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Comparative Effects of Different Co- administration Involving Aqueous Leaf- Extract of *Ficus exasperata*, Chlorpropamide and Metformin on Immunological and Haematological Parameters of Diabetic Wistar Rats

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Abstract

This study investigated the type of pharmacological interaction and its potential beneficial effects on immunological and haematological parameters of diabetic wistar rats when aqueous leaf- extract of *Ficus exasperata* (FEAE) is co-administered with chlorpropamide or metformin. The study was carried out using alloxan model of diabetes. Forty male rats (5 non- diabetic and 35 diabetic) were divided into 8 groups of 5 animals each and treated as follows: Groups 1 and 2 served as Non- diabetic and diabetic controls respectively and received 1ml distilled water, Groups 3, 4 and 5 were treated with Chlorpropamide (20mg/ kg), Metformin (150 mg/kg) and FEAE (400 mg/kg) respectively. Groups 6, 7 and 8 were administered FEAE (400 mg/kg)/ Chlorpropamide (20mg/ kg), FEAE (400 mg/kg)/metformin (150 mg/kg) and chlorpropamide (20mg/kg)/ metformin (150 mg/kg) respectively. All treatments were carried out orally for 28 days and FBS of the rats was monitored weekly during the period of treatment. At the end of the treatment, rats were sacrificed and blood samples collected for the determination of white blood cell, red blood cell, lymphocyte, neutrophil and CD4+ cell counts as well as packed cell volume and haemoglobin concentration. Results showed that white blood cells, lymphocytes and CD4 + cells were significantly ($p < 0.05$) increased in diabetic rats compared to non- diabetic control rats. Treatment of diabetic rats with the aqueous- leaf extract of *Ficus exasperata*, chlorpropamide, metformin and the various co- administerations- extract/chlorpropamide, extract/metformin and chlorpropamide/metformin ameliorated the imbalances in the immunological and hematological parameters caused by alloxan. However, the effect of the extract/chlorpropamide co-administeration was more pronounced when compared to those of other treatments. It was concluded that the extract and chlorpropamide showed synergistic interaction and this could play a key role in the management of immunological and haematological abnormalities associated with diabetes.

Keywords: Immunological, Haematological, *Ficus exasperata*, Chlorpropamide, Metformin

1. 0. Introduction

A drug interaction is said to have occurred when the effectiveness or toxicity of one medication is altered by the administration of another medicine or a substance that is administered either as food or for therapeutic purposes. Herbs are considered to be safe due to their 'natural' origin. Consequently, people consume these herbs with orthodox medicines concurrently in a bid to maximize therapeutic benefits (Idakwoji *et al.*, 2015b). Worthy of note is that not all combinations of herbs and drugs are safe as it has been reported that some may be beneficial while others may be harmful (Amita *et al.*, 2012). When an orthodox drug is co-administered orally with a herb, there is a possibility of alteration in each other's pharmacokinetic profile, that is, absorption, distribution, metabolism, and/or excretion as both orthodox and herbal drugs share the same set of metabolizing enzymes and transporters (Miller, 1998). The outcomes of drug- herb or drug- drug interaction include synergism, additive effects, potentiation and antagonism. The magnitude of the effect seen depends on the mechanism by which the interaction occurs and if the effects are positive, this could form the basis of very important drug combinations for the treatment of chronic diseases such as diabetes mellitus (Idakwoji *et al.*, 2015a).

