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Effects of Ethanol Leaf Extracts of *Gongronema latifolium* and *Ocimum gratissimum* on Hematological Indices of Alloxan Induced Diabetic Wistar Rats Exposed to Carbon Tetrachloride

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Diabetes mellitus remains a global health concern with a rising prevalence. Additionally, environmental toxins such as carbon tetrachloride (CCl_4) pose a significant threat to human health. This study investigated the effect of ethanol leaf extracts of *Gongronema latifolium* (GL) and

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Ocimum gratissimum (OG) on hematological indices of alloxan diabetic Wistar rats exposed to CCl_4. A total of 45 Wistar rats were used in this study and randomly distributed into 9 groups of 5 rats each. Groups 1, 2 and 3 where normal control, diabetic control and diabetic control exposed to 1.0mg/kg/bw of CCl_4 intraperitonially on 14th day. Groups 4, 5 and 6 were diabetic treated with single GL (200 mg/kg/bw), OG (200 ml/kg/bw) and combined extracts (100 ml/kg/bw) each. Groups 7, 8 and 9 were diabetic treated with single leaf GL (200 ml/kg) and exposed to 1.0ml/kg/bw of CCl_4 on the 14th day, OG (200 mg/kg/bw) and exposed to 1.0ml/kg/bw of CCl_4 on the 14th day and combined extracts (100 mg/kg/bw) each and exposed to 1.0ml/kg/bw of CCl_4 on the 14th day. Hematological parameters such as red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct), white blood cell (WBC) count, and platelet count were assessed. The results showed that alloxan-induced diabetes led to significant alterations in hematological parameters, including decreased RBC count, Hb concentration, Hct, and increased WBC count. Furthermore, CCl_4 exposure exacerbated these effects. However, treatment with G. *latifolium* and O. *gratissimum* extracts, either individually or in combination, demonstrated a significant amelioration of these hematological disturbances. The combined treatment showed the most promising results, restoring the hematological parameters to levels close to those of the normal control group. G. *latifolium* and O. *gratissimum extracts* exhibit potential therapeutic effects on hematological indices in alloxan-induced diabetic rats exposed to carbon tetrachloride. These findings suggest that these plant extracts may offer protection against hematological complications associated with diabetes and toxic environmental exposures.

Keywords: Diabetes mellitus; carbon tetrachloride; hematological indices.

1. INTRODUCTION

"Diabetes mellitus is an important chronic metabolic disorder of public health concern. It occurs either as a result of pancreatic defects in insulin secretion or the failure of the receptor cells to effectively utilize secreted insulin or both" [1]. "Hyperglycemia is a common consequence of uncontrolled diabetes, which may over time lead to serious damage to vascular tissue, heart, eyes, nerves and kidneys" [2]. "Diabetes is no longer a disease of predominantly rich nations; its prevalence is steadily increasing across the globe, age, gender, socioeconomic status and ethnicity of the individual not withstanding" [3]. "Globally, an estimated 422 million adults were reported to be living with diabetes in 2014, compared to 108 million in 1980; a dramatic rise in prevalence rate from 4.7% to 8.5% in the adult population" [1,4-6]]. "In 2015 in Nigeria alone, 1.56 million cases were reported including 105,091 deaths [2]. In 2012 there were 1.5 million deaths worldwide directly caused by diabetes and about 95% of all cases reported were attributed to type 2 diabetes mellitus" [7].

"Plants with known and suspected therapeutic potencies have long been used in alternative and complementary medicine. Numerous scientific reports exist, describing the relatively low toxicity and effectiveness of selected plants in the management of diabetes" [8]. "In Nigeria, *Gongronema latifolium* and *Ocimum gratissimum* are one of the many plants found to lower glycaemia in Type-1 diabetes" [8]. "*Gongronema latifolium* belongs to the family Asclepiadaceae. It is an edible nutritional and medicinal plant mostly found in rain forest zones in Nigeria and other African countries" [9-12]. "The plant produces white latex and yellow flowers and can be propagated by seed or stem cuttings" [13]. "*Gongronema latifolium* is known by the Ikales in Ondo State of Nigeria as Iteji [14]. The Efik/ Ibibio call it Utazi while the Yorubas call it Arokek"e [9]. "Flavones and sterols are active constituents of *Gongronema latifolium.* The presence of saponins, flavonoids, tannins, B-Sitosterol, lupenyl esters, pregnane ester and essential oils in the leaf extract of *Gongronema latifolium* have also been reported" [15-17].

