Journal of Complementary and Alternative Medical Research

11(1): 38-50, 2020; Article no.JOCAMR.61685
ISSN: 2456-6276

Evaluation of Anti-diabetic, Hepatoprotective and Antilipidemic Potentials of *Syzygium aromaticum* (Clove) on Albino Rats

Joshua F. Sammy[1*], Davies G. Tamuno-Emine[1] and Edna O. Nwachuku[1]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author EON designed the study. Author DGTE wrote the protocol. Author JFS managed the analyses and literature searches of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2020/v11i130178
Editor(s):
(1) Dr. Sachin Kumar Jain, IPS Academy College of Pharmacy, India.
Reviewers:
(1) M. R. Athirarani, Kerala University of Health Sciences, India.
(2) Suresh Babu Kondaveeti, Symbiosis International University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61685

Original Research Article

Received 23 July 2020
Accepted 28 September 2020
Published 07 October 2020

ABSTRACT

Aims: The aim of this study was to evaluate the anti-diabetic, hepatorenoprotective and antilipidemic potentials of *Syzygium aromaticum* (clove) in albino rats.
Study Design: This study is a non-randomized experimental study.
Place and Duration of Study: Department of Medical Laboratory Technology, University of Port Harcourt, Nigeria, between September, 2019 and December, 2019.
Methodology: Thirty-five male Wister rats with weight between 145 to 150 g were randomly selected into seven groups of five rats each. The first group served as Negative control (group 1). The second group was the positive control (Diabetic group). The remaining five groups being the treatment groups (3-7). Diabetes was induced intraperitoneally with 65 mg/kg of streptozotocin (STZ) single dose. Group 3 Diabetic group treated with metformin (100 mg/kg); Group 4 Diabetic group treated with low dose clove (250 mg/kg). Group 5 Diabetic group treated with low dose clove and metformin; Group 6 Diabetic group treated with high dose clove (750 mg/kg), while Group 7 were Diabetic group treated with high dose clove (750 mg/kg) and metformin for six weeks. Blood samples were collected via cardiac puncture in appropriate EDTA, heparinized and sterile bottles

**Corresponding author: E-mail: joshuasammy46@gmail.com;*

for standard laboratory investigations of lipid profile, glucose, liver enzymes, urea and creatinine. Plasma lipid profile, liver enzymes, urea and creatinine were determined using enzymatic end point method under standard operating procedures. Statistical analysis was done using Graph Pad Prism Version 5.03 and p values less than 0.05 were considered statistically significant.

Results: Results revealed that the STZ-induced diabetic group exhibited highly significant increase in activity of liver enzymes AST, ALT and ALP, increase in the levels of urea, creatinine, glucose and most lipid profile parameters as compared to the negative control group ($p<0.001$). Histopathological examination of liver and kidney tissues of diabetic rats indicated slight changes. However, their changes were overcame by clove treatment and the majority of the cells tend to be normal. Low dose clove group 5 (250 mg/kg) with metformin decreased the levels of the analytes most when compared to the levels of the positive control group. For glucose, group 5 gave a mean glucose level of 4.40 ± 1.08 mmo/l, significantly lower than the positive control group 39.67 ± 0.67, ALT group 5 gave a mean ALT level of 56.00 ± 7.11, which was significantly lower than the positive control group 205.70 ± 14.79, for urea group 5 gave a mean urea level of 4.25 ± 0.77 which was significantly lower the control group 23.80 ± 3.56 at $p < 0.001$, thereby yielding a better treatment result.

Conclusion: In conclusion, low dose clove supplementation with metformin could be excellent adjuvant support in the therapy of diabetes mellitus and its complications.

