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The effect of *Acalypha wilkesiana* leaf extract on the haematology, amylase and lipase activities in Wistar rats exposed to 1, 2-dimethylhydrazine

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Abstract

Acalypha wilkesiana is traditionally used for different ailments. Dimethylhydrazine (DMH) is an environmental pollutant which pose significant risks to human health. Any process or factors that diminish pancreatic blood flow reduces pancreatic secretions and thus impact health. This study was conducted to evaluate the effects of *A. wilkesiana* leaf extract on haematological indices, amylase and lipase activities in Wistar rats exposed to DMH. *A. wilkesiana* leaves were extracted with ethanol using maceration method. Dimethylhydrazine (40 mg/kg) was subcutaneous injected three times a week for 6 weeks to induce colon tumours in rats, followed by oral administration of three doses of *A. wilkesiana*. The rats were divided into six groups with normal control, DMH control, DMH + Xeloda, (DMH + 200; DMH + 400 and DMH + 800) mg/kg. After 6 weeks of treatment, the rats were sacrificed, blood was recovered for haematology and biochemical analysis. Amylase was significantly lower, while lipase increased in DMH ($p < 0.05$) rats compared to normal control. However, amylase and lipase were near normal control levels in the extract treated groups with the higher dose having higher values. Haematology also reflected the ameliorating effects of the leaf extract against DMH. The histology of the pancreas confirmed the toxicity of DMH and the ameliorating effects of the leaf extract. This study, indicates that *A. wilkesiana* ethanol leaf extract influenced the haematological indices and enzyme activities of DMH-induced rats in dose dependant manner.

Keywords: *Acalypha wilkesiana*, amylase, 1,2-dimethylhydrazine, haematology, lipase, pancreas

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INTRODUCTION

The pancreas is a long, accessory digestive gland located in the retroperitoneal space. It produces exocrine secretion (pancreatic juice) and accessory pancreatic ducts and endocrine secretions (glucagon and insulin from the pancreatic islets of Langerhans) that enter the blood (Talathi *et al.*, 2024).

Perforation of the pancreas results in the secretion of digestive enzymes such as amylase and lipase into the abdominal cavity and pancreatic self-digestion (Muratore *et al.*, 2021). The pancreas is highly vascularized, with islet cells receiving 10–20% of the pancreas' blood flow. This blood flow provides nutrients and oxygen to the islet cells, and also transfers hormones to target tissues (Talathi *et al.*, 2024).

Amylase which is from salivary glands and pancreas has the function of digesting starch, glycogen and related polysaccharide and may be elevated in inflammatory conditions such as cholecystitis and peptic ulcer (Basnayake and Ratnam, 2015; Wang *et al.*, 2020). Lipase on the other hand is also synthesized in pancreas and plays a vital role in lipolysis. It may be elevated also in inflammatory conditions (Basnayake and Ratnam, 2015; Hameed *et al.*, 2015; Wang *et al.*, 2020). Research indicates that the level of serum amylase and serum lipase in animals which have been exposed to toxicants is higher than those in non-exposed animals and greater value of lipases than amylase are found in inflammatory conditions (Wang *et al.*, 2020).

The pancreas and blood have a relationship in several ways, including blood supply, blood sugar regulation, and other factors. Other factors that affect the pancreas and blood include: Arginine vasopressin (AVP), a vasoconstrictor that can inhibit pancreatic secretion; Chronic pancreatitis, can cause ductal obstruction, which can lead to tissue hypertension and ischemia; Pancreatitis in severe cases can lead to shock, with low blood pressure and high heart rate (Talathi *et al.*, 2024). An increasing number of studies have demonstrated that disease states of the endocrine or exocrine pancreas aggravate one another, which implies bidirectional blood flow between islets and exocrine cells (Rizk *et al.*, 2023).