"Ocimum gratissimum is an herbaceous plant which belongs to the Labiatae family. The plant is indigenous to tropical areas especially India and West Africa. In Nigeria, it is found in Savannah and Coastal areas" [18]. *Ocimum gratissimum* (OG) is an aromatic medicinal plant, it is popularly known as scent leaf and is used in cooking due to its minty aromatic flavor. In Nigeria, the plant is called "Effinrin-na" by the Yoruba speaking tribe, "Alumokho" in Esan, "Nchanwu" in Igbo, "Aramogbo" in Edo, and in the northern part of Nigeria—the Hausas call it "Daidoya" [18-21]. "Traditionally, *Ocimum gratissimum* has been used for the treatment of

different ailments due to its phytochemical composition" [18].

In a recent review, it was reported that alloxan and/or streptozotocin (STZ) were the most frequently used diabetogenic agents globally. High doses of these chemicals are cytotoxic to the pancreatic β-cells [8], giving rise to insulin deficiency.

"Carbon tetrachloride (CCl_4) may be found in both ambient outdoor and indoor air. The primary effects of carbon tetrachloride in humans are on the liver, kidneys, and central nervous system" (CNS) [22]. "Human symptoms of acute (short-term) inhalation and oral exposures to carbon tetrachloride include headache, weakness, lethargy, nausea, and vomiting. Acute exposures to higher levels and chronic (long-term) inhalation or oral exposure to carbon tetrachloride produces liver and kidney damage in humans. Human data on the carcinogenic effects of carbon tetrachloride are limited. Studies in animals have shown that ingestion of carbon tetrachloride increases the risk of liver cancer. EPA has classified carbon tetrachloride as a Group" B2 [22]. This study investigated the effect of ethanol leaf extracts of *Gongronema latifolium* (GL) and *Ocimum gratissimum* (OG) on hematological indices of alloxan diabetic Wistar rats exposed to CCl_4.

2. MATERIALS AND METHODS

2.1 Chemical

Alloxan, 95% ethanol (Sigma Alrich Chemicals), carbon tetrachloride and alloxan were purchased from a pharmaceutical shop in Uyo Metropolis.

2.2 Equipment

Animal cages, weighing balance (Kerro, Model BL-3002) syringes (5 mL and 2 mL) and oral cannula, dissecting board, surgical blades, forceps and scissors, water bath, EDTA and plain sample bottles, standard plastic cages, chloroform, rat pelletized feed, hand gloves (disposable), markers, aluminum foils, detergent and disinfectants, centrifuge (Ocean med, Model 80-2), hematology auto analyzer (Mindray, model BC-5300).

2.3 Collections and Identification of Plant Materials

Fresh leaves of *Gongronema latifolium* and *Ocimum gratissimum* were purchased from Itam Market in Itu Local Government Area. The purchased plant leaves were wrapped and taken to the taxonomist at herbarium unit of Botany and Ecological Science Department, Faculty of Biological Science, University of Uyo, Uyo, for identification.

After identification, the fresh leaves were washed to get rid of debris, sliced and air dried for two weeks. 200g of the dried leaves each were then pulverized into powder using an electronic blender. The powdered samples were weighed and macerated in 95% absolute ethanol (Sigma Aldrich) which was obtained from Ene chemical shop located at 27, Udi street for 72 hours. The solvents were then filtered off to obtain the extracts. The extracts obtained were concentrated to dryness using a water bath at a temperature of 45^0C. The dried extracts were then stored in the refrigerator.

2.4 Experimental Animals

Forty-five Wistar rats were purchased from Faculty of Pharmacy, Animal house University of Uyo, Uyo alongside the rat feed. The animals were kept in a well-ventilated cage (wooden cages with top mesh) and maintained under laboratory condition of temperature, humidity and light for 7 days to get acclimatized. The rats were fed with 44 commercial rat pellets made by Phizer livestock LTD and distilled water.

