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Research Article

IMMUNOSTIMULATORY AND BIOCHEMICAL EFFECTS OF ETHANOLIC EXTRACT OF *MANGIFERA INDICA* STEM BARK ON DEXAMETHASONE-INDUCED IMMUNOSUPPRESSED MALE RATS

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ABSTRACT

Objective: The study was conducted to investigate theimmunostimulatory effect of the ethanolic extract of *Mangifera indica* stem bark in dexamethasone induced immunosuppred male albino rats relative to the immunoboosting effect of levamisole.

Methods: Male wistar rats (36) were divided into six groups of six animals per group. Group 1 (G1) animals served as the control group, and were administered with food and water only. Group 2 (G2) animals were treated with dexamethasone 5 mg/kg intraperitonially, twice daily for three days. Group 3 (G3) animals were treated with Levamisole 5 mg/kg orally for 21 days. Group 4 (G4) animals were treated with *Mangifera indica* extract 1000 mg/kg orally for 21 days. Group 5 (G5) animals were treated with dexamethasone 5 mg/kg intraperitonially, twice daily on days 1 to 3, followed by Levamisole 5mg/kg orally on days 4 to 21 while Group 6 (G6) animals were treated with dexamethasone 5 mg/kg intraperitonially, twice daily on days 1 to 3, followed by *Mangifera indica* extract 1000mg/kg orally on days 4 to 21.

Results: There was no significant statistical difference in the haematological studies as well as the biochemical parameters in the dexamethasone-induced immunosuppressed rats assayed.

Using information from the neutrophil lymphocyte count ratio (NLCR), levamisole (5 mg/kg) proved to be a better immunostimulant than the *Mangifera indica* extract (1000 mg/kg) as a single or on co-administration. However, a better biochemical profile was observed on treatment with *M. indica* extract in all treatment groups.

Conclusion: The *Mangifera indica* showed a hepato-protective activity, a cholesterol-lowering effect and a stabilising tendency on the Alanine aminotransferase (ALP) concentrations.

Keywords: *Mangifera indica* ,haematological parameters, Biochemical parameters, Hepato-protective activity, Immunostimulatory effect.

INTRODUCTION

The immune system is a system of biological structures and processes within an organism that protects against disease. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases and cancer[1-2]and immunodeficiency. Immunodeficiency occurs when the immune system is less active than normal, resulting in recurring and life-threatening infections. In humans, immunodeficiency can either be the result of a genetic disease such as severely combined immunodeficiency, acquired conditions such as HIV/AIDS, or the use of immunosuppressive medication. Immunosuppressive drugs comprise a large number of drugs that by different mechanisms of action can modulate the immune system[3-4]. Glucocorticoids are the most commonly used drugs, and are widely used for the management of inflammatory diseases. These drugs inhibit various immune functions by affecting gene transcription events[5-6]. They mediate their actions by binding to intracellular receptors, resulting in altered protein-protein interactions and consequently regulation of gene expressions[7].

Mangifera indica (Family: Anacardiaceae) is being used in Ayurvedic and indigenous medical systems for the treatment of various diseases. The bark is reported to contain protocatechic acid, catechin, mangiferin, among other isolates[8]. Mangiferin is the major bio-active constituent, a xanthone glycoside alongside isomangiferin, tannins and gallic acid derivatives. A variety of pharmacological activities include anti-oxidant activity[9-12], significant anti-diarrhoeal activity[13]anti-parasitic activity[14], antibacterial activity[15] and antifungal activity[16] also antispasmodic and antipyretic activity[17-18] and, significant antihyperglyceamic effects have reported. The presence of phytoconstituents such as tannins, terpenoids, sterols and flavonoids may be responsible for the antiulcer activity associated with petroleum ether and ethanolic extracts of leaves of M. indica in the in vivo aspirin induced gastric ulcer assay [19 - 20]. The reduction or increase in Neutrophil-lymphocyte count ratio (NLCR) has been reported to be an important measure of systemic inflammation, bacteremia and neoplasm development. However, little is known and published about neutrophil/lymphocyte count ratio and its relationship with glucocorticoid-induced immunomodulation. Therefore, the current study was conducted to investigate the immunostimulatory potential of the ethanolic extract of *Mangifera indica* stem bark using NLCR as a measure of immunomodulation relative to Levamisole, a standard immunobooster, in immunosuppression induced by glucocorticoids - case study being dexamethasone.