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia (Ugochukwu *et al.*, 2003), lipoprotein abnormalities (Scoppla *et al*., 2001), raised basal metabolic rate (Nawata *et al*., 2004; Okwu *et al.*, 2006), defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances (Unwin *et al.*, 2001; Idakwoji and Uzuazokaro, 2018). The hyperglycaemia in diabetes mellitus can result from an absolute deficiency in insulin secretion (type 1 DM), insulin action (type 2 DM) or both (Ahmed and Goldstein, 2006; Andrade-Cetto *et al*., 2007). In Type 1 diabetes there is a breakdown in immune regulation that lead to expansion of auto-reactive $CD4^+$ and $CD8^+$ T cells, autoantibody- producing B lymphocytes and activation of the innate immune system (Coppieters and Von Herrath, 2010). In type 2 diabetes, there is deterioration of immunity and inflammatory process is enhanced due to increased level of immunoglobulin (Meier- Stiengen and Ziegler, 2011). Type 2, diabetes is also inked by coincident presentation and alterations in Toll- like Receptor (TLR) - dependent B cell cytokine production (Nicolajczky 2010). The adaptive as well as innate immunity are decline in Type 1 diabetes but to a less extent in Type 2 diabetes (Caroll, 2004; Botto *et al.*, 2009) which may herald the susceptibility of patients to life threatening pyogenic infection (Botto *et al.*, 2009) and existence of immune complex disease (Nicollof *et al.*, 2004). There is significant decrease in serum IgM in Type 1and 2 diabetes and this is related to presence of occult chronic infectious disease (Shin *et al.*, 2006). Urinary excretion of IgM is increased and this is associated with an increased risk for cardiovascular mortality and renal failure (Tofik *et al.*, 2009). Haematological changes consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (Comazz *et al.*, 2004). This is presented by alteration in platelet count and activity, coagulopathy, fibronolytic aberration, haemorrhagic factors and changes in endothelial metabolism (McFalarnce, 1997). The underlying cause of the changes in the immunity and hematology in diabetes mellitus is mainly due to oxidative damage (McFalarnce, 1997). A need exists to search for agents that tackle these pathophysiological aspects of diabetes. This study therefore sought to explore the potential beneficial effects of different co-administeration involving aqueous leaf- extract of *Ficus exasperata*, chlorpropamide and metformin on immunological and haematological parameters of diabetic wistar rats.

Ficus exasperata Vahl (Family: Moraceae) otherwise known as Sandpaper leaf (English), "Ewe Ipin or Eepin" (Yoruba-Western Nigeria), "Baure" (Hausa-Northern Nigeria) and "Asesa" (Igbo-Eastern Nigeria) is a deciduous tree which grows up to 18- 20 m tall in evergreen forest or semi-evergreen forests of Nigeria, India, East Africa, Middle East and Sri Lanka. The stem bark is pale to green in colour. The leaf which measures 3-20 by 2-12 cm is simple, alternate and ovate to elliptic. The whole plant has been claimed to have several medicinal uses in African traditional medicine. For instance, the leaf has been used to treat diabetes mellitus, hypertension, rheumatism, arthritis, intestinal pains, colics, epilepsy, bleeding and wounds while the roots have been claimed to be used in managing asthma, dyspnoea and venereal diseases (Chhabra *et al.*, 1990). Studies have implicated *F. exasperata* to have pharmacological activities such as protecting rats from aspirin-induced ulcerogenesis. Nimenibo- Uadia and Osagie, (2001) have reported on the oral effectiveness of the aqueous leaf extract of *F. exasperata* at the single dose of 0.4 g/kg body weight for 13 days on alloxan-induced diabetes mellitus in rats. Nimenibo-Uadia (2003) have also reported on the ability of the aqueous leaf extract of *F. exasperata* at

the dose of 400 mg/kg body weight administered 5 days post- diabetes for 7 days to counter some of the negative effects of diabetes such as hyperlipidemia, hypercholesterolemia and hyperketonaemia.

Chlorpropamide is a first-generation Sulfonylurea. Sulfonylureas acts by stimulating insulin release from pancreatic ß-cells as they bind to specific cell-surface receptors on the ß-cell plasma membrane called Sulfonylureas receptors (SUR). The binding of sulfonylureas to these receptors leads to the closure of adenosine triphosphate (ATP)-sensitive potassium channels, leading to depolarization of the cell membrane. Consequently, the voltage-gated channels open, allowing massive influx of calcium ions and subsequent release of preformed insulin granules. Several drugs interfere with the efficacy of chlorpropamide by influencing its pharmacokinetics and/or pharmacology. Several clinically important drug-herb interactions involving chlorpropamide have also been reported (Elvin-Lewis 2011). An example of such interaction is seen in the enhanced hypoglycemia observed when a meal containing garlic and *Mormodica charantia,* an herb traditionally used in the management of type-2-diabetes was consumed with chlorpropamide (Aslam and Stockley., 1979).

Metformin is a biguanide and it is antihyperglycaemic, not hypoglycaemic (Bailey, 1992). It does not cause insulin release from the pancreas and does not cause hypoglycaemia; even in large doses (Hermann, 1979). Metformin has no significant effects on the secretion of glucagon, cortisol, growth hormone, or somatostatin. It has been shown to increase peripheral uptake of glucose (Hundal *et al.,* 1992) and to reduce hepatic glucose output by approximately 20-30% when given orally (pariello *et al.,* 1994) but not intravenously. Impaired absorption of glucose from the gut has also been suggested as a mechanism of action, but has not been shown to have clinical relevance. Metformin has also been shown to decrease serum triglycerides and fatty acid concentration and slows the rate of lipid oxidation (pariello *et al.,* 1994). These actions indirectly inhibit gluconeogenesis. Metformin treatment is associated with statistically and clinically significant reduction in body weight in obese patients with type 2 diabetes. These are independent of its effect on glycaemic control (Defronzo *et al.,* 1991). Metformin is not metabolized in human tissues, hence metabolic-based drug interactions are not known to occur. Cationic drugs that are eliminated by tubular secretion may compete with metformin for elimination and this may result in clinically significant interactions.