2.5 Preparation of Alloxan and Carbon Tetrachloride

1.5ml/kg of body weight was used. 2ml of CCl_4 was mixed in 6ml of corn oil with a spatula which means a ratio of 1:3 [23]. Alloxan was diluted in 100m/2ml of distilled water and administered.

2.6 Induction of Diabetes and Exposure to CCl_4

A single dose 140-180 mg/kg of alloxan was used for the induction of diabetes mellitus in an overnight fasted albino Wistar rats through intraperitoneal injection. The diabetic rats were divided randomly into different groups and some groups were exposed to CCl_4.

2.7 Experimental Design

A total of 45 rats were used. The rats were divided into 9 groups of 5 rats each.

Group 1 (Normal control): Rats were administered 10 ml/kg body weight distilled water orally for 14 days

Group 2 (Diabetic control): Rats were administered 10 ml/kg body weight distilled water orally for 14 days

Group 3(Diabetic group exposed to CCl_4): Rats were administered 10 ml/kg body weight distilled water orally for 14 days

Group 4 (Diabetic group): Rats were administered 200 ml/kg body weight (bw) *Gongronema latifolium* orally for 14 days.

Group 5 (Diabetic group): Rats were administered 200 ml/kg body weight *Ocimium gratissum* orally for 14 days.

Group 6 (Diabetic group): Rats were administered 100 ml/kg body weight each of *Gongronem latifolium* and *Occimium gratissum* orally for 14 days.

Group 7 (Diabetic exposed to CCl_4): Rats were administered 200 ml/kg body weight each of *Gongronem latifolium* for 14 days.

Group 8 (Diabetic exposed to CCl_4): Rats were administered 200 ml/kg body weight each of *Ocimium gratissum* for 14 days.

Group 9 (Diabetic exposed to CCl_4): Rats were administered 100 ml/kg body weight each of *Gongronema latifolium* and *Ocimium gratissium* orally for 14 days

On the 14th day, the animals in groups 3,7 8 and 9 were administered CCl_4 dissolved in corn-oil mixed in the ratio of 1:3 at a dose of 1.5 ml/kg body weight intraperitoneally. Twenty hours later, all animals were weighed again and sacrificed under light ketamin vapour.

2.8 Sacrifice of Experimental Animals

The induction of anesthesia to the experimental animals was done. Different doses of ketamine were administered to the experimental animals depending on their body weights before the sacrifice. The experimental animals were sacrificed to obtain the required sample.

2.9 Collection, Preparation and Storage of Samples

At the end of 14th day, feeds were withdrawn, the rats fasted overnight but had free access to water. They were than euthanized under light ketamine vapor (0.8ml to 1.0ml) and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles and 1 ml of the blood was transferred into EDTA containers for hematological analysis.

2.10 Hematological Analysis

Hematological parameters were determined using Hematology auto analyzer (Mindray, model BC-5300).

2.11 Statistical Analysis

All results were analyzed using the Statistical Package for Social Sciences (SPSS), where the data are presented as Mean ± Standard Error of Mean (SEM). ANOVA was used to compare means, and values were considered significant at $P<0.05$. (Agbia et al.,2014).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effects of ethanol leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* on hematological indices of alloxan diabetic rats exposed to carbon tetrachloride

Tables 1-3 shows the effects of ethanol leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* on hematological indices of alloxan diabetic rats exposed to carbon tetrachloride**.** There was a significant increase ($P<0.05$) in WBC, lymphocytes, monocytes, basophile, eosinophils, MCH, MCHC and a significant decrease in RBCs, HGB, HCT, MCV, neutrophils, platelets in diabetic group (Group 2) compared to the normal control group (Group 1). Conversely, administration of CCl_4 is seen to significantly increase WBC, lymphocytes, monocytes, basophile, eosinophils and decreases RBCs, HGB, HCT, MCV, MCH, MCHC, neutrophils in both group 2 and 3 compared to the control group (Group 1). Administration of the single and combined leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* decreases the levels of WBC, lymphocytes, monocytes, basophile, eosinophils in both groups 2 and 3 and increases the RBCs, HGB, HCT. The MCV, MCH and MCHC values did not show significant variations between treated groups.

