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Assessment of the Risk of Developing Cardiovascular Disease in Prediabetic and Diabetic Wistar Rats with Insulinaemia, in a Selected Model

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: This examination assessed the risk of developing cardiovascular disease in pre-diabetic and diabetes with insulinaemia in Wistar rats using the HOMA-IR model. The assessment solidified the role of Wistar rats in the evaluation model and took the blood analytes to screen for diabetes.
Methodology: 84 male Wistar rats measuring 175g were isolated into fourteen groups of six animals each and dealt with high fat eating standard and acknowledged with dexamethasone which achieved diabetes. Group 1 animals were given feed and water. Group 2 animals got no treatment. Groups 3-5 were coordinated Glimepiride and Metformin, and Cinnamon independently while Groups 6-14 were administered 100mg/kg, 300mg/kg, and 500mg/kg of Ginger, Aloe Vera, and mix of Ginger and Aloe Vera exclusively. The concentrates were administered orally. Every illustration of blood serum and plasma was explored using Randox and Accubind packs and an autoanalyser to test for various biochemical and hematological limits.
Results: The overall results revealed a colossal differentiation ($p \leq 0.05$) in the limits except for that of Na^+. The 500mg/kg body weight part of the concentrate was ideal while there was much basic development in the HOMA-IR with potential gains of 0.94±0.04, 2.28±0.17 and 3.25±0.44 for the

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three sets. HOMA-IR is enticing over other models, as reported by other investigators. This assessment revealed that HOMA-IR works best in the management of diabetes in connection with various models.
Contributions to Knowledge: HOMA-IR works best at predicting and managing DM.
Administration of varying doses of the extracts to the Wistar rats helped in the control by ameliorating the effect of diabetes as seen in the return of some diabetic markers assayed.
This research used Wistar rats in the evaluation of the HOMA-IR model.
This research compared the blood analytes of non-diabetics, pre-diabetics, and diabetics in order to monitor the onset of diabetes and proffer possible solutions to enhance early detection and manage diabetes.

Keywords: Cardiovascular disease; diabetes mellitus; insulinaemia; obesity.

1. INTRODUCTION

"Insulin is a pancreatic ß-cell hormone released postprandially. It signals a fed state and can directly stimulate glucose disposal into peripheral insulin target tissues, thereby, suppressing hepatic glucose output (HGO). When the action of insulin is interfered with, as may occur during impairment of insulin sensitivity at a peripheral or hepatic level, the plasma glucose elevating effect of the hormone will be altered. Early in the pathophysiological process, insulin impairment may be compensated for by increase in the ß-cell insulin output which maintains normoglycemia. However, when insulin secretion becomes insufficient to sustain normoglycemia, hyperglycemia and diabetes mellitus ensues" [1].

"Insulin resistance entails the reduced ability of insulin to exert its biological effects on target tissues, such as, adipose tissue, skeletal muscle and liver. For blood glucose concentration, insulin resistance is inappropriately high level of insulin for the glycaemia level" [2]. "Both insulin resistance and sensitivity are continuous, thus, it is difficult to ascertain cut-off levels for insulin resistance when considering an individual as lying along the continuum between very high and very low insulin sensitivity. This concept is vital, when considering techniques that assess insulin sensitivity" [2].

"Insulin resistance is commonly regarded as a major risk factor in the etiology of type 2 diabetes mellitus" [3]. Other risk factors include obesity, physical inactivity, body fat distribution, age and hyperinsulinemia. Insulin resistance serves as a predictor to developing type 2 diabetes mellitus, even with normal glucose tolerance (when the peripheral tissues are moderately sensitive to the presence of glucose and utilize same). It is also implicated in the etiology of metabolic syndrome [4] and represents a collection of cardio-metabolic risk factors that encourage the development of cardiovascular diseases and type 2 diabetes mellitus [5, 6], in addition to its characteristic surplus release of insulin to lower plasma glucose levels. The resultant hyperinsulinemia accounts for peripheral reduction of insulin sensitivity [7]. This condition increases the risk of prediabetes, type 2 diabetes mellitus and other health concerns and complications, such as, heart attack, stroke, kidney disease, eye problem and cancer. The symptoms that signal insulin resistance are extreme thirst or hunger, feeling of hunger even after a meal and increased or frequent urination.

Insulin resistance is among the main problems of type 2 diabetes mellitus (T2DM) and its treatment with natural herbs may be associated with lesser side effects compared to available synthetic orthodox oral anti-diabetic agents. Dexamethasone (Dex), a synthetic glucocorticoid with anti-inflammatory and immunosuppressant properties has proffered broad therapeutic applications. "This is not without a setback; as glucose intolerance and reduced insulin sensitivity in vulnerable patients have been reported, but is dependent on the dose and frequency of its administration, which could cause diabetes mellitus" [8]. Apart from its clinical applications, Dex is widely employed to induce insulin resistance in animals and humans in research [9, 10, 11, 12].

