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Haematopoietic effect of methanol extract of Nigerian honey bee (*Apis mellifera*) propolis in mice

Bashir Lawal^{1*}, Oluwatosin Kudrat Shittu¹, Asmau Niwoye Abubakar¹, Garba Muhammed Haruna², Sani Saidu³, Prince Chukwudi Ossai¹

¹Department of Biochemistry, Tropical Disease Research Unit, Federal University of Technology, P.M.B. 65, Minna, Nigeria

²Federal College of wild life Management, New Bussa, Niger State, Nigeria

³Department of Biochemistry, Federal University Ndufu-Alike Ikowo Abakaliki, Ebonyi State, Nigeria

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ABSTRACT

Objective: To investigate the haematopoietic effect of methanol extract of Nigerian honey bee propolis in mice.

Methods: Fifteen white Albino mice were grouped into 3(A-C) of 5 animals each. Group A mice serve as control group, while groups B and C received 300 and 600 mL/kg of honey bee propolis respectively, for 21 days. The haematological parameters were determined using the automated haematologic analyzer Sysmex lx21, (product of Sysmex Corporation, Japan) using standard techniques. The data were analyzed using ANOVA and the level of significance was at $P < 0.05$. Acute oral toxicity study was conducted to determine its safety on acute exposure.

Results; Results showed significant ($P < 0.05$) dose dependent elevations in platelet count, mean platelet volume, plateletcrit, platelet distribution weight, white blood cells, granulocyte count, lymphocytes and mid cell total count. The extract however produce no significantly ($P > 0.05$) alteration to the erythrocytic indices like red blood cells, haematocrite, haemoglobin, mean corpuscular haemoglobin, mean corpuscular volume and red cell distribution width, but increase mean corpuscular haemoglobin concentration in dose related fashion. Acute oral toxicity showed the extract to be relatively safe at a high dose on acute exposure. However, 21-days of treatment with the extract do neither increase nor decrease the body weight of the mice.

Conclusions: Administration of methanol extract of Nigerian honey bee propolis in mice at the doses investigated has brought about leucopoietic and thrombopoietic changes without any significant effect on red blood cells and factors that relate to it, except for the mean corpuscular haemoglobin concentration.

1. Introduction

Anaemia remains one of the major global health problems affecting more than 30% of world's population, with higher prevalence among children and adults of low socio-economic class[1]. Presently, more than half of the world's population will experience some forms of anaemia in their life time due to the presence of many aggravating factors such as poor nutrition, high prevalence of blood parasites *e.g.* plasmodium, trypanosomes and helminthes infestation. It is also known that women are susceptible to anaemia during pregnancy due to high demand from the developing foetus[2]. Hereditary haemoglobinopathies such as sickle cell disease and thalassemia which are associated with recurrent

haemolysis are also important causes of childhood anaemia[3]. A few cases are drug-related particularly with the use of drugs such as sulphonamides, dapsone and methyl dopa. Other rare causes of anaemia include connective tissue disorders such as rheumatoid arthritis, polyarthritis nodosa, Wegener's granulomatosis, and progressive systemic sclerosis[4].

For thousands of years, people have looked to natural means of healing. In developing countries of the world, most the people depend on herbal medical care. The popularity of traditional medicine is due to the belief that some diseases only respond to traditional treatment[5].

Propolis is a natural hive product with a complex chemical composition, consisting of mixture of balsams (resins), beeswaxes, oils, and pollen. It is a sticky resinous substance collected by honey bees (*Apis mellifera*) from buds and barks of different trees[6]. Propolis contains a large number of biologically active components including different flavonoids, polyphenolic esters, terpenoids,

*Corresponding author: Bashir Lawal, Department of Biochemistry, Tropical Disease Research Unit, Federal University of Technology, P.M.B. 65, Minna, Nigeria.
Tel: +234 -8165112378
E-mail: bashirlawal12@gmail.com

steroids, amino acids, caffeic acids and their esters[7]. Propolis have been used as a remedy by humans since ancient times dating back to the times of ancient Greece and Rome, for treating wide spectrum of disorders and diseases[8]. It is used in foods and beverages to improve health and prevent diseases such as inflammation, diabetes, heart disease, and cancer[9]. It has been reported to possess hepatoprotective[10], antibacterial, antiviral, anti-inflammatory, anticancer, antifungal, and anti-tumoral properties[11]. Due to the increasing interest in the characteristics of propolis, the present study was set out to determine the effect of the administration of methanol extract of bee propolis on hematological profile in mice.