This study assessed the type of pharmacological interaction between the extract of *Ficus exasperata* and each of chlorpropamide and metformin when co-administered. The outcome might play a key role in the management of haematological and immunological complications common among diabetics.

2. 0. Materials and Methods

2.1 Materials

2.1.1 Chemicals and drugs

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor. Chlorpropamide (250mg) and metformin (500mg) were purchased from a local pharmacy shop.

2.1.2 Animals

Male adult Wistar rats of weighing 150–200g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

2.2 Methods

2.2.1 Plant Collection and Identification

The leaves of *Ficus exasperata* were collected from a natural habitat in Ajegwu Area of Kogi State, Nigeria. The plants were identified at the herbarium unit of Biological Sciences Department, Federal University, Lokoja and voucher specimens were deposited for future references.

2.2.2 Preparation of Extracts

The leaves of *Ficus exasperata* were shade- dried for five (5) days and pulverized using an electric blender. One thousand and five hundred (1500) gram of the pulverized leaves was soaked in distilled water for 72-hours. The resulting mixture was filtered using Whatmann filter paper (Size No1) and the extract (henceforth reffered to as FEAE) was concentrated using a free- dryer.

2.2.3 Acute Toxicity Study

The oral median lethal dose (LD50) of the extract was determined in rats according to the method of Lorke (1983).

2.2.4 Experimental Design

2.2.4.1 Induction of diabetes

Diabetes was induced in adult male albino rats according to the method of Dunn and Mc Letchie (1943). The animals were fasted overnight and administered intraperitoneally 150 mg/ kg Alloxan monohydrate**.** After 72 h of administeration, rats having Fasting Blood Sugar (FBS) >200 mg/dl were considered hyperglycaemic and hence diabetic and used for the study.

2.2.4.2 Grouping of animals/ Treatment

Forty (40) adult male albino rats (5 non- diabetic and 25 diabetic) were divided into 8 groups of 5 animals each and treated as follows:

Group 1: Non- diabetic control and received 1ml distilled water
Group 2: Diabetic control and received 1ml distilled water
Group 3: Diabetic and received 20mg/ kg Chlorpropamide
Group 4: Diabetic and received 150 mg/ kg Metformin
Group 5: Diabetic and received 400 mg/ kg FEAE
Group 6: Diabetic and received 400 mg/ kg FEAE + 20mg/ kg Chlorpropamide
Group 7: Diabetic and received 400 mg/ FEAE + 150 mg/ kg Metformin
Group 8: Diabetic and received 20mg/ kg Chlorpropamide + 150 mg/ kg Metformin

All treatments were carried out orally for 28 days. Fasting Blood Sugar (FBS) of the rats was monitored weekly during the period of treatment using Fine Test® glucometer and its corresponding strips. At the end of the 28- day treatment, the animals were anaesthetized under chloroform vapour and sacrificed. Blood samples were obtained by cardiac puncture and poured into EDTA sample bottles. The samples were used for analysis within 12 h of collection.

2.2.4.3. Haematocrit determination

The packed cell volume (PCV) was estimated using the method of Alexander and Griffiths (1993). Haematocrit capillary tubes were filled by capillary action to mark with whole blood and bottom end of the tubes were sealed with plasticine. The tubes were centrifuged for 5 min using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along the haematocrit reader until the meniscus of the plasma intersects the 100% line.

2.2.4.4. Haemoglobin estimation

Cyamethaemoglobin (Drabkin) method (Alexander and Grifiths, 1993) of haemoglobin estimation was employed. Twenty microlitres of EDTA anticoagulated whole blood was added to 5 ml of Drabkin reagent mixed and incubated for 5 min at room temperature for the colour to develop. The absorbance was read against reagent blank at 540 nm using optima SP-300 Spectrophometer.