Diabetes mellitus (DM) is a lifestyle non-communicable disease that represents a significant global health challenge afflicting both young and old, irrespective of gender [13]. DM is a metabolic condition that arises from the inability to either produce insulin or utilize available insulin, leading to decline in the quality of human life. Nigeria has the highest number of diabetics in Africa with approximately 4 million cases and prevalence of 4.99% [13]. It is a subtype of the

umbrella disease condition, with the other type being diabetes insipidus. Diabetes mellitus type 2 (DM- 2) accounts for 95% of all reported cases of diabetes [14, 15]. The causes of DM-2 are multi-factorial, encompassing genetic and environmental elements that affect pancreatic β-cell function and insulin sensitivity to peripheral utilization [16,17].

Routine fasting blood sugar (FBS) test is indicative of the present glycaemic state and serves as a common test, especially, in resource-limited climes, but this assay is subject to diurnal fluctuations and is therefore not considered confirmatory. Another assay, glycated haemoglobin (HbA1c) test, alternatively, reveals the FBS level of past 1-3 months. High HbA1c, predisposes to long-term complications (World Health Organization (WHO),[18]. Current research trends favour predictive markers of diabetes mellitus DM. Such measures may detect DM at its pre-diabetic state, implying that once any of such markers begin to rise, the individual could be at risk of developing DM or any cardiovascular disorder (CVD) [19]. It is therefore, valuable to employ such markers to ascertain the onset of DM.

"Different assessment models for insulin resistance exists, such as Homeostasis model assessment of insulin resistance (HOMA-IR) index, Triglyceride and glucose (TYG) index and Triglyceride/high density lipoprotein cholesterol (TG-HDLc) ratio, with individual and collective benefits and shortcomings. This study chose the HOMA-IR index model, due to its advantages over others. Previous studies have associated TyG index with carotid atherosclerosis, coronary artery calcification and high risk of CVD, but associations with TG-HDLc ratio outweighs it" [2,1]. The HOMA-IR index has the benefit of detecting the presence and extent of insulin resistance, making it valuable for both baseline blood sugar and responsive hormone analysis.

Metabolic health lies in the space between insulin and glucose sensitivity [20, 21]. Low HOMA-IR indicates sensitivity to insulin, while increasing HOMA-IR signifies further resistance to insulin. Investigating insulin, HbA1c, glucose, lipid profile, thyroid stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), renal function (sodium, potassium, chloride, bicarbonate, urea and creatinine), full blood count (FBC) and assessment of HOMA-IR, TG/HDL-c ratio and TyG indices, is vital for normal and diabetic subjects, which could be investigated in vivo by employing laboratory animals, which are cost-effective and easier to handle. This necessitated the use of Wistar rats in this study.

2. METHODOLOGY

The study was conducted in Choba community, University of Port Harcourt and the University of Port Harcourt Teaching Hospital (UPTH), all in Obio/Akpor Local Government Area and Aluu community in Ikwerre Local Government Area of Rivers State, Nigeria. The area is located in the Niger Delta region of Nigeria.

The materials used in this study include, cannula (catheter), cotton wool, methylated spirit, micropipette- one hundred (100)µl, 100– one thousand (1000)µl), micropipette tips, pipette (5ml and 10ml), plain bottles, sample containers, syringes and needles, test-tubes and test-tube racks. Other materials used were extracts of aloe vera, ginger and cinnamon, and specific chemicals and reagents adhering the to the manufacturer's instructions in the guide included in the kits.

Plant samples (aloe vera leaves and ginger rhizomes) were obtained from markets at Mile 3 Diobu and Choba in Rivers State. The aloe vera leaf and ginger rhizome samples were identified, collected and deposited in the Departmental herbarium with the voucher numbers UPH/P/184 and UPH/P/185 respectively. Ginger rhizomes were washed with clean sterile distilled water and allowed to air-dry for an hour, then the outer covering was manually peeled off and the ginger washed again and extracted. An aqueous ginger extract was prepared by adhering to previously reported methods by Onyeagba *et al.* [22]. In the method, 100g of fresh, washed ginger cloves was macerated in a sterile, ceramic mortar and the homogenate filtered off with a sterile, muslin cloth.

The plant ginger sample was then blended, dried, and finely ground to powder. 100g of the ground ginger was mixed with one liter of sterile deionized water and kept in a water bath at 60ºC for five hours, then filtered through sterile filter paper "Whatman, UK". The filtrate was exposed at 40 ºC to a hot air oven for evaporation of water. The residue after drying was then kept in a refrigerator at 4 ºC until use [23].

Aloe vera crude extract was prepared by washing the leaves with tap water, followed by weighing. A traditional hand filleting method of processing aloe leaves was used, in which the

lower leaf base, tapering point at the leaf top and short spines located along the leaf margins were removed by sharp blades. The epidermis of the leaves was peeled off and the parenchyma tissue was collected. A colourless, solid mucilaginous gel was cut into pieces. The gel was lyophilized and ground, while the lyophilized gel powder was packed into soxhlet apparatus and extracted with 90% ethanol at 90° C for four (4) hours. The ethanol containing the extract was filtered and concentrated using rotary evaporator and was stored at 90°C.