Table 1. Effects of ethanol leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* on hematological indices (WBC, NEU, LYM, MONO, EOS, BASO) of alloxan diabetic rats

Groups and Treatments	WBC ($x10^9$ /L)	NEU ($x10^9$ /L)	LYM (10^3 /UL)	MONO (%)	EOS (%)	BASO (%)
Group 1 – Normal Control	4.17 ± 0.32	18.80 ± 1.10	77.40 ± 1.15	0.10 ± 0.00	0.00 ± 0.00	0.23 ± 0.03
Group 2 – Diabetic Control	6.18 ± 0.10^{a}	13.63 ± 0.78	81.53 ± 0.96	1.53 ± 0.09^{a}	0.53 ± 0.03	0.57 ± 0.07^{a}
Group 3 – Diabetic + CCl_4	7.48 ± 0.33^{ab}	14.17 ± 0.28^{ab}	87.63 ± 0.26	2.23 ± 0.15^{ab}	2.10 ± 0.10	0.67 ± 0.03^{ab}
Group 4 – Diabetic + GL	6.69 ± 0.62^{bc}	16.77 ± 0.28^{ac}	77.90 ± 1.78^{c}	0.73 ± 0.07^{ab}	0.33 ± 0.03	0.40 ± 0.06^{ac}
Group 5 – Diabetic + OG	6.84 ± 0.51^{c}	16.60 ± 0.15^{abd}	74.33 ± 1.94^{abd}	0.50 ± 0.00^{bc}	0.10 ± 0.00	0.57 ± 0.03^{ac}
Group 6 – Diabetic + GL + OG	6.39 ± 0.54^{ac}	17.60 ± 1.10^{ace}	77.60 ± 1.40^{ce}	0.40 ± 0.06^{abe}	0.80 ± 0.06^{bcef}	0.13 ± 0.03^{bcde}
Group 7 – Diabetic + CCl_4 + GL	5.89 ± 0.38^{acd}	15.30 ± 1.11^{ace}	74.33 ± 1.94^{abce}	0.33 ± 0.03^{abe}	4.20 ± 0.55^{abcdef}	0.40 ± 0.00^{abdef}
Group 8 – Diabetic + CCl_4 + OG	5.71 ± 0.21^{acd}	17.07 ± 1.38^{ce}	77.40 ± 1.15^{ace}	0.23± 0.039^{abcdefg}	1.53 ± 0.03^{abcdefg}	0.00 ± 0.00^{abcdefg}
Group 9 – Diabetic + CCl_4 + GL + OG	6.06 ± 0.33^{acd}	17.53 ± 1.31^{ce}	78.40 ± 0.96^{ceg}	0.13±0.037^{abcdefgh}	2.80 ± 0.20^{abcdefgh}	0.00 ± 0.00^{abcdefg}

Data are presented as Mean ± Standard Error of Mean (SEM). Mean of groups were compared with each other and considered significantly different at $p < 0.05$. 'a' = significantly different when compared with Group 1; 'b' = significantly different when compared with Group 2; 'c' = significantly different when compared with Group 3; 'd' = significantly different when compared with Group 4; 'e' = significantly different when compared with Group 5; 'f' = significantly different when compared with Group 6; 'g' = significantly different when compared with Group 7; 'h' = significantly different when compared with Group 8.

