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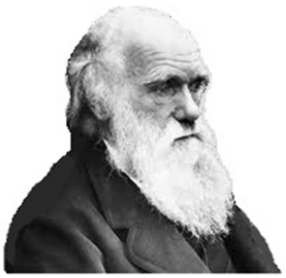

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Effect of Protein Extract of *Mangifera indica* Pollen on the Erythrocytes (Red Blood Cell) of Albino Rats

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ABSTRACT

This study was carried out to show the effect of the protein extract of *Mangifera indica* on the red blood cell (erythrocytes) of albino rats. The *Mangifera* protein was extracted using 500ml of 0.02m (20mM) phosphate- buffered saline (PBS), at pH 7.4, in a large beaker (stirred with a magnetic stirrer for 3 hours at room temperature). The crude extract was saturated to 60% by adding solid ammonium sulphate under constant gentle attiring, and then stored in a refrigerator for 6 hours. Sixteen male albino rats obtained from animal house in Faculty of Biological Science, University of Nigeria Nsukka was used for the study. They were divided into two groups of eight. One group was labeled the experimental group and the other control. The extract was administered to the experimental rats intra nasally for a period of seven (7) days. Data were expressed as mean ± standard error. Means were separated using Duncan's New Multiple Range Test (DNMRT). Blood samples were collected via the orbital plexus of rats to determine the effect of the extract on red blood cell (erythrocytes). The present study demonstrated that the extracted pollen of *Mangifera indica* had no allergic effect on ratsand so would need to be further investigated.

***Keywords*:** Protein extract, *Mangifera indica*, Pollen, Erythrocytes, Albino Rat, Duncan's New Multiple Range Test, red blood cell

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1. INTRODUCTION

Mango (*Mangifera indica* L.) is one of the choicest fruits in the world (Joshi *et al*., 2013). It belongs to the family of *Anacardiaceae*, one of the mist important species of the family and one of the most preferential fruit crops of the tropical and subtropical regions of the world for human consumption (Vasugi *et al*., 2012). Its social and economic impact are most relevant in developing and emerging countries, where mango is a high-valued component in diet, rich in vitamins and minerals (Ribeiro *et al*., 2007).

Various parts of the plant are used as a dentrifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic. According to Galvez-Lopez*et al*.(2010), all parts can be used to treat abscesses, broken horn, rabid dog or jackal bite, tumour, snakebite, stings, acute poisoning due to ingestion of Datura spp, heat stroke, miscarriage, anthrax, blisters, mouth wounds, tympanitis, colic, diarrhoea, glossitis, indigestion, bacillosis, bloody dysentry, liver disorders, excessive urination, tetanus and asthma.

Fruits of *M. indica* can may be used to make juices, mango nectar, or flavouring as a major ingredient in ice cream and sorbets (Bompard, 1993; Mukherjee, 1997). Wood from mango tree is extensively used for low-cost furniture, ceiling boards, window frames, heavy packing cases, match splints, brush backs, oar blades, agricultural implements etc. (Krishna and Singh, 2007).

The pollen grain were originally made for pollination and fertilization of flowers, but a times they do make unscheduled detour from this mission, finding their way into the human nostrils and throats. At this point, the pollen can trigger the activities of the cells of the body resulting to responses like sneezing, coughing, itching, watery eyes etc.This will imply that the structural and biochemical similarities between allergenic proteins and allergenic and non-allergenic groups of rats could explain and determine allergenicity. Along the lines, pollen grains not only release allergens but also non allergenic, bioactive, pollen-associated lipid moderator, which have pro-inflammatory and immunomodulatory effects on the cells involved in the allergic immune response (Chapman *et al.*, 2007).

Pollens are microscopic round or oval grains produced by plant for reproduction. In some species the plant uses the pollen from it own flower to fertilize itself. Other types must be cross-pollinated, that is, in order for fertilization to take place and seeds to form, pollen must be transferred from the flower of one plant to that of another plant of the same species. Insects do this job for certain flowering plants, while other plants rely on wind for transport. The quality and type of pollen and spore that are air borne at a given period are determined by several factors such as the size of plant/fungi community, nature of palynomorph, that is, its morphology – which can be as an index to identify and classify the pollen and spore to their respective groups (genera, family) by means of some distinctive traits, the type of pollen sampler used and meteorological factors like humidity, rainfall, wind direction/velocity, temperature and the biological activities of plants including pollen and spore production, release and deposition (Lyon *et al.*, 1984; Agwu and Osibe, 1992; Agwu, 2001).