2.2.4.5. Total white blood cell count

The estimation of total white blood cells was done by visual method using New Improved Neubauer counting chamber. A 1 in 20 dilution of whole blood was made in Turk's fluid and the counting chamber with its cover glass already in position was filled with the diluted blood using a Pasteur pipette and ensuring that the chamber was filled in one action. The charged chamber was allowed to remain undisturbed for 2 min for the cells to settle. The cells were then counted microscopically using x40 objective lens. Four squares at the corners of the chamber were counted and the result was expressed in cells per litre of whole blood.

2.2.4.6. Red blood cell count

Red blood cells were counted by visual method using new improved Neubauer counting chamber. A 1 in 200 dilution of blood was made in formol citrate solution (Haymen's fluid) and the counting chamber with its cover glass in position was filled with the diluted blood using Pasteur pipette and ensuring that the chamber was filled in one action. The chamber was allowed to settle for 2 min for the cells to settle. Five squares, the four corners and the central squares were counted using x40 objective lens.

2.2.4.7 Differential white cell count

Differential white blood cell count was performed on Leishman's stained thin blood film and read microscopically using immersion oil objective and a differential manual counter. The different white cells were counted and expressed in cells/litre.

2.2.4.8 CD4 + count

The CD4 + lymphocyte were estimated by flow cytometry (Center for Disease Control and Prevention, 1997) using the cyflow automated cell counter (Partec, Germany). Ten microlitres of CD4 + PE antibody (Partec, Germany) was mixed with 50ml of EDTA anticoagulated whole blood in a test tube. The mixture was incubated in the dark chamber for 15 min at room temperature of 22 - 28°C. During incubation, the content of the tube was mixed every five min. Eight hundred microlitres of buffer solution was added, mixed and plugged into the counter. After counting the CD4 + cells, monocytes and noise were separated gated and the result was recorded.

2.2.5. Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Duncan test and difference between means at $P > 0.05$ were considered significant.

3.0.Results

3.1 Acute Toxicity

The results of acute toxicity studies showed no mortality or physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg of aqueous extract of *Ficus exasperata*. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg.

3.2 Effects of Different Co- Administration Involving Aqueous Leaf- Extract of *Ficus Exasperata*, Chlorpropamide and Metformin on Fasting Blood Sugar (FBS) of Rats

The effect of the extract, Chlorpropamide, metformin and the various co- administrations on the FBS of diabetic Wistar rats is presented in Table 1. Groups 3 (chlorpropamide 20 mg/kg), 4 (metformin 150 mg/kg) and 5 (FEAE 400 mg/kg) showed no statistically significant ($P>0.05$) deference in FBS on days 0, 7, 14, 21 and 28 compared to Group 2 (diabetic control). When compared to each other there was no significant ($P>0.05$) difference between the groups. The results also showed that Group 6 (FEAE 400 mg/kg + Chlorpropamide 20 mg/kg) showed no significant reduction ($P> 0.05$) in the FBS on days 0 and 7 but showed significant ($P< 0.05$) reduction in FBS on days 14, 21 and 28 compared to Group 2 (diabetic control). Groups 7 (FEAE 400 mg/kg + Metformin 150 mg/kg) and Group 8 (Chlorpropamide 20 mg/kg + Metformin 150 mg/kg) showed no statistically significant reduction on days 0, 7 and 14 but showed statistically significant reduction on days 21 and 28 compared to Group 2 (diabetic control). Group 6 was found to be statistically significant ($P<0.05$) lower compared to Groups 7 and 8. Compared to Group 1 (non- diabetic control), Group 3(Chlorpropamide 20mg/kg), Group 4 (metformin 150 mg/kg) and Group 5 (FEAE 400 mg/kg) showed statistically significant ($P<0.05$) deference in FBS on days 0, 7, 14, 21 and 28. Group 6 (FEAE 400 mg/kg) showed statistically significant ($P<0.05$) difference in FBS on days 0 and 7 but showed no significant ($P>0.05$) difference on days 14, 21 and 28 compared to Group 1 (non- diabetic control). Groups 7 (FEAE 400 mg/kg + Chlorpropamide 20mg/kg) and 8 (Chlorpropamide 20mg/kg and Metformin 150 mg/kg) showed statistically significant ($P< 0.05$) difference in FBS on days 0, 7 and 14 but showed no statistically significant ($P>0.05$) difference in FBS on days 21 and 28 compared to Group 1 (non- diabetic control). Also there was no significant ($P>0.05$) difference when Groups 7 and 8 are compared to each other. Group 6 showed higher statistically significantly ($P<0.05$) reduction in FBS compared to all the other treated groups.