A total of eighty four (84) male Wistar rats were used for the study. They were weighed and housed in standard cages, with their weights ranging from 150-200g. Humid tropical conditions with 12 hours light were observed and the animals were fed standard diet and water *ad libitium*, and acclimatized with the new housing condition within 14 days. The animals were grouped into a control (group A) and test (group B) groups, with the test group having three subgroups; I, II and III.

The acute toxicity, LD_{50} of the ginger and aloe vera extracts on wistar rats was determined orally using a method described by Alaribe *et al*. (2012). The rats were divided into four groups of four rats each weighing between 150g and 200g. They were subjected to 24 hours fasting (with only water) before the extracts were administered. The extracts was dissolved in 20% between-30 and administered in doses of 100, 300, 500 and 1000mg/kg body weight orally. The fifth group was the control and received only 20% between-30. The rats were further observed for toxicity and fatalities for 72 hours. No mortality was recorded after 72 hours in both categories of extracts all through the four concentrations used, though, paw licking, restiveness, aggressive behaviors and extreme calmness were observed. Loss of weight associated with reduction in food consumption was observed in groups administered with 1000mg/kg doses.

The control group (A) consisted of six (6) rats each and was subdivided into: normal control; treated with only distilled water aside the general feed, negative control; treated with diet and dexamethasone to induce insulin resistance that could result in type 2 diabetes but not treated, while the second group (B) was divided into 4 sub-groups consisting of 6 rats each treated with varying anti-diabetic agents such as glimepiride, ginger, aloe vera and combination of ginger and aloe vera. The third group (C) was also divided into 4 sub-groups consisting of 6 rats each and were given metformin, ginger, aloe vera and combination of ginger and aloe vera. A fourth group (D) was divided into 4 sub-groups which consisting of 6 rats each and given a herb (cinnamon), ginger, aloe vera and combination of ginger and aloe vera.

Following the grouping, the rats were fed for 56 days (28 days for induction, and 28 days for treatment, and were anaesthetized in chloroform and sacrificed 24 hours after the last treatment and after a 12-hour fast. The thoracic region was opened to expose the heart where fresh blood was collected by cardiac puncture and the liver excized for histopathological analysis. The organ was preserved by placing it in a plain bottle with formalin. Serum obtained was analyzed in the laboratory, while the organ was fixed and stained. All samples were assayed in the Chemical pathology and Anatomical pathology laboratories in the UPTH.

3. RESULTS

Table 1 showed a significant difference ($p \leq 0.05$) in the biochemical parameters for the test groups relative to the control groups. The negative control group (untreated) did not show any improvement while the test groups treated with standard drugs and herb (Glimepiride, Metformin, and Cinnamon) showed improvement. The 100mg/kg, 300mg/kg, and 500mg/kg doses of the plants extract (Ginger, Aloe Vera, and their mixtures) also showed a dose dependent ameliorative effect on dyslipidaemia.

Table 2 reveals the renal profile values which shows significant difference ($p<0.05$) in the electrolytes for the test groups relative to the control groups. The negative control group (untreated) showed no improvement while the test groups treated with the standard drugs and herb showed improvement. The doses of the plant extracts also showed improvement with an increase in the level of improvement as the dose increased. There was also significant difference ($p<0.05$) in the level of Urea and Creatinine for the test groups relative to the control groups. The negative control group (untreated) showed no improvement while the test groups treated with standard drugs and herb (Glimepiride, Metformin, and Cinnamon) showed marked improvement. The 100mg/kg, 300mg/kg, and 500mg/kg doses of the plant extracts (Ginger, Aloe Vera, and a mixture of both in equal proportions) also showed marked improvement with an increase in the level of improvement as the dose increased.

Table 1. Lipid profile of rats from the different groups

GROUP	CHOL (mmol/l)	TG (mmol/l)	HDL (mmol/l)	LDL (mmol/l)
NORMAL CONTROL	4.17±0.07^{b,d,e}	1.43±0.10^{b,e}	1.40±0.06^{b,c,d}	2.08±0.07^{b,e}
NEGATIVE CONTROL	7.20±0.47^{a,c,d}	3.38±0.25^{a,c,d}	0.90±0.04^{c,e}	4.73±0.51^{a,c,d}
GLIMEPIRIDE	5.28±0.08^{a,b,d,e}	2.18±0.09^{a,b,e}	0.98±0.03^{d,e}	3.08±0.10^{a,b,d}
METFORMIN	5.55±0.27^{a,b,c,e}	2.28±0.12^{a,b,c,e}	1.10±0.04^{c}	2.83±0.33^{a,b}
CINNAMON	5.98±0.37^{a,b,c,d}	2.53±0.11^{a,b,c,d}	1.24±0.04^{c,d}	2.73±0.30^{a,b}
100mg/kg GINGER	6.48±0.09^{a,b,c}	2.70±0.15^{a,b,c,d}	1.08±0.05^{e}	3.88±0.25^{a,b,c,d}
100mg/kg ALOE VERA	5.88±0.21^{a,b,c,d,}	2.88±0.09^{a,b,c,d}	1.05±0.06^{e}	3.80±0.27^{a,b,c,d}
100mg/kg GINGER + ALOE VERA	5.73±0.31^{a,b,c}	2.55±0.08^{a,b,c}	1.08±0.05^{e}	3.63±0.29^{a,c,d}
300mg/kg GINGER	6.12±0.21^{a,b,c,d}	2.68±0.06^{a,b,c}	1.10±0.04^{e}	3.39±0.17^{a,b,c,d}
300mg/kg ALOE VERA	5.78±0.16^{a,b,c,d}	2.72±0.10^{a,b,c,d}	1.15±0.04^{e}	3.48±0.16^{a,b,c,d}
300mg/kg GINGER + ALOE VERA	5.60±0.25^{a,b}	2.38±0.16^{a,b}	1.18±0.05	3.44±0.31^{a,b,c,d}
500mg/kg GINGER	6.02±0.25^{a,b,c,d,}	2.43±0.07^{a,b}	1.20±0.07^{c,d}	3.15±0.23^{a,b,c,d}
500mg/kg ALOE VERA	5.70±0.24^{a,b,c}	2.40±0.23^{a,b}	1.22±0.06^{c,d}	3.32±0.23^{a,b,c,d}
500mg/kg GINGER + ALOE VERA	5.54±0.11^{a,b,e}	2.23±0.06^{a,b}	1.22±0.05^{c,d}	3.25±0.11^{a,b,c,d}