MATERIALS AND METHODS

Collection of Plant Material

Fresh samples of *Mangifera indica* stem bark were collected at Ilaro in Ogun State during the wet season (April 2012) and then authenticated at the herbarium in the Department of Botany, University of Lagos with a herbarium voucher number LUH1360.

Preparation of Plant extract

The stem bark of *Mangifera indica* , chopped into bits, were oven-air dried at a temperature of 40^{0}C until no observable weight change. The barks were pulverized using grinding machine (Munson Machinery Company). The pulverized stem bark was then subjected to exhaustive cold maceration in ethanol (96%) for 7 days and filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 30°C using a rotary evaporator (944808 Stuat Model RE-300). Further concentration of the extract was effected using a freeze-dryer (Telstar Model: CRYODOS-80) and the freeze-dried extract was labeled appropriately and stored at 10°C.

Experimental animals

Male Wistar mice and rats, in-house bred at the Anatomy Department of the University of Lagos were used for the study. The animals were cared for and used in accordance with the Institute of

Laboratory Animal Research (ILAR) guidelines for care use of animals in experimental studies[21].The animals were acclimatized to laboratory conditions for 10 days, and kept in cages under standard laboratory conditions (light period of 12 hours per day) and temperature of (27±2)°C. They were fed with pelleted feed and had free access to water.

Reagents and chemicals

Copper sulphate pentahydrate, potassium hydroxide and sodium potassium tartarate (NAAFIO Scientific Supplies Ltd, London), Ethanol, iodine, lead acetate, ferric sulphate, formaldehyde, chloroform, sulphuric acid, ethyl acetate, benzene, ammonia (BDH Chemicals Ltd, Poole, England), potassium mercuric iodide solution (Labtech Chemicals, Detroit, Michigan), sodium hydroxide pellets (E. Merck AB, Stockholm, Sweden) and potassium hydroxide pellets (Sigma-Aldrich Laboratory Chemicals, Missouri, United States) were used without further purification.

Experimental immunosuppressant

Dexamethasone Sodium Phosphate Injection (Pemadex®; Hubei Tianyao Pharmaceutical Company Ltd, China) was used to induce immunosuppression. Each millilitre of Pemadex® was labelled to contain 4mg of Dexamethasone Sodium Phosphate.

Standard immunostimulant

Levamisole oral suspension (Retrax®; Reals Pharmaceuticals Ltd, Nigeria) was used for the study. Each 5 ml of Retrax® was labelled to contain 40 mg levamisole.

Phytochemical Screening of Extract

Phytochemical screening for major constituents was carried using standard qualitative methods as described by Sofowora (1993) method[22].

Acute toxicity studies

Swiss albino male mice, fasted for 12 hours were used for this study. The animals were divided randomly into 6 groups of three animals each. Groups 1 to 6 were administered with 50mg/kg, 200mg/kg, 500mg/kg, 2000 mg/kg, 5000 mg/kg and 10000 mg/kg of the ethanolic extract of *Mangifera indica* respectively. The animals were observed for 7 days. Observation of toxic responses within the first 24hours of drug administration was made.

Immunomodulatory activity studies

Male wistar rats (36) were divided into six groups of six animals per group. Group 1 (G1) animals served as the control group, and were administered with food and water only. Group 2 (G2) animals were treated with dexamethasone 5 mg/kg intraperitonially, twice daily for three days. Group 3 (G3) animals were treated with Levamisole 5 mg/kg orally for 21 days. Group 4 (G4) animals were treated with *Mangifera indica* extract 1000 mg/kg orally for 21 days. Group 5 (G5) animals were treated with dexamethasone 5 mg/kg intraperitonially, twice daily on days 1 to 3, followed by Levamisole 5mg/kg orally on days 4 to 21. Group 6 (G6) animals were treated with dexamethasone 5 mg/kg intraperitonially, twice daily on days 1 to 3, followed by *Mangifera indica* extract 1000mg/kg orally on days 4 to 21.

Biochemical and haematological analysis

On the 22nd day, blood was collected by retro-orbital puncture of each rat into EDTA and lithium-heparinised bottles for haematological and biochemical analysis. Haematological parameters such as red blood cells, white blood cells, haemoglobin, packed cell volume, platelets, neutrophils mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration and leucocytes were parameters analysed using the Sysmex Autohaematology Analyser. Biochemical studies were also carried out to analyse low density lipoprotein, high density lipoprotein, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), triglycerides, bilirubin, cholesterol, creatinine and protein using the Roche Hitachi 904 Automated Analyser.