2. Materials and methods

2.1. Experimental animals

A total of thirty five Swiss Albino mice, 23.00 ± 4.00 mean weight were obtained from the Small Animal Holding Unit of the Department of Biochemistry, Federal University of Technology Minna. The mice were kept in clean plastic cages and maintained under standard laboratory conditions: temperature [(22 ± 3) °C]; photoperiod (12 h natural light and 12 h dark); humidity: (40%-45%). The animals were maintained on standard animal feeds (Bendel feeds and flour mills, Edo state, Nigeria) and tap water *ad libitum*. The principles governing the use of laboratory animals as laid out by the Federal university of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review[12], were duly observed.

2.2. Source of propolis

Propolis material was collected from an apiary in Akure, Ondo State, Nigeria. The identity of the propolis was authenticated by an Entomologist in the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria, where a voucher specimen was deposited. The propolis material was chopped into small pieces and air dried in the shade at room temperature for two weeks.

2.3. Preparation of propolis extract

Preparation of the extract of propolis material followed standard procedures[13]. Two hundred grams of propolis pellets were percolated in 1 600 mL of absolute methanol and subsequently allowed to stand in the shade for 48 h before filtration, using Whatman No. 1 filter paper. The extract concentrate obtained was stored in air-tight vials in the refrigerator at 4 °C, until needed for bio-assay.

2.4. Toxicity study (LD_{50}) of the bee propolis

Acute toxicity study of the bee propolis was carried out according to the method of Lorke[14]. The mice were dosed orally with different gradual doses (10–5000 mg/kg body weight). In the first phase, mice were divided into three groups of three mice each and were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight

orally by means of a cannula. They were observed for 24 h for signs of toxicity, mortality and general behaviours. In the second phase, 12 mice were divided into four groups of three mice each and were also administered with the bee propolis at doses of 1000, 1600, 2900, and 5000 mg/kg body weight orally. They were observed for 24 h for signs of toxicity, mortality and general behaviours. The extract was dissolved in dimethylsulfoxide solution and administered once via oral route. The LD_{50} was calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred).

$LD_{50} = \text{Minimum toxic dose} \times \text{Maximum tolerated dose}$

2.5. Animal grouping and extract administration

Fifteen Swiss Albino mice were randomly divided into three groups of 5 rats each Group 1 (control) were orally administered with dimethylsulfoxide (Vehicle for extract administration). Groups 2 and 3 were treated with 300 and 600 mg/kg body weight/day respectively once daily for 21 days.

2.6. Collection of blood sample

Collection of blood sample for analyses was as described previously[15]. Prior to termination of the experiment on day 22, the rats were fasted overnight but distilled water was made available *ad libitum*. Blood samples were collected by cardiac puncture under ether anesthesia. The blood was collected in sample bottles containing ethylene diamine tetraacetic acid for hematological analyses. The samples were analyzed immediately after collection at Center for Genetic Engineering and Biotechnology, Global Institute for Bioexploration Unit, Federal University of Technology, Minna.

2.7. Determination of hematological parameters

The hematological components including haemoglobin, haematocrite, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), granulocyte count (GRA), lymphocytes, platelet count, mean platelet volume (MPV), plateletcrit and platelet distribution weight (PDW) were determined using the automated haematologic analyzer Sysmex kx21, a product of Sysmex Corporation, Japan employing the method described by Dacie and Lewis[16].