Table 2. Renal profile of rats from the different groups

GROUP	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	H_2CO_3 (mmol/l)	Urea (mmol/l)	Creatinine (mmol/l)
NORMAL CONTROL	139.50±1.12^{b,c,e}	3.90±0.09^{d}	97.33±0.71^{b,c,e}	25.67±0.88^{b,c,e}	3.22±0.26^{d}	60.83±1.83^{d}
NEGATIVE CONTROL	132.50±1.85^{a,d}	5.21±0.12^{c,e}	87.25±1.55^{a,d}	18.50±0.65^{a,d}	7.73±0.51^{c,e}	89.00±3.24^{c,e}
GLIMEPIRIDE	135.75±1.11^{b,d}	4.23±0.09	93.25±0.85^{a,b,d}	21.50±0.65^{a,b,d}	4.50±0.21	70.75±2.06
METFORMIN	137.25±1.11^{a,b,c,e}	4.33±0.13^{c}	95.75±1.31^{a,b,c,e}	23.25±0.75^{a,b,c,e}	4.38±0.26^{a,b,c,e}	68.25±2.50
CINNAMON	136.00±0.41^{a,b,d}	4.53±0.15^{c}	94.75±0.85^{a,b,c,d}	22.75±1.03^{a,b,c,d}	5.25±0.19^{c}	71.00±2.12^{c}
100mg/kg GINGER	135.75±0.85^{a,b,d,}	4.70±0.11^{c,e}	90.25±0.63^{a,b,d}	20.50±0.65^{a,b,d}	6.28±0.26^{c,e}	78.25±1.44^{c,e}
100mg/kg ALOE VERA	134.75±0.85^{a,b,d}	4.80±0.10^{c,e}	92.00±0.91^{a,b,d}	21.75±0.41^{a,b,c,d}	6.14±0.28^{c,e}	76.90±1.55^{c,e}
100mg/kg GINGER + ALOE VERA	135.50±0.87^{a,b,d}	4.73±0.17^{c,e}	92.75±0.65^{a,b,d}	21.75±0.48^{a,b,c,d}	6.06±0.20^{c,e}	76.60±2.40^{c,e}
300mg/kg GINGER	136.25±0.63^{a,b,d}	4.63±0.11^{c,e}	93.00±0.71^{a,b}	22.25±0.63^{a,b,c,d}	6.00±0.18^{c,e}	76.95±1.25^{c,e}
300mg/kg ALOE VERA	135.75±1.11^{a,b,d}	4.78±0.11^{c,e}	94.00±1.29^{a,b,c,d}	21.00±0.85^{a,b,d}	5.93±0.27^{c,e}	75.80±2.20^{c,e}
300mg/kg GINGER + ALOE VERA	136.80±0.73^{a,b,d}	4.70±0.07^{c,e}	95.50±0.58^{a,b,c,d,e}	22.20±0.49^{a,b,c,d}	5.80±0.20^{c,e}	73.00±1.81^{c,e}
500mg/kg GINGER	136.25±0.63^{a,b,c,d,e}	4.55±0.11^{c,e}	93.00±0.71^{a,b,d}	23.25±0.63^{a,b,c,e}	5.90±0.18^{c,e}	72.50±1.25^{c,e}
500mg/kg ALOE VERA	136.60±0.75^{a,b,c,d,e}	4.56±0.09^{c,e}	94.20±0.66^{a,b,c,d}	22.60±0.51^{a,b,c,d}	5.68±0.19^{c,e}	73.00±1.77^{c,e}
500mg/kg GINGER + ALOE VERA	137.00±1.29^{a,b,c,d,e}	4.40±0.09^{c}	95.20±0.85^{a,b,c,d,e}	23.75±0.85^{a,b,c,e}	5.50±0.37^{c,e}	72.50±2.40^{c,e}