Secretion is an energy-consuming process such that increased blood flow, supplying oxygen to the tissue, is required during active secretion and factors that diminish pancreatic blood flow reduce pancreatic secretion (Rizk *et al.*, 2023; Baranowska (Sokolowska) *et al.*, 2024). Any process or factors that diminish pancreatic blood flow reduces pancreatic secretions and thus impact health. Dimethylhydrazine (DMH) is a specific colon procarcinogen classified as a toxic environmental pollutant (Claudia *et al*., 2001). Dimethylhydrazine is a potent carcinogen commonly used for the induction of colorectal tumours in the rodent model. Animal studies showed that experimental colonic tumours induced by DMH were of epithelial origin with a similar histology, morphology and anatomy to human colonic neoplasms (Zhou *et al.,* 2022). DMH is an indirect inducer drug and has the ability to promote DNA hypermethylation of colorectal epithelial cells (Olude *et al.*, 2023). The DMH is believed to form active intermediates including azoxymethane and methyl azoxymethanol in the liver, which are transported subsequently into the colon through the bile and blood (Veeresh *et al.,* 2016). Methylazoxymethanol is decomposed to form methyldiazonium ions, which methylate cellular components (Veeresh *et al*., 2016). Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine (Olude *et al.*, 2023). The historic use of natural products in folkloric medicine with low-prices and limited side effects, as opposes to expensive synthetic drugs with several adverse side effects, was the main reason for the development of new drugs from natural sources. *Acalypha wilkesiana*, commonly known as Joseph's coat, is a member of the Euphorbiaceae family (Falodun *et al.*, 2007). The leaves are traditionally used to treat different ailments such as stomach pain, skin infections and sometimes eaten as vegetables to cure hypertension. The leaves are said to be anti-inflammatory, anti-microbial (Olubodun *et al.*, 2020), antioxidant (Olubodun *et al.*, 2021), anti-diabetic (Isirima and Uahomo, 2023) and anti-hypertensive (Omage *et al.,* 2018). There is little or no study on the effect of *Acylpha wilkesiana* leaf extract on the pancreas in rats treated with DMH. This study was designed to evaluate the *effect* of *A. wilkesiana* leaf extract on haematology, amylase and lipase activities in Wistar rats exposed to 1,2, dimethyl hydrazine (DMH) by analysing the heamatological indices, amylase and lipase activities in the serum to understand the effect of DMH in the pancreas.

MATERIALS AND METHODS

Preparation of *Acalypha wilkesiana* extract

The pulverized leaves (300g) were soaked in 1.2 litres of ethanol (95%) for 72 h (3 days). The mixture was occasionally stirred using a magnetic stirrer to ensure proper mixture of the homogenate. The mixture was filtered using a sintered funnel (which is equivalent to four folds of bandage or sheet of cheese cloth). The resultant filtrate was concentrated using rotary evaporator at 40°C and freeze-dried with a freeze dryer. The weight was recorded.

Preparation of DMH and induction of colorectal tumourigenesis (CRT)

A modified method of Aranganathan and Nalini, (2009) was used to induce CRT in the rats. DMH was prepared by dissolving it in 1 mM EDTA immediately before administration and the pH was accustomed to 6.5 by 1 mM NaOH. DMH was subcutaneously administered on the right thigh at 40 mg/kg body weight three times a week, for six weeks.

Experimental design and rat treatment

The rats were quarantined for two (2) weeks in standard laboratory conditions. They were managed following the established guidelines for the care and welfare of research animals and had access to feed and drinking water *ad libitum*. This enabled them to acclimatize before initiation of the experiment. After successful induction of CRT, the rats were randomly divided into six groups of five rats each as follows:
Group 1 rats served as normal control because there was no induction nor treatment given. Group 2 is the DMH control because they were given DMH subcutaneously three times a week for six weeks. After 18 doses of DMH, all administration ceased. Group 3 rats were induced with DMH (40 mg/kg) subcutaneously three times a week for six weeks and orally treated with a standard drug (Xeloda capecitabine) in the morning via gavage every day, for another 6 weeks. Group 4 subcutaneously received DMH (40 mg/kg) three times a week for six weeks and treated with ethanol leaf extract of *A. wilkesiana* (200mg) orally in the morning via gavage every day, for another 6 weeks. Group 5 subcutaneously received DMH (40 mg/kg) three times a week for six weeks and treated with ethanol leaf extract *A. wilkesiana* (400mg) orally in the morning via gavage every day, for another 6 weeks. Group 6 subcutaneously received DMH (40 mg/kg) three times a week for six weeks and treated with ethanol leaf extract of *A. wilkesiana* (800mg) orally in the morning via gavage every day, for another 6 weeks. The rats were euthanized in a chloroform chamber and opened at the abdominal cavity, followed by blood sampling. The pancreas was harvested, two portions of which were, respectively, placed in formalin for histological assessment and tissue homogenate preparation and stored in the freezer for biochemical analyses.