Table 1: Comparative Effects of Different Co- Administration Involving Aqueous Leaf- Extract of *Ficus Exasperata*, Chlorpropamide and Metformin on Fasting Blood Sugar (FBS) of Rats

	Post- Treatment Time in days (d)				
Groups	**0**	**7**	**14**	**21**	**28**
Group 1	83.3±6.77^{a}	89.4±8.88^{a}	79.9±7.78^{a}	82.5±7.65^{a}	80.7±6.91^{a}
Group 2	346.5±40.28^{b}	360.5±56.55^{c}	351.8±77.72^{c}	370.5±99.18^{c}	383.5±75.31^{c}
Group 3	354.4±39.81^{b}	351.3±51.33^{c}	293.5±74.43^{bc}	241.8±82.41^{bc}	238.5±36.18^{bc}
Group 4	330.5±47.28^{b}	291.5±75.48^{bc}	285.7±71.39^{bc}	265.3±61.13^{bc}	240.6±31.13^{bc}
Group 5	339.2±87.19^{b}	293.4±66.56^{bc}	280.4±33.11^{bc}	265.9±43.38^{bc}	200.5±13.22^{b}
Group 6	348.6±77.13^{b}	273.3±81.53^{bc}	150.4±21.23^{ab}	99.7±10.38^{a}	91.9±15.11^{a}
Group 7	342.4±57.55^{b}	300.7±84.35^{bc}	240.1±24.15^{bc}	155.2±67.28^{ab}	154.9±14.43^{ab}
Group 8	350.4±94.28^{b}	281.4±51.82^{bc}	275.9±32.56^{bc}	153.9±24.34^{ab}	100.6±19.33^{a}

Data are presented as mean ± SD. Data were analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns.

Group 1- Non- Diabetic Control (1ml H_2O), Group 2- Diabetic control (1ml H_2O), Group 3 (20 mg/ kg Chlorpropamide), Group 4 (150 mg/ kg Metformin), Group 5 (400 mg/ kg FEAE), Group 6 (400 mg/ kg FXAE + 20 mg/kg Chlorpropamide), Group 7 (400 mg/ kg FXAE + 150 mg/kg Metformin), Group 8 (20 mg/kg Chlorpropamide + 150 mg/ kg Metformin)

3.3 Effects of Different Co- Administration Involving Aqueous Leaf- Extract of *Ficus Exasperata*, Chlorpropamide and Metformin on Serum Haematological Parameters of Diabetic Rats.

The effect of the extract, Chlorpropamide, metformin and the various co- administrations on the haematological parameters of diabetic Wistar rats is presented in Table 2. Groups 3 (chlorpropamide 20 mg/kg), 4 (metformin 150 mg/kg) and 5 (FEAE 400 mg/kg) showed no statistically significant (P>0.05) deference in WBC count compared to Group 2 (diabetic control). When compared to each other there was no significant (P>0.05) difference between the groups. The results also showed that Groups 6 (FEAE 400 mg/kg + Chlorpropamide 20 mg/kg), 7 (FEAE 400 mg/kg + Metformin 150 mg/kg) and 8 (Chlorpropamide 20 mg/kg + Metformin 150 mg/kg) showed statistically significant (P<0.05) reduction in WBC count compared to Group 2 (diabetic control). Compared to Group 1 (non- diabetic control), Groups 6 (FEAE 400 mg/kg+ Chlorpropamide 20mg/kg), 7 (FEAE 400 mg/kg + Chlorpropamide 20mg/kg) and 8 (Chlorpropamide 20mg/kg and Metformin 150 mg/kg) no showed statistically significant (P> 0.05) difference in WBC count. For RBC count, Groups all the groups except Group 6 showed no statistically significant (P>0.05) deference compared to Group 2 (diabetic control). When compared to each other there was no significant (P>0.05) difference between the groups except Group 6. Compared to Group 1 (non- diabetic control), Groups 6, 7 and 8 showed no statistically significant (P> 0.05) difference in RBC count. For Haemoglobin concentration, results showed that Groups 3, 4, 5 and 6 showed no statistically significant (P>0.05) difference in haemoglobin concentration compared to Group 1 (diabetic control). However, Groups 7 and 8 showed statistically significant (P<0.05) difference in haemoglobin concentration compared to Group 2 (diabetic control). For PCV, All the groups showed statistically significant (P<0.05) increase compared to Group 2 (diabetic control) but however, when compared to Group 1(non- diabetic control), there was no significant difference. For platelet count, All the groups showed except Groups 6 and 8 showed statistically significant (P<0.05) increase compared to Group 1 (non-diabetic control).