Anemorphilous grains are pollen grains pollinated by wind. They are produced in copious quantities due to chance factor involved in pollination. Their pollen grains have characteristics that favour wind pollination like small size, light weight and so on (Agwu and Osibe, 1992). Entomophilous pollen grains are grains that are pollinated mainly by insects. They are produced in lesser quantities than anemophilous grains. The grains are large, sticky and have thicker ornamentations than anemorphilous pollen grains. With the aforementioned points, it will be

seen that Aeolian transport of pollen favours anemophilous pollen more than entomophilous pollen grains (Agwu and Osibe, 1992). In general, it is the pollen of arboreal species that are transported some distances from the parent plant because trees release their pollen high in the air and into the wind (King and Kapp, 1962).

The analysis of trapped pollen is the central technique for the reconstruction of vegetation (both past and present). Therefore airborne pollen grains are indicators of the vegetation of a region (Agwu, 2001; Agwu and Osibe, 1992; Hicks *et al*., 1996) and related to the polynomorphs produced in the immediate vicinity of study location and /or catchment area. The flaw in the latter verdict is that most studies have shown that the pollen and spore contribution of plant species in the area under study is relatively small. This is because pollen production and dispersal vary so widely from species to species and the representation of a pollen type is rarely in a 1:1 relationship with the species abundance in the catchment area/vegetation (Hicks *et al*., 1996).

The allergic inflammation has components that can give rise to systemic disease manifestations. Pollen which has aerodynamic size of 15 - 4μm and probably cannot enter the lower thoracic region of the respiratory tract; instead, they affect the nasal or nasopharyngeal mucous membrane. Airborne pollen is one of the most common triggers of allergic disease (Essien and Agwu, 2013). Pollen warnings are meant to be part of guided self-management to prevent system aggregation and help the allergy sufferers take the control of the condition. They increase awareness about the disease and its connection to ambient aeroallergen levels, and thus act in patient education (Knox *et al*., 1997). The first treatment in respiratory allergy is allergy avoidance (Blacklay, 1959). Pollen affects human beings with allergic sensitization since they carry allergens. In sensitized children, these allergens elicits allergic reaction in target organ and give rise to a systemic inflammation (Blacklay, 1959). It has recently been demonstrated that pollen grains, under physiology exposure conditions released not only allergens but bioactive lipids and enzymes that activate human neutrophil and eosinophils *in vitro* (Pope, 1999). Air pollution may be an important factor working synergistically with pollen eliciting symptoms. Air pollutants can both interact with pollen grains, leading to an increased release of antigen characterized by modified allergenicity and affect the airways, by enhancing the contact between allergens and immune active cells, and thus reinforce allergic inflammation (Traidl-Hoffmann *et al*., 2009).

Furthermore, it is plausible that, in spite of aerodynamic principle, a smaller number of grass pollen penetrate into the lower respiratory tract (Behrendt and Beker, 2001), particularly during breathing through the mouth. The main objective of this study was to determine the effect of protein extract of *Mangifera indica* pollen on the erythrocytes (red blood cell) of albino rats.

2. MATERIALS AND METHODS

2. 1. Extraction of Pollen Protein

Protein from fifty grams of anthers of Mango (*Mangifera indica*) flowers was extracted in 500 ml of 0.02 M (20 mM) phosphate-buffered saline (PBS), at pH 7.4, in a large beaker (stirred with a magnetic stirrer for 3hours at room temperature). After filtering through muslin cheese cloth, the filtrate was centrifuged (Harrier 18/80, Japan) at 10,000 revolution per minutes

(rpm), for 20 minutes at 4 ºC. The "crude extract" was saturated to 60% by adding solid ammonium sulphate under constant gentle stirring and then stored in a refrigerator for 6 hours.