Blood and serum tests

The blood samples of the rats were poured in two separate tubes. A tube with EDTA was applied for taking a complete blood count (CBC), while another tube containing no anticoagulant compound was centrifuged at 1000 rpm for 10 min and used to determine serum amylase and lipase on a BIOLIS24i autoanalyzer.

Biochemical analysis of blood and serum

Heamatology

Whole blood was used for determination of hematology. white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and differential WBC count (neutrophils, lymphocytes, eosinophils, monocytes, basophils), were analyzed with an automated analyzer (SYSMEX K-21N: SYSMEX CORPORATION, JAPAN)

Enzyme activity

Serum samples were used for analysing amylase, lipase and total protein (TP) to determine the enzymatic activities of the pancreas of the control groups and the experimental groups. The activity of serum amylase and lipase was measured using commercially available kits from Randox® (Randox Laboratory, UK), according to the manufacturer's instructions.

Histology

The pancreatic tissue was harvested after sacrificing the rats and placed in plain containers which contained 25 mL of normal saline prepared with pH=7.0 phosphate buffer solution and stored in ice (4^0C). One gramme of pancreas was immediately rinsed with sterile normal saline and placed in 10% formalin buffer. Tissue fixation, osmotic dehydration and passage were performed, and paraffin blocks were prepared and cooled. Then, the five-micron sections of tissues were hematoxylin and eosin stained and examined on an optical microscope and the photomicrographs of the tissue samples were recorded (Baker, 1945).

Statistical analysis

Data are presented as mean ± SEM. Statistical analyses were performed using Instat GraphPad based on the one-way ANOVA and Duncan's post hoc tests. While one-way ANOVA was used to determine differences among groups, Dunnett post-hoc test was used to compare treated groups. Values of $p < 0.05$ were considered significant.

RESULTS

Blood parameters:
Haematology

The results of the study recorded varied alterations in the blood parameters such as red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), monocytes (MON), lymphocytes (LYM) and platelet (PLT) in the DMH group with some significant differences ($p < 0.05$). The groups treated with Xeloda and the different doses of *A. wilkisiana* leaf extract also recorded values, some significant and others not significantly different ($p > 0.05$) from DMH control and/or normal control (Tables 1 and 2).

Table 1: Effect of ethanol leaf extract of *Acalypha wilkesiana* on haematological indices in DMH-induced rats

Assays	Groups (mg/kg)					
	Control	DMH Control	500 Xeloda	200 EE	400 EE	800 EE
RBC ($\times 10^6$/µl)	7.40 ± 0.30	6.65 ± 0.39	7.16 ± 1.07	7.46 ± 1.26	7.07 ± 0.56	7.37 ± 1.63
HGB (g/dl)	12.17 ± 0.43	10.55 ± 0.86	10.80 ± 2.00	11.30 ± 1.20	10.97 ± 1.17	10.63 ± 2.52
HCT (%)	37.13 ± 1.28	30.38 ± 1.87	33.75 ± 5.85	35.00 ± 3.70	33.43 ± 3.00	32.87 ± 6.33
MON ($\times 10^{-6}$)	1.45 ± 2.63^a	4.95 ± 0.50	5.25 ± 2.45	4.50 ± 1.50	2.48 ± 0.43	3.77 ± 524^e
MCV (fL)	50.23 ± 1.33^a	45.78± 1.64^b	46.95± 1.15^b	47.45± 3.05^a	47.17± 0.61^a	47.03± 2.03^a
MCH (pg)	16.48 ± 0.42^a	15.50 ± 0.84^b	16.42 ± 0.44^a	16.99 ± .029^a	16.44 ± 0.67^a	16.51 ± 0.57^a
MCHC (g/dl)	32.78± 0:48	33.93± 0:40	31.90 ± 0:40	32.30 ± 0:00	32.67 ± 0.63	31.57 ± 0:68
RDW (%)	15.18 ± 0.63	15.32 ± 0:73	15.13 ± 1.00	16.48 ± 1.28	15.54 ± 0.51	15.29 ± 1.59

EE: Ethanol Extract, DMH: 1,2-Dimethyhydrazine, MON: Monocyte, RBC: Red blood cell, HGB: Haemoglobin. HCT: Haemotocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW: Red blood cell distribution width. Values with different superscripts across the rows are significantly different ($p < 0.05$) and expressed as mean ± SEM ($n = 5$).