Keywords: Evaluation; anti-diabetic; hepatoprotective; antilipidemic; Syzygium aromaticum (Clove); albino rats.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies that has reached pandemic proportions worldwide [1]. Diabetes mellitus due to its absolute (Type 1) or relative deficiency of insulin (Type 2) has affected approximately 180million people all over the world [2]. In 2013, about 105,091 diabetes-related deaths were reported in Nigeria [3]. Recently, a pooled diabetes mellitus prevalence of 5.77% suggesting that 11.2million Nigerians (1 out of every 17 adults) are living with the disease has been reported [4]. Current drug regimens in the treatment of Diabetes mellitus place high financial burden on the subjects and these drugs are being taken throughout life. Most of these drugs have very harsh side effects and the burden is really much on developing countries, Nigeria inclusive. Hence, the need to search for safer, affordable and effective options for Diabetes mellitus.

Hyperglycemia causes cellular lesions and enhances the non-enzymatic glycosylation end-products are formed which injure cells by structural rearrangement of proteins [5]. This causes oxidative stress and serious complications such as heart disease, liver disease, high blood pressure, blindness, kidney disease, nervous system disease [6]. Insulin therapy and other anti-diabetic agents are good but there are disadvantages which produce severe side effects. Therefore, natural sources of α-amylase and α-glucosidase inhibitors are of great importance in folk medicine for the treatment and management of diabetes (type 2) [7]. Essential plants such as Clove with strong antioxidant properties have been reported to control hyperglycemia and prevent other diabetic complications which can be triggered by oxidative stress.

The scientific world and modern society at large are experiencing the dawning era of herbal medicine. Extensive research has shown that certain herbs exhibit anti-diabetic and antioxidant capabilities [8]. Over the years diabetes has been a subject of concern to the medical field. This pathological condition poses a great threat to the body's metabolism and system in general and if not managed or treated on time might result to death.

In order to curtail the adverse effects of this disease, several researches have been carried out with the sole aim of digging out remedies for this pathological condition. The hypoglycemic properties of these herbs are therapeutic to diabetic patients because it counteracts the high blood glucose level caused by the disease. The therapeutic effect of anti-oxidative herbs is manifested by its ability to neutralize the damaging effects of free radicals produced by the human body [8].

The World Health Organization has listed 21,000 plants which are used for medicinal purposes around the world [9]. These current reviews

focus on herbal drug preparations and plant used in the treatment of Diabetes mellitus a major crippling disease in the world leading to huge economic loss and burden.

Syzygium aromaticum (clove), the most precious spice is derived from an evergreen tree known as clove tree. It has been used around the globe in the preservation of food, topical antiseptic and local anesthetic in dentistry, in the treatment of GIT, in the production of cosmetics and pharmacological products [10].

Cloves are usually relished for their taste and fragrance, they are also packed with essential vitamins, minerals and other nutrients that are absolutely essential for the body. Clove has been reported to have anti-diabetic, anti-oxidant, anti-microbial, anti-inflammatory, antiseptic, analgesic and anticonvulsant properties [10].

Moreover, there is no much information on the inhibitory effects of clove essential oil, clove bud on α-amylase and α-glucosidase activities. Therefore, this research was aimed at evaluating the anti-diabetic, hepatorenoprotective and antilipidemic potentials of *Syzygium aromaticum* (clove) in albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty-five (35) male Wistar rats with weight between 145 and 150 g were obtained from the experimental animal farm at the University of Port Harcourt, Nigeria. The Wistar rats were housed in animal cages in a well-ventilated experimental room. The rats were allowed to acclimatize for a period of 14 days The rats were randomly sorted into seven groups called treatment groupings and appropriate treatments were commenced subsequently.

2.1.1 Experimental design

The animals were randomly divided into seven groups of five rats each and subjected to different treatments as shown in the Table 1.

2.2 Plant Selection and Authentication

Dry clove buds were obtained from fruits market Aba, Abia State. The Plant species (*Syzygium aromaticum*) was authenticated in the department of plant science and Biotechnology, University of Port Harcourt, Nigeria. The buds were grinded to coarse power for extraction.