Table 2: Comparative Effects of Different Co- Administration Involving Aqueous Leaf- Extract of *Ficus Exasperata*, Chlorpropamide and Metformin on Serum Haematological Parameters of Diabetic Rats

Treatment	WBC ($x10^9$/L)	RBC ($x10^9$/L)	Hb (g/L)	PCV (%)	Plat ($x10^9$/L)
Group 1	10.3 ± 1.28^{a}	218.6 ± 21.22^{b}	12.8 ± 0.34^{b}	45.5 ± 3.34^{b}	877.5 ± 21.77^{a}
Group 2	16.1 ± 1.42^{b}	139.3 ± 15.44^{a}	09.3 ± 0.41^{a}	30.4 ± 2.22^{a}	999.2 ± 10.56^{b}
Group 3	15.4 ± 1.33^{b}	143.2 ± 21.11^{a}	10.3 ± 0.38^{ab}	45.5 ± 3.21^{b}	900.6 ± 10.23^{b}
Group 4	15.6 ± 2.08^{b}	138.5 ± 16.21^{a}	10.6 ± 0.44^{ab}	41.3 ± 2.27^{b}	912.8 ± 43.26^{b}
Group 5	13.3 ± 2.32^{ab}	147.2 ± 23.21^{a}	10.7 ± 0.36^{ab}	40.4 ± 4.36^{b}	918.4 ± 37.23^{b}
Group 6	10.7 ± 1.63^{a}	198.3 ± 13.04^{b}	10.4 ± 0.39^{ab}	45.5 ± 3.43^{b}	883.4 ± 40.33^{a}
Group 7	11.6 ± 1.21^{a}	182.3 ± 21.17^{ab}	12.2 ± 0.35^{b}	46.4 ± 3.32^{b}	905.3 ± 32.56^{b}
Group 8	11.2 ± 2.11^{a}	185.5 ± 19.16^{ab}	11.9 ± 0.35^{b}	43.2 ± 6.18^{b}	879.3 ± 30.48^{a}

Data are presented as mean ± SD. Data were analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns.
Group 1- Non- Diabetic Control (1ml H_2O), Group 2- Diabetic control (1ml H_2O), Group 3 (20 mg/ kg Chlorpropamide), Group 4 (150 mg/ kg Metformin), Group 5 (400 mg/ kg FEAE), Group 6 (400 mg/ kg FXAE + 20 mg/kg Chlorpropamide), Group 7 (400 mg/ kg FXAE + 150 mg/kg Metformin), Group 8 (20 mg/kg Chlorpropamide + 150 mg/ kg Metformin)

3.4 Effects of Different Co- Administration Involving Aqueous Leaf- Extract of *Ficus Exasperata*, Chlorpropamide and Metformin on Serum Immunological Parameters of Diabetic Rats.

The effect of the extract, Chlorpropamide, metformin and the various co- administrations on the immunological parameters of diabetic Wistar rats is presented in Table 3. A significant ($p< 0.05$) reduction in neutrophil percentage was observed in all the groups compared to Group 1 (Diabetic control). However, there was no statistically significant ($P>0.05$) deference in neutrophil percentage in all groups compared to Group 1 (non- diabetic control). For Eosinophil and Basophil, there was no significant difference compared to both controls. The lymphocytes significantly ($p< 0.05$) increased in all the groups compared to Group 2 (diabetic control) but no difference compared to non- diabetic control. There was a significant ($P< 0.05$) increase in the number of monocytes across all the groups compared to Group 2 (Diabetic control). Comparing with each other, there was no significant ($P>0.05$) difference between the groups except Group 7. All the groups also showed no significant ($P>0.05$) difference in the number of monocytes compared to the non- diabetic control except Group 7. Similarly, there was a significant ($P<0.05$) increase in the $CD4^+$ count across all the groups compared to Group 1 (non-diabetic control). Comparing with each other, there was no significant ($P>0.05$) difference between the groups except Group 7.

Table 3: Comparative Effects of Different Co- Administration Involving Aqueous Leaf- Extract of *Ficus Exasperata*, Chlorpropamide and Metformin on Serum Immunological Parameters of Diabetic Rats.

