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Acalypha wilkesiana Leaf Extracts Influence Heart Disease Risk Factors in 1,2-Dimethylhydrazine (DMH)-induced Rats

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Abstract

Blood is pumped to all parts of the body by the heart muscle to provide oxygen, nutrients, and remove waste products. This is a foundational study to investigate the influence of ethanol leaf extract of *Acalypha wilkesiana* on some serum markers which are risk factors of heart disease, in 1,2-dimenthyhydrazine (DMH)-induced rats. Male albino rats were divided into six groups (A to F) of five rats each. Group A was control while rats in groups B to F were subcutaneously injected with DMH at 40 mg/kg body weight thrice a week for 6 weeks. Group B rats served as DMH control; group C had Xeloda for 6 weeks and groups D to F had graded doses (200, 400 and 800 mg/kg body weight) of the extract orally for 6 weeks. DMH treatment recorded significant increases in LDL-cholesterol, cholesterol, and VLDL-cholesterol and decrease in triglycerides and HDL-cholesterol when compared with control. Doses of the leaf extract recorded significant dose-dependent elevation of cholesterol, HDL-cholesterol and triglycerides but reduction in LDL-cholesterol, glucose and total protein when compared with DMH control. Xeloda treatment did not record any significant change in lipid concentrations when compared with DMH control. The results indicate that *A. wilkesiana* significantly and non-significantly altered the risk factors associated with heart disease factors induced by DMH toxicity.

Keywords: *Acalypha wilkesiana*, Cholesterol, DMH, Ethanol extract, Heart

1. Introduction

The heart pumps and circulates the blood round the body through the vessels of the circulatory system. In the process, oxygen and nutrients are supplied to the tissues and carbon dioxide and other wastes are removed (Lewis and Dutfield, 2022). This is necessary for daily functioning of the body to promote health and sustain life. Any chemical that enters the body also eventually enters the bloodstream and will come into contact with the heart because the heart processes all the blood in the body. There is evidence that exposure to certain chemical substances correlates with heart disease, pulmonary heart disease, stroke, and high blood pressure (Rovira *et al.*, 2020; Meneguzzi *et al.*, 2021; Dai *et al.*, 2022). Many of these chemicals have other toxic effects, contributing to other illnesses such as cancer or respiratory diseases (GBD 2019 Stroke Collaborators, 2021; Ding, 2022). 1,2-Dimethylhydrazine (DMH) as an indirect inducer of carcinogenesis, has been able to represent the mechanisms of development of colorectal cancer that occur naturally in humans. It has become a very useful model in studies that aim to study chemopreventive and chemotherapeutic effects of plants and other substances (Venkatachalam *et al*, 2020).

DMH is activated in the liver and transported to the intestine by bile and blood. Its use promotes the production of free radicals, which are responsible for causing oxidative damage to the DNA of colon and liver cells. It has high degree of specificity for the intestine, the capacity to induce adenomas and adenocarcinomas plus the ability to induce metastasis (Venkatachalam *et al.*, 2020). Environmental toxins/pollutants/stressors like arsenic, cadmium,

lead, pesticides, and other compounds are responsible for Cardiovascular disorders/diseases (CVDs) (Pakiet *et al*, 2019). According to WHO (World Health Organization) arsenic, and cadmium are known carcinogens associated with many serious chronic health diseases (Sevim *et al*, 2020). Poor diet can increase the risk of CVDs such as atherosclerosis, diabetes mellitus, and hypertension (Pakiet *et al*, 2019). Pollutants also play significant roles in the development of CVDs by altering metabolic pathways in blood pressure, lipid vascular functions, and coronary artery disease (Zhang *et al*, 2020). Generally, environmental toxins which include pollutants containing particulate matter and organic substances, such as ammonium, nitrates, sulfate, have been implicated in human health and are considered major contributors to chronic diseases, particularly cardiovascular diseases (CVDs) (Pakiet *et al*, 2019).

