between 180 and 200 g were used. They were allowed free access to water and feed *ad libitum.* Sixteen male rats were divided into four equal groups. The first group received 100 mg/kg BW of the extract, the second group received 300 mg/kg BW of the extract, the third and fourth were given 2% pyrogallol and normal saline respectively. Administration lasted for 21 days after which blood sample was obtained from each of the animals and the serum used for the assay.

SRBC – induced humoral antibody (HA) titer

To specifically assess effects on antibody formation, groups of four rats per treatment were immunized with 0.1ml of sheep Red Blood Cell suspension (1.1 x 10^8 SRBC/ml) intra peritoneal. The day of immunization was referred to as Day 0. Seven days later (day 7), the rats were challenged by injecting 0.1ml of SRBC suspension into the left hind foot pad of the rats. Blood samples were collected from all the animals separately by ocular puncture using glass capillary tubes on Day 7 (after challenge) for measurement of primary antibody titer and on day 14 for measurement of secondary antibody titer. Antibody levels were determined by the method described by Shinde *et al.,* (1999). The collected blood was allowed to clot, then centrifuged to get serum. 25microL was placed into one well of a 96-well micro titer plate. Serial two-fold dilutions of the serum were made using 25microL normal saline each time of transfer across the plate. To the 25 microL diluted serum in each well, 25 microL of 1% v/v SRBC suspension in normal saline was added. The micro titer plate was maintained at room temperature for 1 hour and the content of then examined for haemagglutination. The value of the highest serum dilution showing haemagglutination was defined as the antibody titer for the given rat.

SRBC – Induced Delayed Hypersensitivity (DTH) Response

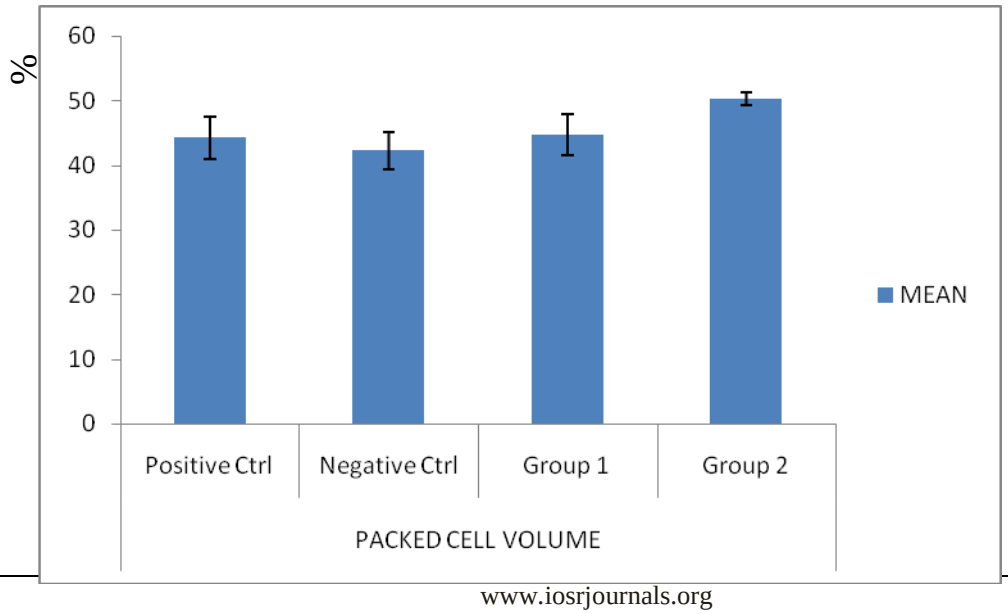
The method of Doherty (1981) was used to assess the effect on DTH responses in the treated rats. Daily treatment with *Ocimum gratissimum* leaf extract began 14 days prior to the challenge (i.e. starting on the same day as immunization with SRBC). The negative and normal control rats received pyrogallol and normal saline respectively each day. On day 0 all rats were immunized. After 14 days of treatment, 0.1ml of SRBC solution was injected subcutaneously into their right hind footpad, the thickness of each rat's left footpad was measured just before the challenge using a Schneltaster caliper that could measure to a minimum unit of 0.01mm. The rats were then challenged by injecting 0.1ml of SRBC solution intra peritoneal into their left hind footpad (deemed time 0). Footpad thickness was the re-measured after. The difference between the thickness of the left footpad just before and 48 hours after challenge (in mm) was taken as a measure of DTH.