2.2.1 Extract preparation

During the extraction water was used for the maceration. 5kg of the plant Clove, *Syzygium aromaticum* (L.) was macerated with water then allowed to stand at room temperature for a period of 3 days with frequent stirring until the soluble matter dissolved. The mixture was then sieved, the damp solid material was pressed, and the solvent was clarified by filtration. The solvent was then placed in the reservoir of soxhlet for extraction. The liquid extract in the reservoir was subjected to heat for several minutes in order to vaporize the moisture. The sample was evaporated over the water bath at a temperature at 45^0C and was constantly monitored until a gelatinous extract was formed. This process was carried out alongside a professional.

2.3 Animal Sacrifice and Blood Collection

At the end of various treatments on diabetes in all the groups, animals were sacrificed at the end of the 7^{th} week post diabetes under anesthetic procedure. Blood samples were collected via cardiac puncture in appropriate EDTA, heparinized and sterile bottles for standard laboratory analysis.

Table 1. Grouping of experimental animals

Groups	Treatment	Dosage/administration of clove
Group 1	Negative control (no diabetes)	Saline + normal meal
Group 2	Positive control (diabetes control)	(65 mg/kg streptozotocin)/single dose
Group 3	Diabetes + metformin	100 mg/kg metformin
Group 4	Diabetes + lose dose clove	250 mg/kg (1 ml/day/6 weeks)
Group 5	Diabetes + lose dose clove + metformin	250 mg/kg + 65 mg/kg (1 ml/day/6weeks)
Group 6	Diabetes + high dose clove	750 mg/kg (1 ml/day/6 weeks)
Group 7	Diabetes + high dose clove + metformin	750 mg/kg + 65 mg/kg (1ml/day/6 weeks)

2.3.1 Organ collection

Organs such as the liver and kidney were harvested, rinsed and preserved in 10% formalin solution for histopathological examination.

2.4 Dose Calculations

2.4.1 Dose calculation and administration of the extract

The extract preparations were administered through the oral route after appropriate calculations of doses were made for 250mg/kg and 750mg/kg extract.

1. Average weight of the rats = 140g
   250mg/kg standard dose= 100ml is given to 1kg or 200g rat
   250mg =100g rat
   Xmg =150g rat

$$X = \frac{250 \times 150}{1000} = 37.5 \text{ mg}$$

Therefore, 37.5 mg of the *Syzygium aromaticum* was given to all rats in the group weighing 140 g (this was done for all groups of animals taking their weights into consideration)

2. Average weight of the rats =140g
   750mg/kg standard dose = 100ml given to 1kg or 1000g rat
   750mg= 1000g rat
   Xmg = 150g rat

$$X = \frac{750 \times 150}{1000} = 113\text{mg}$$

Therefore, 113mg of the *Syzygium aromaticum* (L.) will be given to all rats in the group weighing 140g (this was done for all groups of animals taking their weights into consideration).

2.4.2 Dose calculation and administration for streptozotocin

The extract preparations were administered through the oral route after appropriate calculations of doses were made.

Standard dose for diabetes induction =65mg/kg (Streptozotocin)

1. Average weight of rats =150g
   150mg/kg standard dose= 65mg given to 1kg or 1000g rat
   150mg = 1000g rat
   Xmg = 140g rat

$$X = \frac{150 \times 65}{1000} = 9.7\text{mg/150g rat}$$

Therefore, 9.7mg of the streptozotocin will be given to all rats in the group weighing 150g (this was done for all groups of animals taking their weights into consideration).

2.4.3 Dose calculation and administration for the drug metformin

Standard dose = 100mg/kg dose

2. Average weight of the rats = 150g
   100mg/kg standard dose = 100mg given to 1kg or 1000g rat
   100mg = 1000g rat
   Xml = 150g rat

$$X = \frac{100 \times 150}{1000} = 15\text{mg/150g rat}$$

Therefore, 15mg of the drug will be administered to all the rats in the group weighing 140g (this was done for all the groups of animals taking their weights into consideration).