Statistical analysis

GraphPad Prism Version 5.0 for Windows was used for all statistical analyses. Data are presented as mean ± SEM and analyzed by one-way ANOVA followed by Dunnett's multiple Comparison test (post-test). Analysis at $p \leq 0.05$ showed no statistical significant difference in all analyses.

RESULTS

Phytochemical Screening of *Mangifera indica* Stem Bark Extract

The phytochemical screening of *Mangifera indica* stem bark extract revealed the presence of carbohydrates, reducing sugars, alkaloids, flavonoids, tannins and phlobatannins as shown in Table 1.

Table 1: Phytochemical profile of *Mangifera indica* stems bark extract

Test	Inference
Reducing sugars	present
Alkaloids	absent
Phenols	present
Flavonoids	present
Saponins	present
Tannins	present
Phlobatannins	present

Effect of the *Mangifera indica* on the Haematological profile of the dexamethasone-Induced Immunosuppressed Male Rats

The haematological parameters in the immunosuppressed male rats are as shown in Table 2. There was no significant statistical difference in the parameters when compared to the control.

Table 2: Effect of *Mangifera indica* on the haematology in Dexamethasone induced Immunosuppressed Rats

Parameters	Groups					
	G1	G2	G3	G4	G5	G6
WBC(10^9/L)	6.43 ± 0.44	8.85 ± 1.25	7.10 ± 0.69	6.60 ± 0.55	5.13 ± 2.04	4.73 ± 0.41
RBC(10^{12}/L)	7.47 ± 0.20	6.65 ± 0.92	6.85 ± 0.30	7.30 ± 0.27	7.19 ± 0.41	7.49 ± 0.33
HGB(g/dL)	14.5 ± 0.73	13.1 ± 2.00	12.4 ± 0.52	13.0 ± 0.06	54.7 ± 42.1	13.6 ± 0.44
HCT (%)	48.7 ± 2.64	46.8 ± 6.70	45.2 ± 0.66	47.7 ± 0.77	47.5 ± 1.81	46.2 ± 2.77
PLT(10^9/L)	718.7 ± 95.7	756.0 ± 56.0	711.3 ± 78.7	919.3 ± 51.3	636.0 ± 139.9	717.0 ± 67.0
NEU (%)	23.8 ± 3.67	19.5 ± 7.15	31.9 ± 1.82	27.0 ± 6.41	35.9 ± 0.66	32.3 ± 3.30
LYM (%)	67.0 ± 4.32	71.4 ± 8.70	59.6 ± 2.72	64.2 ± 6.09	54.4 ± 0.69	60.2 ± 3.23
MCV (Fl)	69.7 ± 2.96	70.3 ± 0.30	66.2 ± 2.43	65.6 ± 2.91	66.3 ± 1.43	67.4 ± 0.58
MCH (pg)	18.8 ± 0.46	19.6 ± 0.30	18.1 ± 0.15	65.6 ± 2.91	18.1 ± 0.24	19.0 ± 0.14
MCHC(g/dL)	29.6 ± 1.35	27.9 ± 0.30	27.4 ± 1.01	27.3 ± 0.43	27.4 ± 0.29	30.1 ± 1.42

WBC – White Blood Cells; RBC – Red Blood Cells; HGB – Haemoglobin; HCT – Haematocrit; PLT – Platelets; NEU – Granulocyte; LYM – Lymphocyte; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular Haemoglobin; MCHC – Mean Corpuscular Haemoglobin Concentration; Values are expressed as Mean ± SEM. $p \leq 0.05$ compared against control group

Table 3: Effect of *Mangifera indica* on the Clinical Chemistry in Dexamethasone - induced Immunosuppressed Male Rats