Numerous studies have underscored the harmful effects of environmental toxins on cardiovascular health (Pakiet *et al*, 2019). Epidemiological research has closely examined the connection between these toxins and cardiovascular diseases (CVDs) (Pakiet *et al*, 2019). Substances like aerosols and particulate matter have been linked to various conditions, including dysrhythmia, ischemic heart disease, and myocardial infarction (Omage *et al*., 2018; Pakiet *et al*, 2019). Heavy metals such as lead and environmental toxins like pesticides are known to contribute to CVDs, even with prolonged low-level exposure (Pakiet *et al*, 2019). Mercury, even at low concentrations, can negatively impact multiple organs, including the heart, kidneys, nervous system, and immune system (Pakiet *et al*, 2019). Elevated blood lead levels, exceeding 100 µg% in adults and 60 µg% in children, further pose significant health risks by disrupting various pathways (Omage *et al*., 2018; Pakiet *et al*, 2019). This ultimately leads to cardiac and vascular lesions and functional impairments (Pakiet *et al*, 2019). Cadmium and arsenic exposure particularly in smokers have been well-documented with its cardiotoxic effects, which can result in fatal and irreversible myocardial damage (Omage *et al*., 2018; Pakiet *et al*, 2019; Zhang *et al*, 2020). Many cellular processes are disrupted by pollutant exposure, however, the relationship between DMH and cardiovascular health is unclear.

The harmful effects of pollutants can be treated or managed with drugs or medicinal plants (Waltenberger *et al*, 2016). Medicinal plants are made up of phytochemicals important for therapeutic purposes (Olubodun *et al*, 2020; 2023; Waltenberger *et al*, 2016). Some plants known to treat heart disease are, *Clerodendrum volubile, Amaranthus viridis, Terminalia arjuna, Picrorhiza kurroa, Ginkgo biloba, Salvia miltiorrhiza, Tinospora cordifolia* and *Allium species* (Shah *et al*, 2019). Flavonoids, sterols, polyphenols, and terpenoids are some phytochemicals found in these plants with mechanisms of action ranging from prevention of low-density lipoprotein oxidation, which promotes vasodilatation (Shah *et al*, 2019); to prevention of cardiovascular disease (CVD) by decreasing cholesterol absorption in the blood (Bachheti *et al*, 2022); to decreasing atherosclerotic lesion in the aortic valve (Shah *et al*, 2019; Bachheti *et al*, 2022). For example, the leaves of *Clerodendrum volubile* have been used to treat cardiovascular diseases, while *Allium cepa* and *Allium sativum* have been found to possess cardioprotective activities (Shah *et al*, 2019; Bachheti *et al*, 2022).

Acalypha wilkesiana, is locally called Jacob's coat and Copper leave. The leaves of the plants are used in southern and western parts of Nigeria to treat skin infection in children and inflammations (Olubodun *et al*, 2023). The beta carotene content have been reported to act as antioxidant, aiding in the defense mechanism of the immune system against the invasion of cancerous cells, cataracts, as well as the harmful effects of radiations (Chopra *et al.*, 2022). The hypolipidemic, hypoglycemic and anti-hypertensive properties of the leaves have also been reported with the leaves being eaten as vegetables (Olubodun *et al*, 2023). Accumulating evidence suggests that *Acalypha* species could be an effective chemo-preventive agent against chronic diseases (Omage *et al.*, 2018). However, the influence of the leaves in ameliorating risk factors of heart/cardiovascular disease in chemical toxicity has not been reported. This foundational study sought to evaluate the influence of ethanol leaf extracts of *A. wilkesiana* on some blood markers that are risk factors of heart disease, in 1,2-dimenthyhydrazine-induced rats.