Hematological Profile

After 21 days of oral administration, blood was collected from each rat via ocular puncture under light anesthesia. Various parameters such as total white blood cell (TWBC), haemoglobin (HB) levels and packed cell volume (PCV) were estimated using standard haematological techniques as described by Ochei and Kolhartkar (2008)

III. Results

4.0.1 Effect Of Methanolic Extract Of *Ocimumgratissimum* On Packed Cell Volume



Group 1 = 100mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

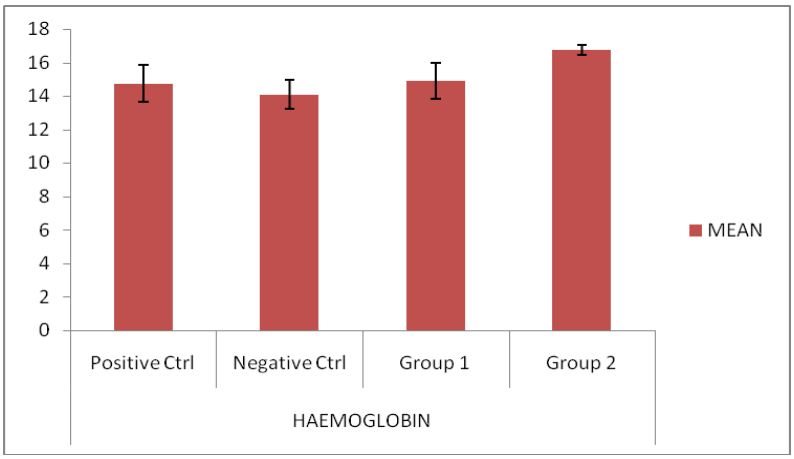
Group 2 = 300mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

Negative Ctrl = 100mg/kg bwpyrogallol administered.

Positive Ctrl = Normal saline administered

The diagram above shows that groups 1 and 2 increases non-significantly (p>0.05) when compared with the negative control.

4.0.2 EFFECT OF METHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* ON HAEMOGLOBIN



Group 1 = 100mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

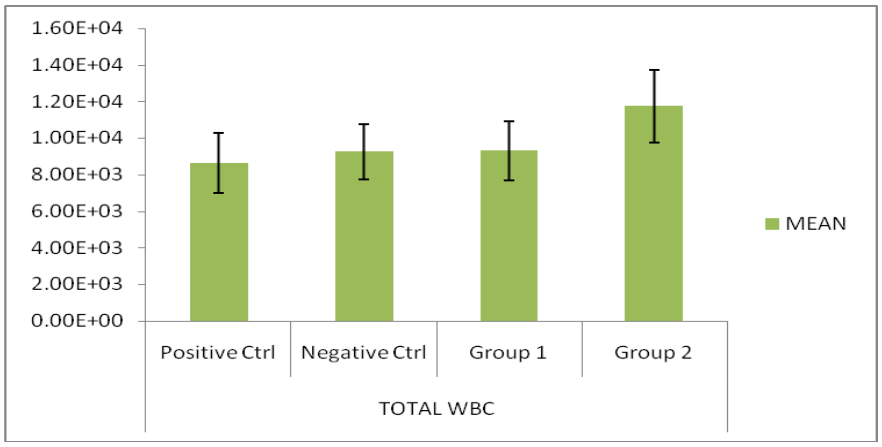
Group 2 = 300mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

Negative Ctrl = 100mg/kg bwpyrogallol administered.

Positive Ctrl = Normal saline administered

Groups 1 and 2 showed a non-significant (p>0.05) increase when compared against the control group.

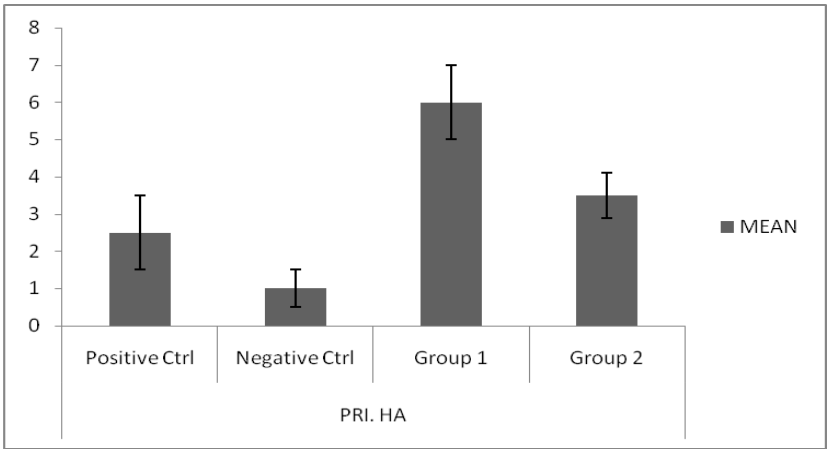
FIG. 3 EFFECT OF METHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* ON TOTAL WBC



Group 1 = 100mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.
Group 2 = 300mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.
Negative Ctrl = 100mg/kg bwpyrogallol administered.
Positive Ctrl = Normal saline administered

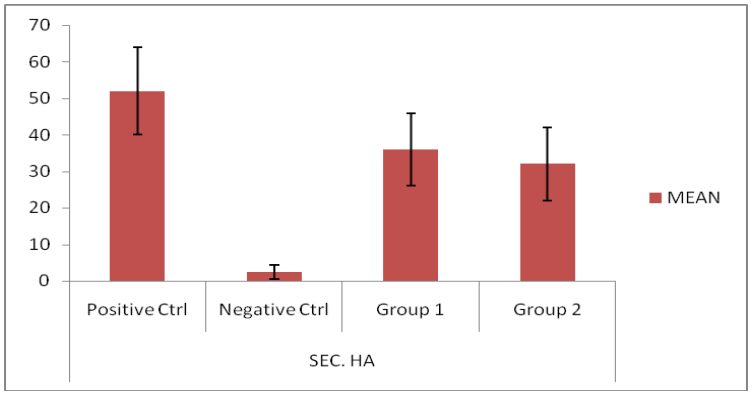
The figure above shows that group 2 increased significantly ($p<0.05$) when compared to the control group, while group 1 shows a non-significant ($p>0.05$) difference when compared against the control group.