Enzyme activity

The results of the study recorded significant increases ($p < 0.05$) in amylase and lipase activities in the serum of DMH control when compared with control but the treated groups recorded no significant differences ($p > 0.05$) when compared with normal control (Table 3)

Table 2: Effect of ethanol leaf extract of *Acalypha wilkesiana* on white blood cell (WBC) and Platelets in DMH-induced rats

Assays	Parameters				
	MPV (fL)	WBC ($\times 10^3$ /µl)	PCT (%)	Lymphocyte (%)	Platelets ($\times 10^3$ /µl)
Control	5.13 ± 1.709[a]	6.43 ± 1.19[a]	0.25 ± 0.100[a]	77.75 ± 2.839	0.51 ± 0.081
DMH Control	6.89 ± 0.246	15.88±1.82	0.33 ± 0.88	78.60 ± 1.656	0.50 ± 0.138
500 mg/kg Xeloda	7.55 ± 0. 050	22.85 ± 3.15[b]	0.36 ± 0.015	82.20± 0.100[b]	0.47 ± 0.018
200 mg/kg EE	8.10 ± 0.700	13.45 ± 4.95	0.34 ± 0.65	81.45 ± 0.150[c]	0.43 ± 0.119
400 mg/kg EE	7.70 ± 0.611	16.20 ± 2.29	0.27 ± 0.32	79.10 ± 0.436[d]	0.37 ± 0.07
800 mg/kg EE	7.27 ± 0.247	19.27 ±:4.11[e]	0.41 ± 0.26	82.49 ± 2.531[e]	0.47 ± 0.033

EE: Ethanol Extract, DMH: 1,2-Dimethyhydrazine, WBC: White blood cell, LYM: Lymphocyte, PLT: Platelet, MPV: Mean platelet volume, PCT: Procalcitonin test (Plateletcrit). Values with different superscripts across the rows are significantly different ($p < 0.05$) and expressed as mean ± SEM (n = 5).

Table 3: Effect of ethanol leaf extract of *Acalypha wilkesiana* on serum amylase and lipase in DMH-induced rats

Groups	**Parameters**			
	Groups	**Amylase (U/L)**	**Lipase (U/L)**	**TP (mg/mL)**
Control	1	2.11±0.08[a]	0.114 ± 0.17[a]	15.99 ±2.30
DMH Control	2	6.15±0.48[b]	0.316 ± 0.34[b]	12.77 ±5.46
500 mg/kg Xeloda	3	4.25±1.04[b]	0.135 ± 0.21[a]	18.96 ±0.86
200 mg/kg EE	5	4.27±0.49[bc]	0.145 ± 0.23[a]	18.50 ±0.85
400 mg/kg EE	6	3.50±0.93[a]	0.141 ± 0.38[a]	18.84 ±2.10
800 mg/kg EE	7	2.84±0.68[a]	0.128 ± 0.62[a]	19.16 ±3.30

Values with different superscripts across the rows are significantly different ($p < 0.05$) and expressed as mean ± SEM (n = 5).

Histology of the pancreas

The results of the histology of control and rats treated with DMH and ethanol leaf extracts of *A. wilkesiana* is shown in Figures 1 to 6. The histological sections of the pancreatic tissues of the control rats showed a normal structure. In other words, it revealed normal architecture. (Figure 1; group 1).

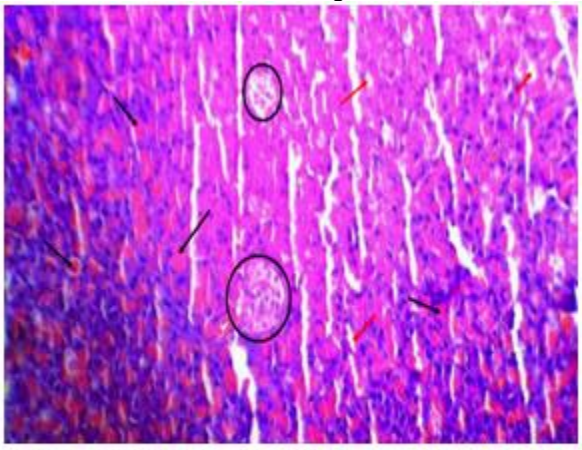

Figure 1: Photomicrograph of a section of the pancreas of the control (group 1) showing normal histo-architecture with Islet of Langerhans (encircled), closely-packed acini (black arrow), interlobular ducts (red arrow) (H&E; 100×)