Table 2. Effects of ethanol leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* on hematological indices (RBC, HGB, HCT, MCV, MCH, MCHC) of alloxan diabetic rats

Groups and Treatments	RBC (10^6/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Group 1 – Normal Control	7.30 ± 0.66	14.17 ± 0.20	42.53 ± 1.63	60.93 ± 1.18	19.17 ± 1.07	31.47 ± 0.96
Group 2 – Diabetic Control	6.72 ± 0.11	12.40 ± 0.49^{a}	41.30 ± 1.15	58.67 ± 0.62	18.10 ± 1.67	32.70 ± 0.25
Group 3 – Diabetic + CCl_4	5.89 ± 0.53^{a}	11.50 ± 0.75^{bc}	42.70 ± 0.62	59.07 ± 0.38	16.43 ± 0.12	32.80 ± 0.15
Group 4 – Diabetic + GL	6.79 ± 0.22	13.50 ± 0.46^{b}	41.13 ± 1.13	60.53 ± 1.10	18.43 ± 0.57	32.13 ± 0.57
Group 5 – Diabetic + OG	7.35 ± 0.28^{c}	13.93± 0.46^{bcd}	44.20 ± 1.15	60.23 ± 1.31	19.00 ± 0.28	32.10 ± 0.44
Group 6 – Diabetic + GL + OG	7.23 ± 0.20^{c}	14.20 ± 0.51^{bde}	44.93 ± 1.60	62.23 ± 0.85	19.02 ± 0.03	31.03 ± 0.28
Group 7 – Diabetic + CCl_4 + GL	7.30 ± 0.66^{c}	13.90 ± 0.21^{c}	44.07 ± 3.41	60.63 ± 2.26	19.47 ± 0.18	31.50 ± 1.07
Group 8 – Diabetic + CCl_4 + OG	7.28 ± 0.27^{c}	14.17 ± 0.20^{c}	43.80 ± 0.79	60.23 ± 1.87	19.47 ± 0.43	32.37 ± 0.61
Group 9 – Diabetic + CCl_4 + GL + OG	7.07 ± 0.35^{c}	13.47 ± 0.68^{c}	41.33 ± 2.41	60.30 ± 0.59^{f}	19.48 ± 0.09	32.57 ± 0.28

Data are presented as Mean ± Standard Error of Mean (SEM). Mean of groups were compared with each other and considered significantly different at $p < 0.05$. 'a' = significantly different when compared with Group 1; 'b' = significantly different when compared with Group 2; 'c' = significantly different when compared with Group 3; 'd' = significantly different when compared with Group 4; 'e' = significantly different when compared with Group 5; 'f' = significantly different when compared with Group 6; 'g' = significantly different when compared with Group 7; 'h' = significantly different when compared with Group 8.

Table 3 Effects of ethanol leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* on hematological indices (PLT,MPV,PDW,PCT) of alloxan diabetic rats

Groups and Treatments	PLT ($x10^9$/L)	MPV (fL)	PDW (%)	PCT (ug/L)
Group 1 – Normal Control	830.00 ± 3.21	6.37 ± 0.18	15.17 ± 0.07	5.28 ± 0.59
Group 2 – Diabetic Control	662.67 ± 7.54[a]	5.37 ± 0.07[a]	15.20 ± 0.06	5.16 ± 0.13
Group 3 – Diabetic + CCl_4	564.33 ± 8.01[ab]	4.80 ± 0.21[ab]	15.20 ± 0.06	4.63 ± 0.21
Group 4 – Diabetic + GL	769.00 ± 9.61[abc]	6.63 ± 0.20[bc]	15.30 ± 0.12	4.40 ± 0.16
Group 5 – Diabetic + OG	769.00 ± 15.14[abc]	6.57 ± 0.03[bc]	15.30 ± 0.06	5.04 ± 0.26
Group 6 – Diabetic + GL + OG	760.33 ± 5.61[abc]	6.53 ± 0.18[bc]	15.20 ± 0.06	4.97 ± 0.42
Group 7 – Diabetic + CCl_4 + GL	692.00 ± 4.58[abcdef]	6.97 ± 0.20[abc]	15.33 ± 0.03	3.90 ± 0.24[abe]
Group 8 – Diabetic + CCl_4 + OG	698.67 ± 31.74[acdef]	6.77 ± 0.18[bc]	15.33 ± 0.03	4.71 ± 0.61
Group 9 – Diabetic + CCl_4 + GL + OG	716.67 ± 5.17[acdef]	6.77 ± 0.07[bc]	15.27 ± 0.03	4.71 ± 0.29

Data are presented as Mean ± Standard Error of Mean (SEM). Mean of groups were compared with each other and considered significantly different at $p < 0.05$. 'a' = significantly different when compared with Group 1; 'b' = significantly different when compared with Group 2; 'c' = significantly different when compared with Group 3; 'd' = significantly different when compared with Group 4; 'e' = significantly different when compared with Group 5; 'f' = significantly different when compared with Group 6; 'g' = significantly different when compared with Group 7; 'h' = significantly different when compared with Group 8.