2.0 Materials and methods

2.1 Procurement and Preparation of Animals, DMH and Plant Leaves

Male Wistar rats of average weight of about 90g were obtained from the Department of Animal Science, Faculty of Agriculture, University of Benin, Nigeria. The leaves of the plants were collected from gardens in and outside Benin City. It was authenticated as *Acalypha wilkesiana* at the Department of Plant Biology and Biotechnology, University of Benin, Benin City and given herbarium number UBH-A549. The leaves were separated to remove unwanted and spread out on a flat surface to dry at room temperature. The dried leaves were milled into a fine powder and kept before use. The rats were managed following the established guidelines for the care and welfare of research animals.

2.2 Preparation of Plant Extract

Ethanol (95%) was used to soak the pulverized leaves (300g) for 72 hours (3 days), during which the mixture was intermittently stirred with a magnetic stirrer to ensure thorough homogenization. It was then filtered through a sintered funnel, equivalent to four layers of bandage or cheesecloth. The resulting filtrate was concentrated using rotary evaporator at 40°C and freeze-dried with a freeze dryer. The weight was recorded. Graded doses of 200, 400 and 800 mg/kg body weight of the extracts used for the study was selected based on previous studies on dose response to ascertain the effective and therapeutic doses. Also, the exact volume of dose administered was based on the body weight of the rats

2.3 Preparation of DMH and Induction of Colorectal Tumourigenesis

A modified method of Aranganathan and Nalini (2013) was used to induce colorectal tumourigenesis 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.

2.4 Experimental Design

The rats were quarantined for two (2) weeks for acclimatization before initiation of the experiment. After successful induction of colorectal tumourigenesis, the rats were placed into six groups of five rats each as described below:
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) via gavage for another six weeks.
Group 4 subcutaneously received DMH (40 mg/kg) three times a week for six weeks and treated with ethanol leaf extract *Acalypha wilkesiana* (200mg) orally via gavage 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 via gavage 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 *A. wilkesiana* (800mg) orally via gavage for another 6 weeks. After 12 weeks, all the rats survived. The rats were euthanized in a chloroform chamber and opened at the abdominal cavity, followed by blood sampling. The heart was harvested; two portions placed in formalin for histology and tissue homogenate preparation and stored for biochemical analyses.

2.5 Blood Collection and Serum Tests

Blood samples were withdrawn through cardiac puncture and from the abdominal aorta. Approximately 1 ml and 4 ml of blood were drawn from each rat into bijou bottles with or without EDTA (for serum), using syringe needles. The 1 ml blood was thoroughly mixed with EDTA to prevent coagulation and was used for haematological testing. The 4 ml sample was left to clot at room temperature and the serum was collected after centrifugation at 1000 rpm for 10 min to assess serum markers.

2.6 Biochemical Assays

Triacylglycerol, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL), and glucose were measured in a spectrophotometer using commercial kits of enzymatic colorimetric methods. Bradford method was used to determine protein using bovine serum albumin as the standard.

2.7 Statistical analysis

Mean ± S.E.M (n = 5) were used to represent the data. One-way variance of analysis (ANOVA) was used to analyse the significance of the results, and the significant difference between groups was scrutinized through Dunnett's multiple test using the GraphPad Instat Version 6 (GraphPad Software Inc. San Diego, California U.S.A.). Statistical significance was set at $p < 0.05$.

3.0 Results and Discussion

3.1 The effects of ethanol leaf extracts of *Acalypha wilkesiana* on LDL-cholesterol, HDL-cholesterol, cholesterol, and VLDL-cholesterol in DMH-induced Wistar Rats.

Numerous chemicals in the environment may be instrumental to the development of cardiovascular disease. Once a chemical enters the body, it eventually reaches the bloodstream and interacts with the heart, as the heart circulates

all the blood throughout the body (Omage *et al*., 2018; Pakiet *et al*, 2019; Shrivastav *et al*, 2024). There is evidence that exposure to certain chemical substances in the environment correlates with heart disease, pulmonary heart disease, stroke, and high blood pressure (Omage *et al*., 2018; Pakiet *et al*, 2019; Shrivastav *et al*, 2024). Chronic inflammation can reduce HDL-cholesterol levels (Ni *et al*, 2014). A reduction in HDL-cholesterol is said to be a greater risk factor than high triglycerides (Omage *et al*., 2018). However, since increase in triglycerides result in significantly altered composition of all plasma lipoproteins, the contribution of other pathways may be a possible cause to increased ricks factors to heart disease (Omage *et al*., 2018; Pakiet *et al*, 2019; Shrivastav *et al*, 2024).