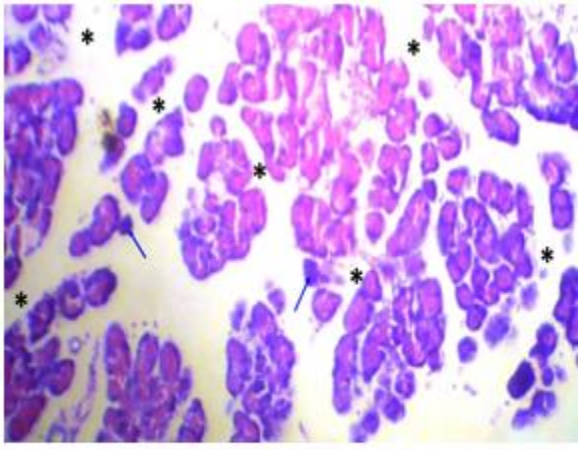

Figure 2: Photomicrograph of a section of the pancreas of the DMH-induced group (group 2) showing acute pancreatitis marked by necrotic acini (blue arrows) and severe pancreatic oedema evidenced by dilated intralobular and interlobular spaces (*) (H&E; 100×)

Histological sections of the DMH control (Figures 2) were markedly oedemic but extract-treated rat pancreas for Xeloda and doses of 200, 400 and 600 mg/kg body weights did not reveal any marked pathological changes (Figures 3 to 6).

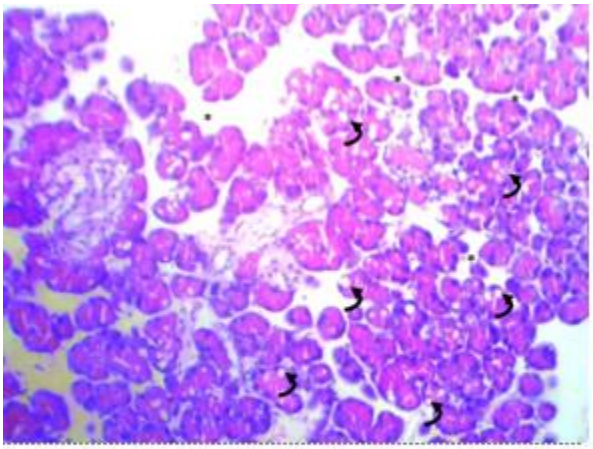

Figure 3: Photomicrograph of a section of the pancreas of the DMH + Xeloda group (group 3) showing vacuolations in the acini (curved arrow) and reduced pancreatic oedema (*) (H&E; 100×)

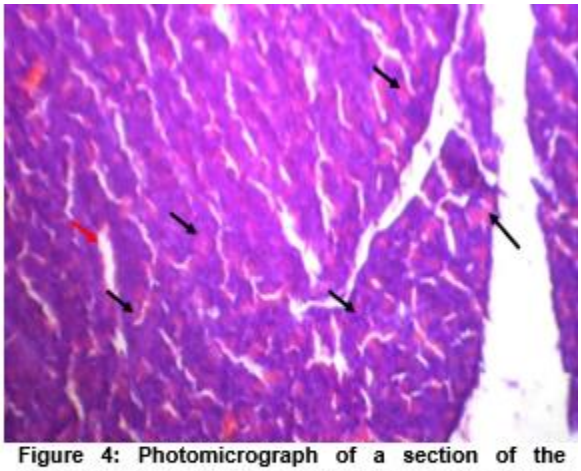

Figure 4: Photomicrograph of a section of the pancreas of the group 4 induced with DMH before low dose of extract showing histological features similar to the control group with closely-packed acini (black arrow), interlobular ducts (red arrow) (H&E; 100×)

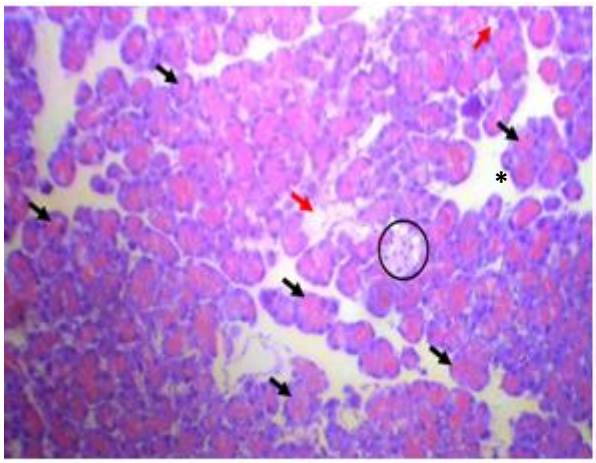

Figure 5: Photomicrograph of a section of the pancreas of the group 5 induced with DMH before medium dose of extract with Islet of Langerhans (encircled), closely-packed acini (black arrow), interlobular ducts (red arrow) (H&E; 100×)