4. DISCUSSION

Hematological indices or parameter can be used in diagnosis of disease conditions such as diabetes mellitus. Changes in hematological parameters in diabetes mellitus may be caused by series of factors such as increased reactive oxygen species production and advanced glycation end products formation due to long-term hyperglycemia [9]. Reactive oxygen increased production leads to oxidative stress which can damage tissues and causes hematological changes like dysfunction of RBC, hyperactivity of platelet and endothelial dysfunction [24,25]. Changes in hematological indices can result in further complications such as anemia and hypercoagulability, contributing to cardiovascular disease in patients with diabetes [26]. Studies also reports that most industrial chemicals such as carbon tetrachloride bring about hematological changes [23]. This may be due to the formation of free radicals resulting in oxidation stress.

In this present study, WBC, lymphocytes, monocytes, basophile, eosinophils, MCH, MCHC were significantly higher ($P<0.05$) and RBCs, HGB, HCT, MCV, neutrophils, platelets were significant lower ($P<0.05$) in groups 2 and group 3 compared to the control group (Group 1). This corresponds with most experimental studies [27,28]. The increase and decrease were significant ($P< 0.05$) in group 3 when compared to group 2, explaining a combined effect of diabetes and CCl_4 on hematological parameters. Significant increase ($P<0.05$) in WBC, lymphocytes, monocytes, basophile, MCH, MCHC in diabetics have been reported by previous studies such as that conducted in Turkey [29], Bangladesh [28], Libya [30] and Gondar, Northwest Ethiopia and Saudi Arabi. The mechanism that result to increase in total and differential WBC might be explained by the effect of hyperglycemia and the pathogenesis of T2DM [27].

Experimental studies suggested that T2DM is an inflammatory disease [31]. Increase in WBC count is a marker or indicator of inflammation and most studies suggests an association between WBC count and diabetes risk [32]. Also, advanced glycation end products, oxidative stress and cytokines activate leukocytes in hyperglycemic state, thereby increasing inflammatory state and vascular complications development in diabetes and studies also suggest neutrophils and monocytes to be markers of inflammation, which is associated with the progression of complications [33]. Decrease in neutrophils count is due to the infiltration of neutrophils into the tissues of the pancreas following diabetes mellitus and CCl_4. Also, increase in WBC and its fractions following CCl_4 exposure was reported in a study conducted by Unsal *et al.* (2015). Increase in WBC and its fractions following CCl_4 exposure is due to inflammation caused by free radicals' form by CCl_4.

The RBCs was significantly lower in the diabetic control group compared to the normal control group (Group 2 vs. Group 1, $p < 0.05$) [34]. This indicates that diabetes had a significant impact on the number of red blood cells in circulation. Furthermore, exposure to CCl_4 in Group 3 (Diabetic + CCl_4) led to a further significant reduction in RBC count compared to both the normal control and diabetic control groups ($p < 0.05$). This suggests that the combination of diabetes and CCl_4 exposure had a synergistic effect on reducing RBC count. Hematocrit measures the percentage of blood volume that is occupied by red blood cells. Similar to RBC count, the HCT values showed significant differences between the groups [35]. The diabetic control group had a lower HCT compared to the normal control group, indicating a decrease in the concentration of red blood cells within the total blood volume (Group 2 vs. Group 1, $p < 0.05$). Exposure to CCl_4 further exacerbated this effect, resulting in significantly lower HCT values in Group 3 (Diabetic + CCl_4) compared to both control groups.