Parameters	Groups					
	G1	**G2**	**G3**	**G4**	**G5**	**G6**
AST(U/L)	148.1 ± 77.7	202.0 ± 98.6	249.4 ± 152.3	96.7 ± 60.7	70.4 ± 68.2	155.6 ± 76.9
ALT(U/L)	34.1 ± 15.5	30.0 ± 2735	43.6 ± 19.9	15.3 ± 13.8	21.4 ± 20.2	28.0 ± 16.4
ALP(U/L)	184.0 ± 26.7	111.7 ± 108.7	108.5 ± 52.1	54.4 ± 46.0	83.4 ± 81.9	184.4 ± 92.7
BIL(µmol/L)	2.00 ± 0.36	1.35 ± 0.05	1.63 ± 0.16	1.13 ± 0.14	1.10 ± 0.10	2.16 ± 0.26
URE(mmol/L)	5.93 ± 2.9	5.55 ± 4.55	4.93 ± 1.82	2.16 ± 1.86	2.90 ± 2.15	5.53 ± 2.02
ALB(g/L)	26.7 ± 13.5	15.7 ± 15.1	24.7 ± 12.3	4.60 ± 4.10	11.9 ± 11.2	27.8 ± 13.1
TP(g/L)	43.7 ± 20.2	33.5 ± 31.5	51.8 ± 24.3	26.6 ± 24.9	21.6 ± 19.2	53.5 ± 24.8
HDL(mmol/L)	0.03 ± 0.03	0.00 ± 0.00	0.03 ± 0.03	0.03 ± 0.03	0.10 ± 0.10	0.06 ± 0.03
LDL(mmol/L)	0.16 ± 0.08	0.20 ± 0.20	0.26 ± 0.14	0.10 ± 0.10	0.43 ± 0.43	0.26 ± 0.13
CHO(mmol/L)	1.02 ± 0.87	1.13 ± 1.04	1.55 ± 0.75	0.84 ± 0.46	0.62 ± 0.55	1.54 ± 0.64
TG(mmol/L)	0.71 ± 0.15	0.63 ± 0.33	0.65 ± 0.19	0.75 ± 0.45	0.49 ± 0.19	0.76 ± 0.23
CRE(µmol/L)	49.1 ± 7.78	44.8 ± 1.48	52.7 ± 9.26	45.4 ± 3.32	48.2 ± 4.95	58.9 ± 8.22

AST – Aspartate amino transferase; ALT – Alanine amino transferase; ALP – Alkaline phosphatase; BIL – Bilirubin; ALB – Albumin; TP – Total Protein; HDL – High Density Lipoprotein; LDL – Low Density Lipoprotein; CHO – Cholesterol; TG – Triglycerides; CRE – Creatinine.

Values are expressed as Mean ± SEM. p≤0.05 against control group

Effect of *Mangifera indica* on the Clinical Chemistry in Dexamethasone-Induced Immunosuppressed Rats

Results for the clinical chemistry in the dexamethasone-induced immunosuppressed rats are as shown in Table 3. There was no significant statistical difference in all the biochemical parameters assayed.

DISCUSSION

The present study was performed to investigate the immunostimulatory activity of *Mangifera indica* stem bark in dexamethasone-induced immunosuppressed male albino rats, relative to the immunoboosting effect of levamisole. The phytochemical results showed the Mangifera indica stem bark contain starch, reducing sugars, alkaloids, phenols, flavonoids, saponins, tannin and phlobatannins. The oral minimum lethal dose (LD_{50}) of the aqueous ethanolic extract of *M. indica* stem bark estimated as > 5000mg/kg suggests that the extract may have low toxicity[23]. Earlier studies established that any substance with LD50 estimate greater than 2000 mg/kg body weight by oral route may be considered of low toxicity and safe in humans[24-25].