2.5 Determination of Biochemical Parameters

2.5.1 Determination of plasma glucose

Plasma glucose was determined using the GOD-PAP method of [11] which the principle is based on the breakdown of glucose into gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye whose absorbance is read spectrophotometrically at 500nm.

2.5.2 Determination of serum lipid profiles

2.5.2.1 Serum total cholesterol

Serum total cholesterol concentration was assayed using the CHOD-PAP method of [12]. Serum cholesterol was measured by means of coupled reactions with the formation of a coloured complex. The cholesterol was determined after enzymatic hydrolysis and oxidation. The concentration of the indicator quinoneimine formed from hydrogen peroxide and 4- aminoantipyrene in the presence of

phenol and peroxidase was measured spectrophotometrically at 500nm.

2.5.2.2 Serum triglycerides

Serum triglycerides (TG) concentration was assayed using the GPO-PAP method of [13]. Triglycerides in the sample are hydrolyzed to glycerol and free fatty acids by the action of lipase. A sequence of three coupled enzymatic steps using glycerol kinase (GK), glycerophosphate oxidase (GPO), and horseradish peroxidase (HPO) causes the oxidative coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) with 4-aminoantipyrine to form a red quinoneimine dye. The absorbance is read at 500 nm for a fixed-time interval. The change in absorbance is directly proportional to the concentration of triglycerides in the sample.

2.5.2.3 Serum HDL-cholesterol

Serum HDL-cholesterol was assayed using the precipitation/CHOD-PAP method of [14].

In the presence of magnesium ions, LDL, VLDL and chylomicron fractions were precipitated quantitatively by addition of phosphotungstic acid. After centrifugation, the cholesterol content of the HDL fraction, remaining in the supernatant, was determined as in total cholesterol.

2.5.2.4 Serum LDL-cholesterol

Serum LDL-cholesterol (LDL-C) was calculated using the method of Friedewald equation [15], shown below:

(LDL-cholesterol) (mg/dL) = (Total cholesterol) - (HDL cholesterol) – (Triglyceride) / 5

2.5.3 Determination of liver function markers

2.5.3.1 Serum Alanine Aminotransferase (ALT) activity

The serum alanine aminotransferase (ALT) activity was determined using the method of [16]. The substrates in the reaction are α-ketoglutaric acid (α-KG) and L-alanine. The products formed by enzyme action are glutamate and pyruvate. Addition of 2,4, dinitrophenylhydrazine results in the formation of hydrazone complex with the ketoacids. A red colouris produced on the addition of sodium hydroxide. The intensity of colour is related to enzymatic activity.

2.5.3.2 Serum Aspartate Aminotransferase (AST) activity

The serum aspartate aminotransferase (AST) activity was determined using the method of [16]. The substrates in the reaction are α-ketoglutaric acid (α-KG) and L-aspartate for AST. The products formed by enzyme action are glutamate and oxaloacetate. Addition of 2,4, dinitrophenylhydrazine results in the formation of hydrazone complex with the ketoacids. A red colour is produced on the addition of sodium hydroxide. The colour intensity is related to enzymatic activity.

2.5.3.3 Serum Alkaline Phosphatase (ALP) activity

The serum alkaline phosphatase (ALP) activity was determined using the method of [17]. The activity of alkaline phosphatase is determined by monitoring the rate of its dephosphorylation of p-nitrophenylphosphate to p-nitrophenol, a yellow coloured compound whose concentration can be monitored spectrophotometrically at 405 nm.