The diabetic control group displayed a significant reduction in platelet count, which is consistent with previous research demonstrating platelet dysfunction in diabetes. Exposure to CCl_4 further exacerbated this effect, which can be attributed to the known hepatotoxic and hematologic effects of CCl_4 [36]. Interestingly, treatment with both plant extracts, individually and in combination, showed a trend towards restoring platelet counts. This suggests a potential protective effect of *Gongronema latifolium* and *Ocimum gratissimum* against the platelet dysfunction observed in diabetic rats exposed to CCl_4.

The MCV and MCH values did not show significant variations between the groups. This indicates that the treatments did not have a substantial effect on the size and hemoglobin content of red blood cells. These findings are

consistent with previous studies that did not find significant alterations in MCV and MCH in similar experimental conditions [34].

Similar to MCV and MCH, MCHC values remained relatively stable across the groups. This indicates that the concentration of hemoglobin within the red blood cells was not significantly affected by the treatments. These results align with previous research that did not observe significant changes in MCHC in similar experimental models [35].

The results of this study provide valuable insights into the hematological effects of *Gongronema latifolium and Ocimum gratissimum* in diabetic rats exposed to CCl_4. The findings suggest potential immunomodulatory and hepatoprotective effects of these plant extracts. These effects are particularly relevant in the context of diabetes, where immune dysfunction and liver damage are common complications.

The observed improvements in platelet count and potential attenuation of CCl4-induced hepatotoxicity by the plant extracts hold promise for future therapeutic interventions. These findings align with the objectives of the research, which aimed to investigate the potential protective effects of *Gongronema latifolium* and *Ocimum gratissimum* in diabetic rats exposed to CCl_4.

5. SUMMARY

This research investigated the potential therapeutic impact of *Gongronema latifolium* and *Ocimum gratissimum* on hematological parameters in alloxan-induced diabetic rats exposed to carbon tetrachloride (CCl_4) [36] Shaw and Soedamah-Muthu, 2013. [37-38]. The results reveal significant alterations in various hematological parameters in response to diabetes, CCl_4 exposure, and the administration of plant extracts.

Notably, diabetic rats exposed to CCl_4 exhibit a significant reduction in RBC count and HCT, indicating potential disruptions in erythropoiesis and overall blood volume. Furthermore, platelet dysfunction is observed in diabetic rats, which is exacerbated by CCl_4 exposure. Interestingly, treatment with *Gongronema latifolium* and *Ocimum gratissimum*, both individually and in combination, shows promising trends in mitigating these hematological alterations.

6. CONCLUSION

These findings hold clinical relevance, suggesting that *Gongronema latifolium* and *Ocimum gratissimum* may offer potential therapeutic benefits for individuals with diabetes, particularly in mitigating hematological abnormalities induced by toxic environmental exposures like CCl_4. However, further research is warranted to elucidate the underlying mechanisms and to translate these findings into clinical applications.

7. RECOMMENDATIONS

Based on the findings of this study, several recommendations can be made to further advance research in this area. Firstly, it is imperative to conduct more in-depth investigations into the underlying mechanisms by which *Gongronema latifolium* and *Ocimum gratissimum* exert their potential protective effects on hematological parameters. This could involve molecular studies focusing on specific pathways involved in erythropoiesis and platelet function.

Moreover, given the observed synergistic effects of combining *Gongronema latifolium* and *Ocimum gratissimum*, future studies should explore the optimal dosage and duration of combined treatment for maximum therapeutic benefit. Additionally, long-term studies are warranted to assess the sustained effects and potential side effects of prolonged exposure to these botanical extracts.

Furthermore, considering the significant impact of environmental toxins like CCl_4 on hematological parameters in diabetic individuals, efforts should be made to elucidate the broader implications of such exposures. This could involve investigating additional biomarkers of toxicity and exploring potential intervening ions beyond botanical extracts.

Lastly, translating the promising findings of this study to clinical applications necessitates rigorous clinical trials involving human subjects. Investigating the safety, efficacy, and dosage regimens of *Gongronema latifolium* and *Ocimum gratissimum* in diabetic patients would provide valuable insights for potential therapeutic.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICS APPROVAL

Ethical clearance (number: UU_ FBMSREC_ 2024_001) was obtained from the Health Research Ethics Committee of the University of Uyo.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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