2.8. Statistical analysis

Data were analyzed using SPSS version 16 and presented as mean \pm SEM. Comparisons between different groups was done using ANOVA and Duncan's multiple range test. Values of $P < 0.05$ were considered as statistically significant as described by Yalta and Talha[17].

3. Results

3.1. Acute toxicity (LD_{50})

The LD_{50} of methanol extract of bee propolis was found to be more than 5000 mg/kg body weight. No death or sign of toxicity

were recorded within 24 h after treatment with the extract (Table 1). Mice at dose of 5000 mg/kg body weight show behaviors changes of rubbing of nose and mouth on the floor of the cage this however disappear within 24 h of extract administration.

Table 1

Acute toxicity profile of methanol extract of bee propolis in mice

Dose (mg/kg body weight)	Numbers of mice	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3
1600	3	0/3
2900	3	0/3
5000	3	0/3

3.2. Clinical observations

Clinical observations shows that the bee propolis did not produce any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma, at dose investigated.

3.3. Hematological parameters

Table 2 shows effect of 300 and 600 mg/kg body weight/day of methanol extracts of bee propolis on haematological parameters in treated mice. Results showed that 21-day oral treatment with the two doses of the extract significantly ($P < 0.05$) increased thrombocyte indices including; platelet count, MPV, plateletcrit and PDW (Table 2). The extracts also significantly ($P < 0.05$) increased leucocytic indices including WBC count, granulocyte, lymphocytes and mid cell total count (Table 2). The extracts however produce no significantly ($P > 0.05$) alteration to the erythrocytic indices like RBC counts, haematocrite, haemoglobin, MCH, MCV and red cell distribution width but increase MCHC in dose related fashion.

Table 2

Effects of 21-day administration of methanol extract of propolis on haematological parameters of mice.

Parameters	Control rats	300 mg/kg propolis	600 mg/kg propolis
WBC ($\times 10^9/L$)	3.90 \pm 1.11 ^a	7.38 \pm 1.18 ^b	11.29 \pm 1.90 ^c
Granulocyte (%)	0.84 \pm 0.70 ^a	0.62 \pm 0.24 ^a	2.59 \pm 0.80 ^b
LY ($\times 10^9/L$)	2.87 \pm 0.60 ^a	4.47 \pm 0.58 ^b	7.86 \pm 2.79 ^b
MCTC ($\times 10^9/L$)	0.19 \pm 0.08 ^a	0.11 \pm 0.005 ^a	0.58 \pm 0.87 ^b
RBC ($\times 10^{12}/L$)	2.78 \pm 0.10 ^a	2.90 \pm 0.05 ^a	2.91 \pm 0.003 ^a
Hematocrite (L/L)	0.23 \pm 0.01 ^a	0.21 \pm 0.00 ^a	0.21 \pm 0.003 ^a
Hemoglobin (g/L)	98.33 \pm 1.20 ^a	95.33 \pm 1.85 ^a	95.00 \pm 5.03 ^a
MCH (pg)	30.96 \pm 1.59 ^a	32.20 \pm 0.35 ^a	31.67 \pm 1.51 ^a
MCHC (g/L)	399.33 \pm 2.74 ^a	436.00 \pm 3.60 ^b	429.00 \pm 1.98 ^b
MCV (fL)	98.33 \pm 1.20 ^b	95.33 \pm 1.85 ^b	73.33 \pm 0.33 ^a
RCDW-cv (fL)	42.13 \pm 0.08 ^a	36.43 \pm 3.16 ^a	41.43 \pm 0.93 ^a
RCDW-sd (fL)	17.00 \pm 0.36 ^a	16.60 \pm 0.11 ^a	17.00 \pm 0.36 ^a
PC ($\times 10^9/L$)	338.67 \pm 36.08 ^a	807.33 \pm 6.83 ^b	959.00 \pm 29.50 ^c
MPV (fL)	9.20 \pm 0.09 ^a	9.81 \pm 0.35 ^a	13.01 \pm 0.30 ^b
Plateletcrit (L/L)	0.57 \pm 0.15 ^a	1.51 \pm 0.15 ^b	1.78 \pm 0.17 ^b
PDW (%)	18.56 \pm 0.41 ^a	18.65 \pm 0.03 ^a	26.23 \pm 1.11 ^b