Table 3 shows there was significant difference ($p<0.05$) in the level of Glucose for the test groups relative to the control groups. The negative control group (untreated) showed no improvement while the test groups treated with standard drugs and herb (Glimepiride, Metformin, and Cinnamon) showed marked improvement. The 100mg/kg, 300mg/kg, and 500mg/kg doses of the plant extracts (Ginger, Aloe Vera, and a mixture of both in equal proportions) showed marked improvement with increase in dose. HOMA-IR, TYG and TG-HDL indices, and Insulin (INS) also showed significant difference ($p<0.05$) in the biochemical indices for the test groups relative to the control groups. Insulin for the Normal control group was 0.88±0.34 mIU/L, while that for the Negative control (Induced but not treated) was 11.65±0.51 mIU/L. The HOMA-IR Index for the Normal control was 0.19±0.07, while that for the Negative control was 6.67±0.14. Both Insulin and HOMA-IR for the Negative control were significantly different from the Normal control indicating a significant increase in insulin resistance as is found in Type 2 Diabetes.

The TYG index and TG-HDL index of the Negative control were also statistically different from that of the Normal control; 3.75±0.02 and 4.54±0.02 for TYG index, and 1.03±0.06 and 3.80±0.43 for TG-HDL index, respectively for the Normal control and Negative control groups. The test groups treated with standard drugs and herb (Glimepiride, Metformin, and Cinnamon) showed improvement in the indices.

Table 4 reveals that groups treated with the standard drugs and herb showed improvement. The doses of Ginger, Aloe vera, and a mixture of both, showed improvement with decrease in levels as the dose increased.

Table 5 of some haematological parameters showed that red blood cell (RBC) count, packed cell volume (PCV), and haemoglobin (Hb) count were significantly higher ($p<0.05$) in negative control and test groups relative to normal control group. Platelet (PLT) count on the other hand was statistically higher in the negative control and test groups than the normal control group. The test groups treated with standard drugs and herb (Glimepiride, Metformin, and Cinnamon) showed improvement. The 100mg/kg, 300mg/kg, and 500mg/kg doses of the plant extracts (Ginger, Aloe Vera, and a mixture of both in equal proportions) also showed progressive improvement with increase in dose of extract. PLT count levels decreased as doses of extracts increased.

Table 6 shows the white blood cell (WBC), neutrophil (NEU) and lymphocyte (LYMP) count for the animals. WBC and LYMP counts are higher ($p<0.05$) for the test groups relative to normal control group while the NEU count is higher ($p<0.05$) in the test groups compared with the normal control group. The test groups treated with standard drugs and herb (Glimepiride, Metformin, and Cinnamon) showed improvement. The 100mg/kg, 300mg/kg, and 500mg/kg doses of the plant extracts (Ginger, Aloe Vera, and a mixture of both in equal proportions) also showed improvement. It also showed significant difference ($p<0.05$) in Monocyte (MONO) and Eosinophil (EOS) levels for the test groups relative to the normal control group. The test groups treated with standard drugs and herb (Glimepiride, Metformin, and Cinnamon) showed improvement. The 100mg/kg, 300mg/kg, and 500mg/kg doses of the plant extracts (Ginger, Aloe Vera, and a mixture of both in equal proportions) also improved. There was no statistical difference between Basophil (BAS) level of test and control groups both before and after treatment with standard drugs and herbs or extracts.

4. DISCUSSION

Due to the complexity in determining an individual prone to insulin resistance, it becomes also difficult to interpret the animal model of insulin resistance as an inference to standardize that of humans. According to Aleixandre *et al.* (2009), the usefulness of rat model of non-insulin dependent diabetes mellitus (NIDDM) is nevertheless questionable, and there is no clear experimental model of hypertension. This study did not consider risk factors for DM-II such as such as genetics, ethnicity and age, apart from obesity. Type 2 diabetes directly correlates increased risk of visceral fat deposition (Simmons, 2019). This agrees with this study. Groups fed with high fat diet showed increased LDL, CHOL and TG levels where the levels of LDL, CHOL and TG levels of the induced and untreated and even the treated groups were much higher than that of the normal control groups. With increased glucose production, reverse feedback mechanism occurs leading to increase in the lipid profile parameters. HDL levels were lower in the diabetic than normal rats but increased as treatment proceeded; the HDL levels increased as doses increased.