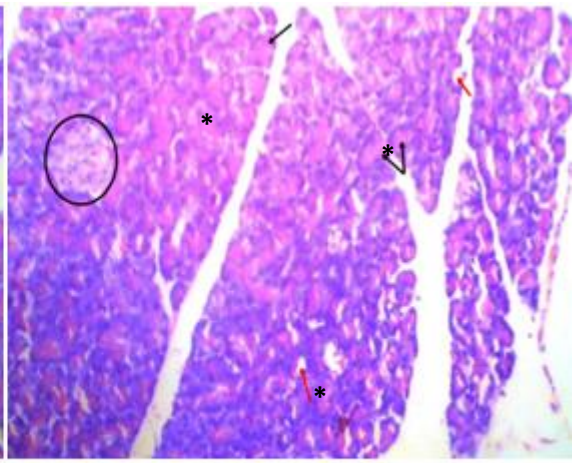

Figure 6: Photomicrograph of a section of the pancreas of the group 6 induced with DMH before high dose of extract showing histo-architecture similar to the control group with Islet of Langerhans (encircled), closely-packed acini (black arrow), interlobular ducts (red arrow) (H&E; 100×)

DISCUSSION

Haematological indices are linked to health indicators and hold diagnostic importance when assessing individual's overall health during routine clinical evaluations. Evaluating haematological indices may help to gauge the harmful impact of foreign substances, such as toxins and plant extracts, on blood components (Ashafa *et al.,* 2009). DMH and its metabolites as carcinogens have been reported to induce inflammatory responses (Reis *et al.*, 2022; Salehi *et al*, 2022) and cause free radical generation in blood and oxidative stress in erythrocytes. A therapeutic compound should have high antioxidant activity to reduce free blood

antioxidants for preventing oxidative stress in erythrocytes (Salehi *et al.*, 2022). The surge in stress to cope with increased phagocyte and lymphocyte may disturb the immune system leading to inflammation, tumourigenesis and neoplasm (Salehi *et al.*, 2022). The study recorded significant increase in WBC in the DMH group and a decrease in RBC when compared with control (Tables 1 and 2). Salehi *et al.* (2022) observed similar effects of propolis for dimethylhydrazine-Induced colorectal cancer in Wistar Rats. However, the study differs from Olude *et al*. (2023), who observed increase in RBC of *Annona muricata* in 1,2-Dimethylhydrazine-induced rats. Reduced levels of RBC, hemoglobin and hematocrit may indicate anemia as a result of bleeding, destruction of RBCs, or other causes (Olude *et al.*, 2023; Olubodun *et al.*, 2023). White blood cells (WBC) or immune responses are provoked when certain illnesses or conditions are encountered in the body system, leading to an increase or decrease in the quantity of WBCs in circulation. The significant increase in the WBCs may be due to administration of DMH as well as the extracts which have been shown to have immune modulating properties (Abu *et al.*, 2021). WBCs carry out the many tasks required to protect the body against disease-causing microbes and abnormal cells (Usunobun and Okolie, 2015). A study by Zhu *et al* (2021), confirmed that some patients with elevated WBC were more at risk of death than patients with reduced WBCs (Awoke *et al.*, 2023). The non-significant alteration observed for LYM, PLT, MPV and PCT in DMH control ae well as extract treated groups when compared with normal control, may indicate that the DMH and/or administered doses of the extract did not have any effect on the rats. This result agrees with the report recorded by other researchers with the same or different species at different treatment protocols (Obiandu *et al*., 2022; Olubodun *et al.*, 2023; Olude *et al.*, 2023). The other haematological parameters recorded non-significant alterations in DMH control except HCT and MCV which recorded significant decreases and MON which recorded significant increase when compared with normal control. The MCV is an index which reflects how small or large the RBCs are relative to normal. The significant decrease recorded in MCV in DMH control is at variance with other researchers who observed increases (Ikewuchi *et al.*, 2011; Olubodun *et al*., 2023), though some were relatively not significant but agreed with the report of Iniaghe *et al*. (2013).