LY: Lymphocytes; MCTC: Mid cell total count; RCDW-cv: Red cell width coefficient of variation; RCDW-sd: Red cell width standard deviation; PC: Platelet count; Values are mean \pm SEM of 5 determinations. The values along the same row with different superscripts are significantly different ($P < 0.05$).

3.4. Body weight

Twenty one days of treatment with the extract does neither

increase nor decrease the body weight of the mice, *i.e.* there were no significant ($P > 0.05$) difference between the body weight of the mice before extract administration and after 21 days of administration while the control group shows a significant increase in body weight after the 3 weeks of the study (Table 3).

Table 3

Weight changes of mice administered methanol extracts of bee propolis. g.

Parameters	Initial weight	Final weight	Weight gain
Control rats	23.45 \pm 2.47 ^a	27.40 \pm 2.12 ^b	3.95
300 mg/kg propolis	22.14 \pm 3.03 ^a	23.01 \pm 1.03 ^a	-
600 mg/kg propolis	23.02 \pm 4.21 ^a	23.45 \pm 0.90 ^a	-

Values are mean \pm SEM of 5 determinations..

4. Discussion

The examination of the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutrition and pathological status of an organism[18]. Results of the present study showed that chronic administration of methanol extract of bee propolis to mice resulted in significant ($P < 0.05$), dose related elevations in the hematological parameters investigated except the erythrocytes parameters. It has been established that oral ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters[19]. These alterations could either be positive or negative. In this study, most of the effects recorded for the extract were positive except for its suppressive effects on the MCHC.

The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. The significant increases ($P < 0.05$) in the total WBC, lymphocytes and GRA count reflect leucopoietic and possible immunomodulatory effects of the extract which augmented the production of more WBC, lymphocytes and GRA. This will increase the animal's capability of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions[20].

Blood platelets are implicated in blood clotting. The significant increase in platelet count, MPV, plateletcrit and PDW following chronic administration of bee propolis is a desirable property as high platelet concentration suggests that the process of clot-formation (blood clotting) will be fast and adequate resulting in minimal loss of blood in the case of injury. It is possible that the bee propolis methanol extract possesses active principle(s) containing haematopoietin-like principle(s) or contains active biological principle(s) stimulating haematopoietins (leucopoietin, thrombopoietin) synthesis or release[3].

According to Peters, *et al.*[21], previous reports stated that haematocrite, haemoglobin and MCH are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce RBC as in mammals[22].

The non-significant effect of the extract on the RBC and indices

relating to it is an indication that there was no destruction of matured RBC and no change in the rate of production of RBC (erythropoiesis). It further shows that the bee propolis does not have the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production[23]. The non-significant effect on the RBC and haemoglobin also implies that there was no change in the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following the extract administration since RBC and haemoglobin are very important in transferring respiratory gases[24]. However, the decrease in the MCHC in this study was an indication of erythrocytes swelling. This suggests that the extract may be selectively toxic to MCHC among other erythrocyte lineages. Result of acute oral toxicity of the extract suggests that the extract could be relatively safe on acute exposure to it, even at a high dose. This study has also revealed the ability of the extract to stabilize the weight of treated mice for a period of 3 weeks. This can be recommended for peoples who are cautions of their weight gain.

In conclusion, administration of methanol extract of Nigeria honey bee propolis in mice at the doses investigated has brought about leucopoietic and thrombopoietic changes without any significant effect on RBC and factors that relate to it, except for the MCHC. However, isolation of the active principles in the extract and elucidation of their mechanisms of inducing the observed effects would constitute areas of further studies

Conflict of interest statement

We declare that we have no conflict of interest.

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