Table 1 shows the effects of ethanol leaf extracts of *Acalypha wilkesiana* on LDL-cholesterol, HDL-cholesterol, cholesterol, and VLDL-cholesterol in DMH-induced Wistar Rats. The DMH control presented high total cholesterol, LDL-cholesterol and VLDL-cholesterol and low HDL-cholesterol concentrations when compared with control value. Whereas, treatment with graded doses (200, 400 and 800 mg/kg body weight) of *A. wilkesiana* leaf extract recorded significant ($p < 0.05$) dose-dependent elevation of HDL-cholesterol and total cholesterol, when compared with DMH control. Low Density Lipoprotein-cholesterol and VLDL-cholesterol when compared with DMH control presented significant and non-significant increase relative to normal control but decrease with respect to DMH control. Interestingly, treatment with Xeloda (500 mg/kg) did not record any significant change in lipid levels when compared with DMH control.

Table 1. Effects of ethanol leaf extracts of *Acalypha wilkesiana* on Cholesterol, HDL-Cholesterol, LDL-Cholesterol, and VLDL-Cholesterol in DMH-induced Wistar Rats

Assays	Groups	Parameters (mg/dl)			
		Cholesterol	HDL-C	LDL-C	VLDL-C
Control	1	31.71 ± 1.74^{a}	38.12 ± 0.46^{a}	17.65 ± 2.42^{a}	4.54 ± 0.19^{a}
DMH Control	2	59.67 ± 3.04^{b}	20.23 ± 1.61^{b}	32.52 ± 5.24^{b}	7.09 ± 0.83^{b}
500 mg/kg Xeloda	3	59.00 ± 8.92^{b}	27.53 ± 1.45^{b}	25.13 ± 3.22^{c}	7.28 ± 0.82^{b}
200 mg/kg EE	5	63.67 ± 3.04^{b}	29.22 ± 1.62^{b}	23.65 ± 5.22^{c}	7.54 ± 0.46^{b}
400 mg/kg EE	6	70.99 ± 4.30^{c}	30.81 ± 2.34^{b}	20.07 ± 4.32^{c}	5.36 ± 0.29^{a}
800 mg/kg EE	7	76.27 ± 8.74^{c}	33.62 ± 3.41^{a}	21.53 ± 3.23^{c}	4.80 ± 1.24^{a}

EE: Ethanol Extract, DMH: 1,2-Dimethyhydrazine, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, VLDL-C: LDL= Very Low-Density Lipoprotein-Cholesterol of rats are expressed as means ± SEM of n = 5. Means of the same column with different superscripts are different statistically ($p < 0.05$).

The result of high cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol and low HDL-cholesterol concentrations when compared with normal control and DMH control is in agreement with the result of Omage *et al*., (2018) who reported high triglyceride and LDL-cholesterol in rabbits treated with high salt-loaded diets but at variance with the report by Bekusova *et al*., (2017). Whereas, treatment with graded doses (200, 400 and 800 mg/kg body weight) of *A. wilkesiana* leaf extract recorded significant ($p < 0.05$) dose-dependent elevation of HDL-cholesterol and total cholesterol, when compared with DMH control showing the suppressive effect of ethanol leaf extract of *A. wilkesiana* on rick factors of heart disease in rats (Table 1).

Low Density Lipoprotein-cholesterol and VLDL-cholesterol when compared with DMH control presented significant and non-significant increase relative to normal control but decrease with respect to DMH control. Interestingly, treatment with Xeloda (500 mg/kg) did not record any significant change in lipid levels when compared with DMH control. But scientifically, the basis for explaining the molecular mechanism of the cardio protection potential of A. species has not been studied (Shah *et al*, 2019).