Table 3. Biochemical indices from the different groups

GROUP	GLUCOSE (mmol/l)	INS (mIU/L)	HOMA-IR	TYG INDEX	TG-HDL INDEX
NORMAL CONTROL	4.95±0.18[b]	0.88±0.34[b]	0.19±0.07[b]	3.75±0.02[b]	1.03±0.06[b]
NEGATIVE CONTROL	12.95±0.65[a,c,d,e]	11.65±0.51[a,c,d,e]	6.67±0.14[a,c,d,e]	4.54±0.02[a,c,d,e]	3.80±0.43[a,c,d,e]
GLIMEPIRIDE	6.88±0.21[a,b,d]	7.85±0.24[a,b,d,e]	2.74±0.06[a,b,d,e]	4.13±0.01[a,b,d]	2.23±0.09[a,b,d,e]
METFORMIN	6.58±0.18[a,b]	7.63±0.18[a,b]	2.57±0.10[a,b,e]	3.94±0.02[a,b]	2.07±0.10[b]
CINNAMON	8.65±0.21[a,b,c,d]	6.83±0.17[a,b]	2.05±0.04[a,b]	4.20±0.03[a,b,c,d]	2.11±0.10[a,b,d]
100mg/kg GINGER	9.98±0.27[a,b,c,d,e]	9.35±0.17[a,b,c,d,e]	4.89±0.02[a,b,c,d,e]	4.33±0.01[a,b,c,d]	3.15±0.12[a,b,c,d,e]
100mg/kg ALOE VERA	9.53±0.36[a,b,c,d,e]	9.34±0.14[a,b,c,d,e]	4.83±0.04[a,b,c,d,e]	4.31±0.02[a,b,c,d,e]	3.00±0.11[a,b,c,d,e]
100mg/kg GINGER + ALOE VERA	9.73±0.19[a,b,c,d,e]	9.50±0.16[a,b,c,d,e]	4.86±0.02[a,b,c,d,e]	4.29±0.02[a,b,c,d,e]	2.94±0.09[a,b,c,d,e]
300mg/kg GINGER	9.60±0.44[a,b,c,d,e]	9.04±0.23[a,b,c,d,e]	4.30±0.08[a,b,c,d,e]	4.27±0.02[a,b,c,d,e]	2.87±0.10[a,b,c,d,e]
300mg/kg ALOE VERA	9.42±0.17[a,b,c,d,e]	8.70±0.27[a,b,c,d,e]	4.23±0.06[b,c,d,e]	4.23±0.04[a,b,c,d,e]	2.80±0.10[a,b,c,d,e]
300mg/kg GINGER + ALOE VERA	9.63±0.38[a,b,c,d,e]	8.98±0.10[a,b,c,d,e]	4.29±0.03[a,b,c,d,e]	4.21±0.02[a,b,c,d,e]	2.78±0.14[a,b,c,d,e]
500mg/kg GINGER	9.05±0.25[a,b,c,d,e]	8.63±0.15[a,b,c,d,e]	4.28±0.05[a,b,c,d,e]	4.24±0.02[a,b,c,d,e]	2.77±0.19[a,b,c,d,e]
500mg/kg ALOE VERA	9.38±0.58[a,b,c,d,e]	8.53±0.29[a,b,c,d,e]	4.19±0.13[a,b,c,d,e]	4.22±0.04[a,b,c,d,e]	2.70±0.29[a,b,c,d,e]
500mg/kg GINGER + ALOE VERA	9.14±0.30[a,b,c,d,e]	8.38±0.38[a,b,c,d,e]	4.11±0.08[a,b,c,d,e]	4.20±0.01[a,b,c,d]	2.60±0.13[a,b,c,d,e]

Table 4. Glucose and Lipid profile on day 0 and 14 from the different groups

GROUP	GLU (mmol/l)		CHOL (mmol/l)		TG (mmol/l)	
DAYS	0	14	0	14	0	14
NORMAL CONTROL	5.32±0.39[d]	5.00±0.24[e]	4.11±0.27	4.23±0.10[a]	1.47±0.08	1.50±0.09[b]
NEGATIVE CONTROL	10.50±0.20[c]	11.55±0.45	7.00±0.20	7.32±0.45	2.88±0.20	3.07±0.21
GLIMEPIRIDE	7.18±0.41	7.22±0.40	5.73±0.20	5.30±0.18	2.35±4.03	2.52±0.15[a]
METFORMIN	7.10±0.40	7.03±0.25[d,e]	5.25±0.19[b]	5.63±0.18	2.37±0.15	2.52±0.10[b]
CINNAMON	7.85±14.03[a]	7.40±0.32	5.72±0.39	5.40±0.29[d,e]	2.35±0.15	2.48±0.14[d]
100mg/kg GINGER	9.12±0.49	9.02±0.38	6.83±0.23	6.80±0.14	2.68±0.14	2.87±0.13
100mg/kg ALOE VERA	9.77±0.43[c]	8.90±0.34	6.87±0.27[c]	6.40±0.13[d]	2.70±0.18	2.82±0.08[a]
100mg/kg GINGER + ALOE VERA	9.65±0.56	9.07±0.43	6.70±0.32	6.42±0.20	2.68±0.09	2.80±0.15
300mg/kg GINGER	9.03±0.44	8.53±0.31	6.68±0.37	6.63±0.23	2.67±0.19	2.78±0.11
300mg/kg ALOE VERA	9.48±0.49[a]	8.63±0.21	6.75±0.31[a]	6.30±0.21	2.65±0.16	2.75±0.15
300mg/kg GINGER + ALOE VERA	9.17±0.64	8.68±0.21	6.57±0.31	6.32±0.41	2.63±0.16	2.73±0.09
500mg/kg GINGER	8.25±0.33[b]	8.33±0.58	6.45±13.35	6.55±0.21	2.63±0.19	2.60±0.14
500mg/kg ALOE VERA	9.15±1.07	8.37±0.36	6.40±10.32[b]	6.15±0.18	2.55±0.15[c]	2.70±0.16
500mg/kg GINGER + ALOE VERA	8.20±0.28[b]	8.47±0.33	6.27±0.23	6.23±0.26	2.60±0.12	2.65±0.15