FIG. 4 EFFECT OF METHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* ON PRIMARY ANTIBOY TITRE



Group 1 = 100mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.
Group 2 = 300mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.
Negative Ctrl = 100mg/kg bwpyrogallol administered.
Positive Ctrl = Normal saline administered
Fig 4 shows a significant ($p<0.05$) increase in primary antibody titre of rats given both low and high doses of the extract compared to the negative control.

FIG. 5 EFFECT OF METHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* ON SECONDARY ANTIBODY TITRE



Group 1 = 100mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

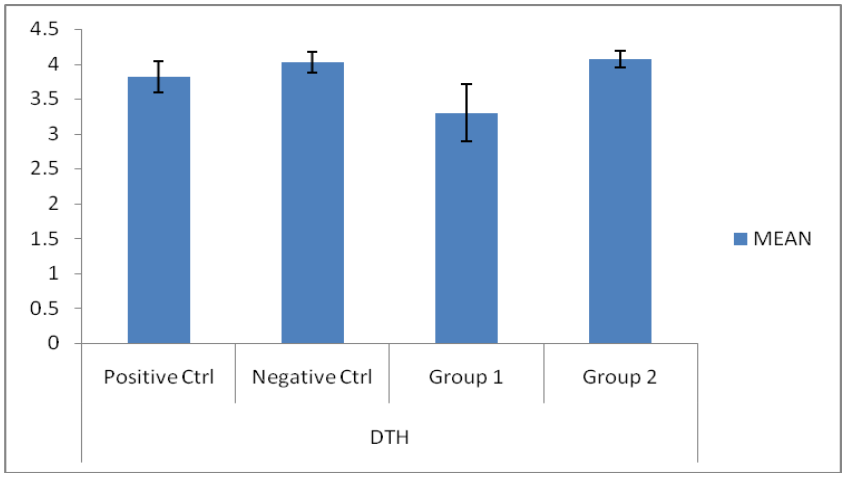
Group 2 = 300mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

Negative Ctrl = 100mg/kg bwpyrogallol administered.

Positive Ctrl = Normal saline administered

Groups 1 and 2 showed significant ($p<0.05$) increase in secondary titre when compared against the negative control.

Fig. 6 EFFECT OF METHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* ON DELAYED TYPE HYPERSENSITIVITY



Group 1 = 100mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

Group 1showed a non-significant ($p>0.05$) decrease when compared against the negative control while group 2 showed a non-significant ($p>0.05$) difference when compared against the negative control.

Group 2 = 300mg/kg bw methanol extract of *Ocimumgratissimum* + pyrogallol administered.

Negative Ctrl = 100mg/kg bwpyrogallol administered.

Positive Ctrl = Normal saline administered

IV. Discussion

This study investigated the possible effect of the methanol leaf extract on immunological indices in rats. The results obtained showed a significant ($p<0.05$) increase in total white blood cells (TWBC) count and non significant ($p>0.05$) increase on packed cell volume (PCV) and haemoglobin (HB) concentration in test rats compared to the negative control and the normal control in some cases. *Ocimum gratissimum* has been shown to stimulate the activity of the bone marrow (Salminen *et al.*, 2008) and this obviously should be responsible for the increase in total white blood cell count. Lymphocytes are usually depleted during active infection. The results equally is consistent with the report of Alada (2000) who had earlier observed increase in haematological indices of rats fed leaf extract of *Ocimum gratissimum.* The use of *Ocimum gratissimum* as a vegetale soup is predicated upon its capacity to serve as immune booster when it is taken continuously (Ezekwesili *et al.*, 2004) The result of delayed type hypersensitivity (DTH) and humoral antibody titre showed a significant ($p<0.05$) increase in the rat treated with the extract compared to the negative control. DTH is characterized by large influxes of non-specific inflammatory cells in which the macrophage is a major participant, (Dunnet, 1994). DTH is agreed to be very important in host defence against parasites and bacteria that live and proliferate intracellularly. The extract in this study enhanced delayed type hypersensitivity reaction as seen in the increase in paw diameter of sensitized rats. Primary and secondary humoral antibody (HA) mean values shows a significant ($P<0.05$) increase in the test groups when compared to the negative control group. Antibody mediated immune response is an important part of acquired immunity. B-cell activation is a large part of the humoral immune response. The B-cells are usually activated to produce antibodies against antigens by T-cell dependent mechanism. In conclusion, this study has established a multi approach influence of the extract on the immune response and may be of great benefit in managing immune compromised conditions.

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