Serum triglycerides concentrations of the DMH-induced rats were higher than that of control. Treatment with the extract further increased the concentrations of triglycerides but this increase was not significant ($p > 0.05$) when compared with the control. High triglyceride concentration is the cause of hypertriglyceridemia, a condition known to increase the risk of coronary heart disease. High triglycerides are a characteristic shared by numerous illnesses, such as lipase deficiency (Balasubramanian *et al*, 2023) and carcinogenesis (Pakiet *et al*, 2019; Fernández *et al*, 2020; Bouzas *et al*, 2022) and centrally mediated obesity, hypertension, insulin resistance, and thickening of the artery walls (arteriosclerosis) (Micheal *et al.,* 2011). Plaque deposits on artery walls, which narrow the lumen and hinder blood flow, have also been linked to high levels of VLDL cholesterol. The alterations observed in the increase in triglycerides and VLDL-cholesterol concentrations, made it unlikely that the potential hazards of

coronary heart disease may arise in this study. When comparing the levels of very low-density lipoprotein and serum triglycerides in each group before and after treatment, there were no significant alterations (Bouzas *et al*, 2022; Shrivastav *et al*, 2024).

In comparison to the control, the extract resulted in a dose-dependent increase in the concentration of HDL-cholesterol, but it also increased the concentrations of total cholesterol and lowered the concentration of LDL-cholesterol ($p < 0.05$). Increase in total cholesterol is a risk factor for heart-related conditions. Research suggest that low concentration of HDL-cholesterol raises the risk of coronary heart disease (Omage *et al*, 2018).

The risk of coronary heart disease may grow rather than decrease with continued administration of the extract, which may further lower HDL cholesterol levels and raise concentrations of total cholesterol and LDL-cholesterol. When lipid and/or cardiovascular profile is imbalanced in an organism, the organism would be adversely affected (Shrivastav *et al*, 2024). High-density lipoprotein-cholesterol, as opposed to "bad" LDL-cholesterol, is thought to be good for cardiovascular health (Omage *et al*, 2018; Pakiet *et al*, 2019).

Reducing serum LDL- and VLDL-cholesterol is considered a strategy that may help delay the on-set of chronic conditions related to hyperlipidemia in humans (Omage *et al*, 2018). *Acalypha wilkesiana* may be beneficial in this regard, as treatment with the leaf extracts has shown decrease in serum LDL-cholesterol levels and a corresponding increase in HDL-cholesterol (Omage *et al*, 2018), though not significant. Considering the receptor hypothesis of low-density-lipoprotein (LDL), high concentration of LDL-cholesterol in the blood may lead to the development of atherosclerosis (Omage *et al*, 2018). *Acalypha wilkesiana* may have suppressed the onset of atherosclerosis, by lowering LDL-cholesterol and as such, prevented heart disease in the rats.

3.2 The effects of ethanol leaf extract of *A. wilkesiana* on albumin, glucose, total protein and triglycerides in DMH-induced Wistar rats.

Table 2 shows the effects of ethanol leaf extract of *A. wilkesiana* on albumin, glucose, total protein and triglycerides in DMH-induced Wistar rats. The groups induced with DMH recorded decrease in the serum albumin, glucose and total protein but the decrease was not significant ($P > 0.05$). The study also recorded increase in triglycerides levels (mg/dl) but the increase was not significant when compared with normal control after 6 weeks. Administration of graded doses of ethanol leaf extract of *A. wilkesiana* and Xeloda at 500 mg/kg body weight over 6 weeks period, presented non-significant increases in albumin, and triglycerides relative to DMH control and significant increase in total protein relative to normal and DMH control. Serum glucose level were decrease in all groups however the decrease what not significant relative to the normal or DMH control.