	Parameters					
Groups	**Neutrophil (%)**	**Eosinophil (%)**	**Basophil (%)**	**Lymphocytes (%)**	**Monocytes (%)**	**$CD4^+$ Count ($x10^9$/L)**
Group 1	37.9±2.31^a	2.8±0.04	0.0±0.00	56.8±3.33^b	2.5±0.09^c	1.23 ±0.25^a
Group 2	57.6±4.11^b	2.0±0.03	0.0±0.00	40.0±3.14^a	0.4±0.19^a	1.80±0.38^b
Group 3	38.4±3.18^a	2.2±0.02	0.0±0.00	58.4±4.17^b	1.0±0.18^b	1.65±0.44^b
Group 4	37.5±3.67^a	2.1±0.04	0.0±0.00	59.0±2.13^b	1.4±0.14^b	1.67±0.51^b
Group 5	39.0±4.21^a	2.8±0.01	0.0±0.00	57.0±3.11^b	1.2±0.12^b	1.59±0.62^b
Group 6	38.6±3.31^a	2.3±0.03	0.0±0.00	57.9±2.22^b	1.2±0.20^b	1.40±0.34^{ab}
Group 7	36.0±3.67^a	2.6±0.01	0.0±0.00	59.0±2.24^b	2.4±0.18^c	1.30±0.45^a
Group 8	39.5±4.56^a	2.3±0.06	0.0±0.00	57.0±2.31^b	1.2±0.17^b	1.43±0.45^{ab}

Data are presented as mean ± SD. Data were analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns.

Group 1- Non- Diabetic Control (1ml H_2O), Group 2- Diabetic control (1ml H_2O), Group 3 (20 mg/ kg Chlorpropamide), Group 4 (150 mg/ kg Metformin), Group 5 (400 mg/ kg FEAE), Group 6 (400 mg/ kg FXAE + 20 mg/kg Chlorpropamide), Group 7 (400 mg/ kg FXAE + 150 mg/kg Metformin), Group 8 (20 mg/kg Chlorpropamide + 150 mg/ kg Metformin)

4.0. Discussion

Herbal products have increasingly been incorporated into Western health care as various reports suggest a high contemporaneous prevalence of herb-drug use in developing and developed countries (Vidushi, 2013). Herb-drug interaction is the single most important clinical consequence of this practice (Fasinu *et al.,* 2012). Despite these consequences, a standard system for interaction predictions and evaluation is non-existent as most researchers concentrate on potential therapeutic effects and mechanism of action of medicinal plants and often ignoring their potential interactions with conventional drugs (Idakwoji *et al.,* 2015a). Consequently, the mechanisms underlying herb-drug interaction remain an understudied area in pharmacology (Brantley *et al.,* 2013). The knowledge of the interactions of herbs with prescription and/or over-the-counter drugs is essential for maximizing therapeutic benefits and minimizing clinical risks (Alissa, 2014; Idakwoji *et al.,* 2015b).

In the management of diabetes mellitus, a number of herbs have been observed to interact with oral hypoglycaemic drugs (Idakwoji *et al.,* 2015c). These include enhanced anti-hyperglycemia observed with co-administration of aqueous leaf extract of *Vernonia amygdalina* and metformin (Adikwu *et al.,* 2010) and co-administration of the fruit juice of *Mormodica charantia* with glibenclamide (Lal *et al.,* 2011). Similar observation was made when *Mormodica charantia* was consumed with chlorpropamide (Aslam and Stockley, 1979). Clinical studies have also shown that dietary gums such as the gum from the guar plant (*Cyamopsis tetragonobulus*) reduce the absorption of metformin and glibenclamide, consequently reducing hypoglycaemic effect (Izzo, 2004). Scientific identification of potential herb-drug interactions is of importance for effective therapy as this will provide data to justify or discourage the co-use of a particular herb and an orthodox drug (Idakwoji *et al.,* 2015c). Hence, this study assessed the potential beneficial effects of different co- administeration involving aqueous leaf- extract of *Ficus exasperata*, chlorpropamide and metformin on immunological and haematological parameters of diabetic wistar rats.

Untreated diabetes is usually associated with a number of haematological and immunological disorders. In Type 1 diabetes there is a breakdown in immune regulation that lead to expansion of auto-reactive $CD4^+$ and $CD8^+$ T cells, autoantibody- producing B lymphocytes and activation of the innate immune system (Coppieters and Von Herrath, 2010; Meier-Stiengen and Ziegler, 2011). In type 2 diabetes, there is deterioration of immunity and inflammatory process is enhanced due to increased level of immunoglobulin (Coppieters and Von Herrath, 2010. Type 2 diabetes is also inked by coincident presentation and alterations in Toll- like Receptor (TLR)- dependent B cell cytokine production (Nicolajczky, 2010). The adaptive as well as innate immunity are decline in Type 1 diabetes but to a less extent in Type 2 diabetes (Caroll, 2004; Botto *et al.,* 2009). Haematological changes consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (Comazz *et al.,* 2004). This is presented by alteration in platelet count and activity, coagulopathy, fibronolytic aberration, haemorrhagic factors and changes in endothelial metabolism (McFalarnce, 1997).