The resulting precipitate was collected by centrifugation at 10,000 rpm for 20 minutes, dissolved in a minimum volume of pre-cooled distilled water (50 ml) and dialyzed against distilled water for 24 hours at 4 ºC. After cooling and centrifugation for 10minutes at 10,000 rpm, the clear supernatant thus obtained was designated as the "crude protein solution", which was then freeze-dried and the lyophilized (Taitec-VD-800F Freeze Dryer, Japan) extracts were stored at 80 ºC for use in all experiment.

2. 2. Experimental Design

Sixteen male albino rats obtained from Faculty of Biological Science animal house were assigned into two groups of eight (8) rats per group. The rats were allocated to acclimatize for two (2) weeks before the commencement of the experiment. The rats in group one were not administered with Mango (*Mangifera indica*) extract. The rats in group two received 6 drops of liquid Mango (*Mangifera indica*) pollen extract intranasally two times a day for seven days. On the seventh day 3 ml of blood was collected from each rats into EDTA bottle for hematological, (Red blood cell (RBC) count analysis.

2. 3. Haematological Determinations of Packed Cell Volume

The packed cell volume (PCV) was determined by the microhaematocrit method. Micro-capillary tubes were almost filled with anti-coagulated blood samples and one end sealed with plasticine. The filled tubes were centrifuged at 10,000 revolutions per minute for 5 minutes using a microhaematocrit centrifuge. The PCV was seen as a percentage on the microhaematocrit read.

2. 4. Determination of Haemoglobin Concentration

The haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method. The blood sample (0.02 ml) was added to 5 ml of Drabkin's reagent in a clean test tube. This was mixed gently and kept at room temperature for 20 reagents in a clean test tube. The absorbance of both sample and standard were read, against a working reagent blank at wavelength of 540 nm using a spectrophotometer (Lab-tech, India). The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with factor derived from the absorbance and concentration of the standard.

2. 5. Erythrocyte Count

The erythrocyte count was determined by the haemocytometer method (Thrall and Weiser, 2002). Blood sample (0.02 ml) was added to 4ml of red blood cell diluting fluid (sodium citrate, formaldehyde solution and distilled water) in a clean test tube, to make a 1:200 dilution. A drop of the diluted blood was charged onto the Neubaeur counting chamber and allowed to settle for 2-3 minutes.

The high dry objective (× 40) of the light microscope was used in carrying out the erythrocyte count, in the five groups of 16 small squares. The number of erythrocytes enumerated for each sample was multiplied by 10,000 to obtain the erythrocyte count per microliter of blood (Thrall and Weiser, 2002).

2. 6. Statistical Analysis

All data obtained were subjected to statistical analysis using Student's t-test using Statistical Package for Social Sciences (SPSS for windows, version 12.0). Data were expressed as mean ± standard error. Means were separated using Duncan's New Multiple Range Test (DNMRT).

3. RESULTS

3. 1. Effect of the Protein Extract of *Mangifera indica* Pollen on the Red Blood Cell Counts (RBC) of Experimental Albino Rats as Compared to those of the Control Group

The RBC results of rats fed with pollen extract of *Mangifera indica* is represented in Table 1. The RBC results did not differ significantly among the fields in both experimental and control rats respectively ($p > 0.05$). The RBC results of fields 1 and 3 of the control rats were higher than the experimental, but with no significant difference ($p > 0.05$). Interestingly, the experimental and control RBC results in field 2 recorded the same values (7.12 ±0.12).

Table 1. Effect of protein extracts of *Mangifera indica* on the red blood cell count (RBC) ($\times 10^{12}$/L) of experimental albino rats as compared to those of the control group.

Fields	Experimental	Control	T	p-value
1	7.12 ±0.12^{a}	7.25 ±0.16^{a}	-1.000	0.351
2	7.12 ±0.12^{a}	7.12 ±0.12^{a}	0.000	1.000
3	7.00 ±0.00^{a}	7.25 ±0.16^{a}	-1.528	0.170
Total	7.08 ±0.06^{a}	7.21 ±0.08^{a}	-1.366	0.185

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$). Level of significance along rows: * $p < 0.05$; ** = $p < 0.01$.

3. 2. Effect of the Protein Extract of *Mangifera indica* Pollen on the Packed Cell Volume (PCV) of Experimental Albino Rats as Compared to those of the Control Group

There were variations among the field in the experimental and control PCV results (Table 2). The differences observed showed no significant difference ($p > 0.05$). The control results were significantly higher than the experimental in all the fields including the overall result ($p < 0.01$).