Table 5. Haematological profile

GROUP	RBC ($\times10^6$cells/cmm)	PCV (%)	Hb (g/dL)	PLT ($\times10^9$/L)
NORMAL CONTROL	3.83±0.14[b]	35.07±1.08[b,c]	11.27±0.39[b]	141.50±6.00[b]
NEGATIVE CONTROL	4.22±0.12[a]	37.20±1.08[a,]	12.28±0.37[a]	175.25±9.62
GLIMEPIRIDE	4.15±0.17[a]	36.65±2.02[a,b]	11.90±0.68[a]	155.25±3.30[a,b]
METFORMIN	4.10±0.13[a]	35.95±0.60[b]	11.75±0.52[b,c]	153.75±4.78[a]
CINNAMON	4.05±0.16[a,b]	36.80±1.30[a]	11.65±0.41[b]	158.50±4.33
100mg/kg GINGER	4.18±0.13[a]	37.75±1.38	12.08±0.44[a]	163.80±4.16[a]
100mg/kg ALOE VERA	4.14±0.07[a]	36.20±0.71[a,b]	12.14±0.22[a]	164.60±3.20[b]
100mg/kg GINGER + ALOE VERA	4.13±0.07[a]	36.09±0.71[a,b]	12.04±0.22[a]	168.60±4.49[a]
300mg/kg GINGER	3.96±0.09[a,b]	36.09±0.29[a,b]	12.00±0.44[a]	162.00±7.59[b]
300mg/kg ALOE VERA	3.90±0.25[b]	36.75±0.03[a,b]	11.88±0.35[b]	163.25±4.91[a]
300mg/kg GINGER + ALOE VERA	3.90±0.15[b,c,d]	35.90±2.04[b]	11.58±0.68[b]	164.25±5.02[a,b]
500mg/kg GINGER	3.85±0.10[b,c,d]	35.40±2.20[b]	11.63±0.72[b]	161.25±3.20[b]
500mg/kg ALOE VERA	3.88±0.25[a,e]	36.00±2.48[b]	11.53±0.83[b]	160.75±4.64[b]
500mg/kg GINGER + ALOE VERA	3.87±0.29[b,c,d,e]	35.50±1.04[b]	11.43±0.37[b]	162.25±7.55

Table 6. WBC count and WBC component from different groups

GROUP	WBC ($\times10^9$/L)	NEU ($\times10^9$/L)	LYMP ($\times10^9$/L)	MONO ($\times10^9$/L)	EOS ($\times10^9$/L)	BAS ($\times10^9$/L)
NORMAL CONTROL	5.52±0.37[b]	29.17±1.45	55.83±1.99[b]	2.83±0.60[b]	1.03±0.49[b]	0.00±0.07
NEGATIVE CONTROL	6.40±0.69[a,c]	44.00±8.16[a,c,d,e]	62.75±7.68[a,c,d,e]	3.50±0.29[a,c,d]	1.35±0.48[a]	0.00±0.07
GLIMEPIRIDE	5.92±1.08[a,b]	35.00±2.89[b,d]	60.00±3.32[a,b]	3.10±0.58[a,b]	1.20±0.58[a]	0.00±0.07
METFORMIN	6.00±1.75[a,b]	34.00±1.00[a,b]	59.50±1.32[a,b]	3.00±0.65[a,b]	1.20±0.07[a]	0.00±0.07
CINNAMON	6.09±0.76[a,c]	35.50±2.87[a]	59.00±2.71[a,b]	3.15±0.25[a,b]	1.25±0.25[a]	0.01±0.29
100mg/kg GINGER	6.23±0.83[a]	39.75±3.52[c,d,e]	61.00±3.24[a,b]	3.35±0.41[a]	1.35±0.25[a]	0.00±0.07
100mg/kg ALOE VERA	6.10±0.64[a]	39.80±5.10[c,d,e]	62.15±5.51[a,c,d,e]	3.25±0.48[a]	1.30±0.41[a]	0.00±0.07
100mg/kg GINGER + ALOE VERA	6.22±0.57[a]	42.60±4.32[b,d]	61.05±5.00[a,b,c]	3.24±0.40[a]	1.30±0.24[a]	0.00±0.07
300mg/kg GINGER	5.96±1.25[a,b]	38.20±2.87[b,d,e]	60.55±2.63[a,b]	3.10±0.40[a,b]	1.29±0.24[a]	0.00±0.20
300mg/kg ALOE VERA	5.80±0.39[a]	39.50±1.74[b,d,e]	61.35±1.47[a,b]	3.09±0.55[a,b]	1.30±0.24[a]	0.00±0.07
300mg/kg GINGER + ALOE VERA	5.82±0.73[a]	41.00±6.25[a,b,c,e]	60.25±6.26[a,b]	3.10±0.29[a,b]	1.27±0.48[a]	0.01±0.05
500mg/kg GINGER	5.90±0.92[a,b]	37.25±3.33[b,c,e]	59.75±3.33[a,b]	3.00±0.25[a,b]	1.25±0.25[a]	0.01±0.07
500mg/kg ALOE VERA	5.85±0.10[a,b]	38.00±4.57[b,c,e]	59.67±4.33[a,b]	3.07±0.29[a,b]	1.20±0.25[b]	0.00±0.07
500mg/kg GINGER + ALOE VERA	5.80±1.24[b]	40.25±2.78[b,d,e]	59.60±2.92[a,b]	3.00±0.50[a,b]	1.19±0.07[a,b]	0.01±0.05