In this study, Results obtained showed that the aqueous extract of *Ficus exasperata* leaves produced significant reduction in blood glucose level in alloxan-induced hyperglycaemic. The therapeutic actions of *Ficus exasperata* has been attributed to the relatively high antioxidant activity of its leaves. Hypoglycaemic activity of kaemferol derivatives from many medicinal plants has been reported by Desokky and Youssef (1997). These antioxidants in the extract might have played a role in inhibiting and scavenging the free radicals generated by alloxan consequently, the regeneration of the beta-cells which led to the release of insulin and reduction in glycaemia (Idakwoji *et al.,* 2016). The extract might have also produced anti-hyperglycaemic activity through the release of insulin by inhibiting the ATP-sensitive potassium channels in the membrane of the residual beta-cells just like sulfonylureas and meglitinides. It is also possible that the extract might have potentiated the action of insulin to stimulate glucose uptake and utilization by tissues, especially by the liver, skeletal muscle, and adipose tissue (Gerich, 2000). The anti-hyperglycaemic activity of the extract/chlorpropamide co-administeration was greater when compared to that of chlorpropamide or metformin alone and extract/ metformin and chlorpropamide/ metformin co-administeration. This suggests that the extract and chlorpropamide showed synergistic anti-hyperglycaemic activities. The implication of this is that, a diabetic patient could be placed on a reduced dose of chlorpropamide (which also implies lower adverse effect such as weight gain) while being encouraged to take more of *F. exasperata* extract. Therefore, this particular co- administeration can play a significant role in the management of diabetes.

In this study, diabetic rats were observed to have alterations in hemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) count. The significant ($P<0.05$) reduction in the hemoglobin, and RBC cell and an increase in platelet and CD4+ cells and the differential white blood cells count, neutrophil of the diabetic untreated rats (diabetic control) compared to the normal control. As it pertains to hematology of the diabetic, terms such as anaemia in diabetes, atherosclerosis resulting from increased platelet aggregation, glycosylation of hemoglobin and of recent, even white blood cells have been discussed extensively (Saliu *et al.,* 2012). Table 2 shows that there was a significant increase in the WBC count of the diabetic control rats compared to the normal control and the diabetic treated rats. The increased immune cell counts may be the manifestations of the low grade inflammatory reactions associated with the atherosclerotic complications of diabetes mellitus (Hansson, 2005). Elevations in platelet count in diabetic control and treated- diabetic groups compared to the non- diabetic group. Platelets have been prominently and critically implicated in the onset and pathogenesis of cardiovascular diseases (CVD) either of diabetics or of other causes. Treatment with the extract, chlorpropamide, metformin alone and their co-administeration compared showed restoration of the haematological parameters. Also, the effect of the extract/chlorpropamide co-administeration was more pronounced when compared to that of chlorpropamide or metformin alone and extract/ metformin and chlorpropamide/ metformin co- administration.

Table 3 shows that untreated diabetic rats had expanded auto-reactive CD4+ cells. Treatment with the extract, chlorpropamide, metformin alone and their co-administeration resulted in reduction of circulating neutrophil, total lymphocyte and CD4+. Although the mechanism of this effect is not well known, anti-inflammatory activities have been reported for *Ficus exasperata* leaf extract. The extract effects were attributed to inhibition of immune cells migration and phagocytosis, particularly for macrophages and neutrophil in respect to inflammatory stimuli. The effect of the treatments might also have resulted from

inhibition of the induction of inducible nitric oxide synthase, prostaglandin E2 (PG E2) and interleukin 1 (IL - I) productions thus controlling the increased vascular permeability associated with inflammatory reactions.

5.0. Conclusion

Treatment of diabetic rats with the aqueous- leaf extract of *Ficus exasperata*, chlorpropamide, metformin and the various co- administerations- extract/chlorpropamide, extract/metformin and chlorpropamide/metformin ameliorated the imbalances in the immunological and hematological parameters caused by alloxan. However, the effect of the extract/chlorpropamide co-administeration was more pronounced when compared to that of chlorpropamide or metformin alone and extract/ metformin and chlorpropamide/ metformin co- administeration. The interaction between the extract and chlorpropamide can be said to be synergistic and this could play a key role in the management of immunological and haematological abnormalities associated with diabetes.

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