The white blood cells (leucocytes) and the differential leucocytes count are principally the haematological indicators of immunity. Table 2 shows the dexamethasone treated group exhibited a higher level of WBC count (8.85 ± 1.25 x10^9/L) than all the treated groups. Immunosuppression can be a consequence of acute and chronic stress which can increase the susceptibility to a range of infectious diseases as their results indicated the potential for haematology and immune function assays to reflect elevated activity of the hypothalamic-pituitary-adrenocortical axis in cattle when treated with dexamethasone[25]. The WBC levels in all the treated groups trends as follows: G2 > G3 > G4 > G1 > G5 > G6. A high WBC counts has been suggestive of increase in disease-fighting cells known as leucocytes. It therefore follows that the lowering of the immune system of the dexamethasone-treated subjects, resulted in a high susceptibility for infections. Hence, the high WBC levels in the group even though the elevation is not statistically significant when compared with the control group. Our findings are in consonance with those of Koffour *et al*., (2011), which showed that levamisole produced an elevation in WBC count in Sprague-Dawley rats, compared to control[26]. The difference in both studies, however, is in the significance. While previous studies by Koffour *et al*. (2011) showed a significant increase in the Sprague-Dawley rats, the increase was not statistically significant in male albino rats[27]. Our study in effect might substantiate the genetic variation in drug metabolism, particularly as it concerns levamisole. The blood neutrophil to lymphocyte count ratio (NLCR) ratio is a simple marker of subclinical inflammation that can be easily obtained from the differential white blood cell count. The N/L ratio has been used to predict outcomes in patients with cancer and coronary artery disease[28-29]. The NLCR ratio integrates information on two different immune pathways – the neutrophils that are responsible for the ongoing inflammation and the lymphocytes that represent the regulatory pathway[30-31]. The NLCR values of the groups are: G1 (0.355), G2 (0.272), G3 (0.535), G4 (0.421), G5 (0.66) and G6 (0.536). This shows that levamisole served a better immunostimulatory role than the *M. Indica* extract when compared to the baseline NLCR levels. Expectedly, the very low level of NLCR of the dexamethasone-treated group is suggestive of the immunosuppressive action of dexamethasone. Also, following prior immunosuppression by dexamethasone in G5 and G6, levamisole again showed better immunoboosting effects than the ethanolic stem bark extract of *M. Indica.* So, given as a single preparation or intended after a glucocorticoid-induced immunosuppression, *M. Indica* showed an appreciable immunostimulatory potential though less favourable than levamisole at the tested doses.

Results obtained for the RBC count show that the dexamethasone + *M. indica* treated group gave the highest value (7.49 ± 0.33 x10^{12}/L). This was closely followed by the control group (7.47 ± 0.20 x10^{12}/L) and the *M. indica* treated group (7.30 ± 0.27 x10^{12}/L). Although not statistically significant, this agrees with the studies by Ogbe*et al* (2012) which reported that the observed significant increase in PCV, RBC counts, and MCHC of the test rats may suggest that the extract of *M. Indica* stem bark has haematopoietic property and might enhance erythropoietic processess and may increase resistance to oxidative damage to red blood cells membranes[23].

All clinical chemistry parameters showed no statistical significant difference from the control group. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been established as markers of hepatocellular injury while alkaline phosphatase (ALP) is a marker of cholestasis[32-33]. Several studies have shown that elevations in serum ALT are more specific for liver-related injuries or diseases while AST is less specific than ALT. The control group produced AST levels of 148.1 ± 77.7 U/L, while the levamisole treated group produced the highest with 249.4 ± 152.3 U/L, thus indicating that levamisole exerted the highest degree of damage to the hepatocytes on chronic administration. This was followed closely by the dexamethasone treated group, with a value of 202.0 ± 98.8U/L, also indicating high hepatocellular injury on acute exposure. According to Iniaghe *et al*. (2008), the increase of ALT and AST levels in serum may be due to leakage from hepatocytes through peroxidative damage of their membranes[34]. However, the *M. indica* treated group presented with a low levels of aspartate aminotransferase (96.67 ± 60.7U/L), thus implying hepatoprotective potential of the *M. indica* stem bark extract. ALT values indicate that the levamisole treated group produced the highest level, while the *M. indica* group produced the lowest. ALP results showed that the control group and the dexamethasone + *M. indica* treated group produced similar results, (184.0 ± 26.7) and (184.4 ± 92.7) U/L respectively, suggesting a possible countering effect on the ALP elevation by the ethanolic *M. indica* stem bark.

The *M. indica* treated group was observed to have reduced cholesterol levels (0.84±0.46 mmol/L) compared to control (1.02 ± 0.87 mmol/L). The hypocholestereamic effect is in agreement with Dineshkumar *et al*. (2010) when compared to control rats at doses of 10 and 20 mg/kg, i.p[35].

CONCLUSIONS

Our study showed quite definitive conclusions, one of which is the use of the neutrophil-lymphocyte count ratio (NLCR) to scale the immunostimulatory potential of the ethanolic extract of *M. Indica* stem bark relative to levamisole, a standard immunostimulant in dexamethasone-induced immunosuppression. Using information from the NLCR, Levamisole (5 mg/kg) proved to be a better immunostimulant than the extract (1000 mg/kg) as a single or on co-administration. However, a better biochemical profile was observed on treatment with *M. indica* extract in all treatment groups. The *Mangifera indica* showed a hepato-protective activity, a cholesterol-lowering effect and a stabilising tendency on the Alanine aminotransferase (ALP) concentrations.

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