Table 2. Effects of ethanol leaf extracts of *Acalypha wilkesiana* on Albumin, Glucose, Total Protein and Triglycerides in DMH-induced Wistar Rats

Assays	Groups	Parameters (mg/dl)			
		Albumin	Glucose	Total Protein	Triglycerides
Control	1	3.70±1.24	106.92±9.17	15.99±2.30	28.72±0.61[a]
DMH Control	2	2.97±0.58	104.60±11.7	12.77 ±5.46	32.94±4.57[ab]
500 mg/kg Xeloda	3	3.51±0.17	102.40±11.0	18.96 ±0.86	35.82±3.61[ab]
200 mg/kg EE	5	3.10±0.24	100.00±10.0	18.50 ±0.85	33.65±1.82[ab]
400 mg/kg EE	6	3.43±0.15	98.81±5.43	18.84 ±2.10	28.72±0.61[a]
800 mg/kg EE	7	3.60 ±0.30	98.40±5.44	19.16 ±3.30	32.94±4.57[ab]

EE: Ethanol Extract, DMH: 1,2-Dimethyhydrazine, Total Protein, of rats are expressed as means ± SEM of n = 5. Means of same column with different superscripts are different statistically ($p < 0.05$).

The serum glucose concentrations, as well as total proteins and albumin of the DMH-induced rats were lower than that of control however, the difference were not significant. This study agrees with that reported by Omage, *et al*, 2018 but is at variance with the report of Bekusova *et al*., (2017) who recorded increase in glucose in DMH-induced mice. The decrease in glucose may signify increased energy metabolism in the rats. Treatment with *A. wilkesiana* extract recorded further reduction in the concentration of glucose and significantly high triglycerides in the serum, when compared with DMH-control and normal control. *A. wilkesiana* may have a sparing effect on triglyceride's breakdown with consequent increase in glucose utilization (Omage *et al*, 2018; Fernández *et al*, 2020).

The non-significant alteration observed in serum glucose concentration may confirm the hypoglycemic and/or anti-diabetic property of the plant extract. Proteins perform a wide range of biological functions, acting as enzymes, regulator of metabolism, as antibodies and component of complement system (Omage *et al*, 2018). Plasma proteins help maintain osmotic pressure and facilitate the transport of drugs, hormones and other substances, often serving as reservoirs for their controlled release (Omage *et al*, 2018). Changes in protein concentration in disease conditions result from acute phase reaction proteins such as albumin, transferrin, among others (Omage *et al*, 2018). DMH induction resulted in non-significant decrease in albumin concentration which increased non-significantly with administration of *A. wilkesiana* leaf extract (Table 2).

The reduction in albumin concentration due to DMH induction may represent an effort by the rats' homeostatic mechanisms to compensate for the decrease in oncotic pressure (Omage *et al*, 2018). Albumin helps to transport small molecules like bilirubin, calcium, drugs and progesterone through the blood (Omage *et al*, 2018), and plays an important role in keeping the fluid from the blood from leaking out into the tissues. However, treatment with the extract led to non-significant increases in the serum albumin levels. The extract has been reported to possess electrolytes which may have caused an internal redistribution of fluid from the intra- to extracellular compartment and synthesis of albumin to balance the oncotic pressure to ensure normal physiology (Omage *et al*, 2018).

4.0. Conclusion

DMH treatment showed significant ($p < 0.05$) increases in all risk factors for cardiovascular diseases. Treatment with varying doses of *A. wilkesiana* leaf extract showed significant ($p < 0.05$), dose-dependent rise in cholesterol levels, HDL-cholesterol and triglycerides but reduction in LDL-cholesterol, glucose and total protein when compared with DMH control. Treatment with Xeloda (500 mg/kg) did not record any significant change in lipid concentrations when compared with DMH control. The results indicate that *A. wilkesiana* significantly and non-significantly altered the risk factors associated with heart disease factors induced by DMH toxicity. Since, this is a foundational study, more details are required for future investigations to unravel the molecular mechanism involved in the influence of the action of *A. wilkesiana* observed and at higher doses.

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