Table 2. Effect of the Protein Extract of *Mangifera indica* Pollen on the Packed Cell Volume (%) (PCV) of Experimental Albino Rats as Compared to those of the Control Group.

Fields	Experimental	Control	T	p-value
1	10.12 ±0.12[a]	30.12 ±0.12[a]	-105.830	0.0001
2	10.37 ±0.18[a]	30.25 ±0.16[a]	-87.717	0.0001
3	10.00 ±0.19[a]	30.00 ±0.00[a]	-105.830	0.0001
Total	10.17 ±0.10	30.12 ±0.07	-177.763	0.0001

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$). Level of significance along rows:*$p < 0.05$; ** = $p < 0.01$.

3. 3. Effect of the Protein Extract of *Mangifera indica* Pollen on the Mean Cell Haemoglobin Concentration (MCHC) of Experimental Albino Rats as Compared to those of the Control Group

Table 3 represents the MCHC results of rats fed with different doses of *Mangifera indica*. Fields 1, 2 and 3, recorded concordant MCHC values in experimental and control rats respectively ($p < 0.05$). The MCHC results of the control rats in all the fields were significantly higher than the experimental ($p < 0.01$).

Table 3. Effect of the Protein Extract of *Mangifera indica* Pollen on the Mean Cell Haemoglobin Concentration (MCHC) (g/dl) of Experimental Albino Rats as Compared to those of the Control Group.

Fields	Experimental	Control	T	p-value
1	43.50 ±0.19[a]	45.37 ±0.18[a]	-6.355	0.0001
2	43.37 ±0.26[a]	45.25 ±0.16[a]	-6.355	0.0001
3	43.00 ±0.33[a]	45.25 ±0.16[a]	-9.000	0.0001
Total	43.25 ±0.15	45.29 ±0.09	-12.558	0.0001

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$). Level of significance along rows: *$p < 0.05$; ** = $p < 0.01$.

3. 4. Effect of the Protein Extract of *Mangifera indica* Pollen on the Mean Cell Haemoglobin (MCH) of Experimental Albino Rats as Compared to those of the Control Group

The MCH results of rats given different doses of protein extract of pollen of *Mangifera indica* was shown in Table 4. The MCH results of fields 1, 2 and 3 recorded equal in experimental and control rats respectively. No significant difference existed in the MCH results among the fields in both experimental and control rat ($p > 0.05$). The same MCH value (17.00 ±0.00) was recorded in both control and experimental rats in field 1. There was no significant difference in the MCH results between the control and experimental rats in fields 2 and 3.

Table 4. Effect of the Protein Extract of *Mangifera indica* Pollen on the Mean Cell Haemoglobin (MCH) of Experimental Albino Rats as Compared to those of the Control Group.

Fields	Experimental	Control	T	p-value
1	17.00 ±0.00[a]	17.00 ±0.00[a]	-	-
2	17.00 ±0.00[a]	17.25 ±0.16[a]	-1.528	0.170
3	17.12 ±0.12[a]	17.25 ±0.16[a]	-1.000	0.351
Total	17.04 ±0.04	17.16 ±0.08	-1.813	0.083

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$).Level of significance along rows: * $p < 0.05$; ** = $p < 0.01$.

3. 5. Effect of the Protein Extract of *Mangifera indica* Pollen on the Mean Cell Volume (MCV) of Experimental Albino Rats as Compared to those of the Control Group

Table 5 shows the MCV results of rats fed with different doses of *Mangifera indica*. The experimental results differed significantly, with field 2 showing higher MCV values (44.37 ±0.42) compared to other fields. The same MCV value (38.00 ±0.00) was recorded in fields 1 and 2 of the control rats. However, no significant difference was observed among the fields in the control group ($p > 0.05$). The experimental values were significantly ($p < 0.01$) higher than the controls in all the fields.

Table 5. Effect of the protein extract of *Mangiferaindica* pollen on the mean cell volume (MCV) counts of experimental albino rats as compared to those of the control group.