"A number of factors contribute to becoming obese, such as eating high calorie diet (high fat diet), not getting enough physical exercise, genetics, medical conditions and being on medications. Obesity triggers changes to the metabolism of the body. These changes cause adipose tissue to release fat molecules into the blood which affect insulin responsiveness in cells and lead to reduced insulin sensitivity. Obesity causes pre-diabetes, a metabolic condition that usually results in type 2 diabetes" [3].

"Cinnamon has been known to increase insulin sensitivity while Glimepiride and Metformin have been known to decrease insulin resistance and the risk of type 2 diabetes" [1]. "Type 2 diabetes affects the homeostasis acid-base regulation. High glucose concentration results in an osmotic force that draws water to the extracellular space. This dilutes extracellular sodium and results in lower blood sodium level" [24]. "In our result, a decrease in blood sodium level was observed as we moved from normal, to pre-diabetic and diabetic subjects, though the decrease were not statistically significant at $p<0.05$. Potassium levels are also altered in diabetes. High plasma glucose concentrations result in potassium efflux to the extracellular space, causing hyperkalemia" [24]. This was observed in this study. Bicarbonate (H_2CO_3) degrades to carbon (IV) oxide and water, and anion gap acidosis results. This is observed in the significantly lower Bicarbonate levels in the pre-diabetic and diabetic groups. The chloride values also follow the same trend. Generally, the diabetic rats are at increased risk of assay-based disturbance and electrolyte disturbances. "This risk is due to the diseased state of diabetes itself and the associated disruptions in glucose homeostasis, drugs used to treat diabetes, and the organ damage associated with diabetes" [24]. The urea and creatinine levels of the pre-diabetic and diabetic groups were higher than that of the non-diabetics. This is in tandem with other studies that reported hyperglycaemia as one of the major causes of progressive renal diseases (Bamanikar *et al.*, 2016). Approximately 20% to 30% of diabetics will develop abnormal kidney function, represented by a reduced glomerular filtration rate and a rise in serum urea and creatinine. Administration of Glimepiride and Metformin ameliorated the condition. Cinnamon also had a similar effect as the standard drugs. There was also an improvement with the extracts of Ginger and Aloe Vera in different concentrations though to a lesser degree. In this study, we found that insulin resistance was increased significantly in the pre-diabetic and diabetic groups as depicted by the HOMA-IR index. This is expected and agrees with other studies of insulin resistance as a major risk factor and predictor of type 2 diabetes [3].

"Analysis of the haematological parameters in the test groups showed alterations in haematological indices in the diabetic state. Diabetes is a metabolic disease that is characterized by hyperglycaemia, dyslipidemia, hypertension, and impaired hematological indices. Several hematological changes affecting RBCs, WBCs and coagulation factors are directly associated with DM" [25]. "Other hematological abnormalities reported in the DM patients include RBCs, WBCs, and platelet dysfunction" [26]. In this study, PCV, Hb and RBC were all higher in the test models and is similar to the negative control group relative to the Normal control group. This may be partly due to increased HbA1c in the diabetic state (Marar, 2011).

Treatment of the animal test groups with the standard drugs and herbs led to reduction in insulin resistance as measured by HOMA-IR. This improved the adverse effects of the disease condition as shown by the improvement in the HbA1c levels, Lipid profiles, Renal Profiles, Thyroid Function and the Haematological parameters. Treatment with the plant extracts also led to mild improvements in these indices.

5. CONCLUSION

Insulin resistance is generally accepted to be a major risk factor in the etiology of type 2 diabetes mellitus [3]. Several risk factors (obesity, physical inactivity, body fat distribution, age and hyperinsulinemia) may be considered markers of insulin resistance. Insulin resistance is a predictor for the development of type 2 diabetes mellitus even in individuals with normal glucose tolerance. Therefore, it is important to recognize insulin resistance in the pre-disease stage when therapeutic intervention is likely to be more successful than in manifest disease [27].

This study aimed at proposing a new system where normal and diabetic subjects coming to assess fasting blood glucose can assess insulin resistance level and so, be able to assess their HOMA-IR, so that they can see if it works better than other available indices to predict and monitor or controlling DM, while hypoglycaemic drugs, commonly used herb and plant extracts.

The animal models were designed and used to assay various biochemical and haematological parameters and the findings largely corroborated previous studies with few exceptions. it is conclusive that ginger and aloe vera are very effective plant extracts when combined with glimepiride, metformin and cinnamon upon inducement with dexamethasone and high fat diet.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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