The observed reduction in HCT concentration in DMH control may imply that DMH contain anti-erythropoietic property that depressed erythropoiesis. However, treatment with different doses of the extract and Xeloda resulted in non-significant increases. Obeagu *et al* (2014) and Olubodun *et al* (2023) recorded elevation in HCT when they used the leaf extracts of *Telfairia* and *A. wilkesiana* respectively on rat species. Obinna *et al* (2018) reported that an alteration in any one parameter may alter other haematological parameters. The significant increase observed in MON agrees with earlier report on effect of DMH on monocytes (Olubodun *et al.*, 2023). Monocytes (MON) are critical component of the innate immune system. They are the source of many other vital elements of the immune system, such as macrophages and dendritic cells. MON play a role in both the inflammatory and anti-inflammatory processes that take place during an immune response. The increase in MON may have been due to DMH because MON can increase by infection and not necessarily by any health challenge. The non-significant increase observed after treatment with *A. wilkeseina* ethanol leaf extract may indicate ameliorating effects of the leaf extract or non-alteration of the production and destruction of the blood cells (Olubodun *et al*., 2023).

Amylases and lipases present in pancreatic secretions and participate in digestion and metabolism (Qiu *et al.*, 2015; Wang *et al.*, 2020). Serum amylase and serum lipase are pancreatic enzymes used to diagnose acute pancreatitis (AP). When pancreatic enzyme is activated, pancreatic acinars are damaged and a large number of pancreatic enzymes are released to cause pancreatic tissues into self-digest, edema, and bleeding, which eventually lead to local inflammatory reactions (Wang *et al.*, 2020).

The increase in amylase and lipase in the serum, though not a correlation to pancreatitis, indicate that the pancreas may have been compromised (Wang *et al.,* 2020). Serum amylase has been reported to rapidly increase within few hours in some conditions with a half-life of 12 hours and may return to normal concentration within 24 hours (Wang *et al.*, 2020). This may explain the non-significant alteration recorded for the serum amylase in the studied groups. Researches also reported that elevated serum levels of amylase and/or lipase may or may not be present in some

chronic conditions, in contrast to acute conditions (Qiu *et al.*, 2015; Oh *et al.*, 2017), where serum lipase is almost always elevated. However, treatment with the leaf extracts resulted in non-significant alterations in both amylase and lipase relative to control, an indication that the plant extract has ameliorating properties.

The DMH control group exhibited severe pancreatic edema characterized by spaced out acini. This severe edema indicates the detrimental effects of DMH on the pancreas, potentially linked to inflammation and tissue damage (Wang *et al.,* 2020)

The group treated with 200 mg/kg extract after DMH induction, their pancreas maintained a histological structure similar to the control group. This suggests that the low extract may potentially be curative to DMH-induced histological alterations in the pancreas. The groups treated with Xeloda and 200 mg/kg extract experienced mild pancreatic edema, it may imply that the induction of DMH and treatment with Xeloda or 300 mg/kg extract may not offer significant protection against pancreatic edema. The observed edema may be indicative of inflammation and fluid retention within the pancreas, possibly related to DMH exposure (Kingsley and Marshall, 2014; Qiu *et al.*, 2015; Wang *et al.*, 2020).

The group treated with 800 mg/kg extract, after DMH induction, maintained the normal histo-architecture of the pancreas with closely-packed acini. This suggests that a higher extract dose is more effective in curing the effects of DMH in the pancreas. Generally, the results highlighted the curative potential of the extract, especially at higher doses, against DMH-induced pancreatic alterations. The extract potential as a therapeutic agent for pancreatic health may be attributed to its antioxidant, anti-inflammatory and apoptotic properties (Kingsley and Marshall, 2014).

CONCLUSION

The results of the study suggest that DMH induced rats experienced significant ($p < 0.05$) and non-significant ($p > 0.05$) disturbances in the haematology, amylase and lipase activities in relation to the pancreas. However, *A. wilkesiena* ethanol leaf extract was able to alter the values to levels that were not significant ($p > 0.05$) relative to normal control, a confirmation in the histology of treated groups. Further investigation of the therapeutic role of *A. wilkesiana* is advised in relation to organ functions in DMH toxicity.

Conflict of Interest

Authors have no conflict of interest to declare.

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Author contribution

OSO initiated and supervised the project. HEU, EM, FDO and ONB performed the experiments. HEU wrote part of the draft manuscript, while OSO completed and edited it.

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