Fields	Experimental	Control	T	p-value
1	43.12 ±0.35[a]	38.00 0.00[a]	14.627	0.0001

2	44.37 ±0.42[a]	38.00 0.00[a]	15.181	0.0001
3	44.12 ±0.40[ab]	38.12 ±0.12[a]	12.000	0.0001
Total	43.87 ±0.24	38.04 ±0.04	22.430	0.0001

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$). Level of significance along rows: *$p < 0.05$; ** = $p < 0.01$.

3. 6. Effect of the Protein Extract of *Mangifera indica* Pollen on the Haemoglobin (Hb) of Experimental Albino Rats as Compared to those of the Control Group

The haemoglobin (Hb) results of rats fed with different doses of *Mangiferaindica* were represented in Table 6. Equal haemoglobin values (14.00 ±0.00) were recorded among the fields in the experimental rats, but with no significant difference ($p > 0.05$). Comparatively, the haemoglobin results of the experimental and control rats did not differ significantly in all fields ($p > 0.05$).

Table 6. Effect of the protein extract of *Mangifera indica* pollen on the haemoglobin (Hb) of experimental albino rats as comparedto those of the control group.

Fields	Experimental	Control	T	p-value
1	14.00 ±0.00	14.12 ±0.12[a]	-1.000	0.351
2	14.00 ±0.00	14.25 ±0.16[a]	-1.528	0.170
3	14.00 ±0.00	14.25 ±0.16[a]	-1.528	0.170
Total	14.00 ±0.00	14.21 ±0.08	-2.460	0.022

Mean values with different alphabets as superscripts in a column differ significantly ($p<0.05$).Level of significance along rows:*$p<0.05$;**=$p<0.01$.

4. DISCUSSION

The red blood cell results did not differ significantly among the fields in both experimental and control rats respectively ($p > 0.05$). The RBC results of fields 1 and 3 of the control rats were higher than the experimental, but with no significant difference ($p > 0.05$). The experimental and control on RBC results in field 2 recorded the same values (7.12 ±0.12).

There were variations among the field in the experimental and control PCV results .The differences observed showed no significant difference ($p > 0.05$).

The Mean Cell Haemoglobin Concentration (MCHC) of experimental albino Rats as Compared to those of the Control Group showed no significant difference statistically ($p > 0.05$). No significant difference existed in the MCH results among the fields in both

experimental and control rats ($p > 0.05$). The same MCH value (17.00 ±0.00) was recorded in both control and experimental rats in field 1. There was no significant difference in the MCH results between the control and experimental rats in fields 2 and 3.

Also, the Mean Cell Volume (MCV) of experimental Albino Rats as Compared to those of the Control Group did not differ significantly ($p > 0.05$). The same MCV value (38.00 ±0.00) was recorded in fields 1 and 2 of the control rats. However, no significant difference was observed among the fields in the control group ($p > 0.05$). The experimental values were significantly ($p < 0.01$) higher than the controls in all the fields.

The haemoglobin (Hb) results of experimental rats are represented in Table 6. Equal haemoglobin values (14.00 ±0.00) were recorded among the fields in the control rats, but with no significant difference ($p > 0.05$). Comparatively, the haemoglobin results of the experimental and control rats did not differ significantly in all fields ($p > 0.05$).

Pollen grains and spores are transported from one place to another. Variations in monthly count of airborne palynomorphs have been reported by some authors in Nigeria (Njokuocha and Osayi, 2005; Njokuocha, 2006; Adeniyi *et al*., 2014; Njokuocha *et al*., 2018). Njokuocha (2006), reported that high numbers of pollen grains are observed during the late rainy season-early dry season/harmattan (September to December). These variations in the monthly palynomorphs counts of families and individual palynomorphs types at different locations also suggest that the abundance of palynomorphs is influenced not only by the meteorological factors but also the existing vegetation type as well as flowering phenology of the plants among others (Agwu and Osibe, 1992; Agwu, 1997; Essien, 2014). Apart from the meteorological factors, the concentrationof atmospheric pollen content was as well considered to be greatly affected by the geographical distribution of the pollen producers and the period of main pollen release (Calleja *et al*., 1993)

5. CONCLUSION

From the above findings, it is evident that theprotein extract of *Mangifera indica* pollen had no effect on the erythrocytes (red blood cell) of albino rats. The pollen, spores and other bio-particles are periodically released and distributed in the atmosphere. Their presence and abundance play major roles in the spread of human allergic reactions and plant diseases.

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