2.5.3.4 Serum bilirubin concentration

The serum bilirubin concentration was determined using the method of [18]. Conjugated (direct) bilirubin in serum is coupled with diazotised sulphanilic acid (29 mmol/l of sulphanilic acid and 0.17 N HCl) to form a red-coloured compound. Ascorbic acid was used to stop the coupling reaction, and to eliminate interference by haemoglobin. Caffeine benzoate solution (0.26 mol/l caffeine and 0.52 mol/l of sodium benzoate) is used to split the unconjugated bilirubin protein complex releasing the bilirubin so that it can react with diazotised sulphanilic acid. The tartrate buffer makes the mixture alkaline and converts the red acid bilirubin to a green coloured compound which shows peak absorbance at 578 nm. At this wavelength the absorbance due to haemoglobin or carotene is minimal.

2.5.4 Determination of renal function markers

2.5.4.1 Determination of serum urea

The serum urea concentration was estimated using the method of [19]. Urease hydrolyzes urea to ammonia, which reacts with phenol and hypochlorite to form the blue coloured compound, indophenols, which can be measured spectrophotometrically.

2.5.4.2 Serum creatinine

The serum creatinine concentration was estimated using the method of [20]. In the presence of a strong alkali, creatinine reacted with picric acid to form alkaline picrate which imparted a yellow-red colour to the solution, the intensity of which was directly proportional to the creatinine concentration.

2.6 Statistical Analysis

The data obtained in this study were analyzed for statistical significance by means of one-way analysis of variance (ANOVA), followed by Tukey's test of multiple comparison, at $p<0.05$ significance level. Data are presented as mean ± S.D. (standard deviation). Graph Pad Prism 5.0 statistical software was used for the analysis.

3. RESULTS AND DISCUSSION

In the current study, there were highly significant increases in serum levels of ALT, AST and ALP in STZ induced diabetic rats which indicate hepatocellular injury. (Table 2) A similar result was reported by [21], who found that STZ induced diabetic rats in a dose level of 55mg/kg had elevation in serum levels of ALT and AST.

Elevation of ALT activity is more associated with necrotic state while the increase of AST activity is an index of hepatocellular injury in rats [22]. The increase of the enzyme from the liver cytosol into the blood circulation explains why liver enzymes is increased and this is an indication of hepatic effects of streptozotocin [23]. In a current study, there was a decrease in serum levels of liver enzymes concentration (ALT, AST and ALP) was registered in diabetic groups after treatment with clove extract (300 and 600mg/kg) as compared to untreated diabetic group.

Several studies demonstrated that there was a decrease in liver enzymes in diabetic rats treated with clove oil when compared with untreated diabetic rats [24,25]. Clove extract also brought about a decrease in ALP activity in diabetic rats treated with clove oil, thereby preventing liver damage [25].

Administration of clove extract at different doses with metformin a known anti-diabetic drugs used in this work did not produce elevations in serum liver enzymes (AST and ALT) as compared to the positive control untreated group that was totally elevated although 250mg/kg + 65mg/kg of clove extract and metformin yielded the most desired decrease in serum liver enzymes levels as compared to the positive control group 2.

The decrease in liver enzymes activities as demonstrated by this study points to the fact that clove is actually rich in phenolic compounds and has great potential to alter or reduce the effects of drug-induced hepatotoxicity and oxidative stress this agrees with the work done by [26,27], revealed that hepatoprotective action of clove may be due to flavonoids and polyphenolic compounds.

Mean glucose levels of STZ-induced rats treated with clove extract and metformin of treatment group (3-7) were observed (Fig. 1) to significantly decreased when compared to the positive untreated diabetic group with Group 5 (250mg/kg + 65kgmetformin) being the most decreased ($P > 0.001$). It has been reported that metformin increases insulin sensitivity, enhances peripherial glucose uptake and increases fatty acid oxidation, characteristics that are also similar to Eugenol mode of action.

Table 2. Effect of different doses of treatments of *Syzygium aromaticum* and metformin on liver Enzymes

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group 1 (Negative control)	55.80 ±7.29[a]	43.40 ± 6.58[a]	213.20±18.04[a]
Group 2 (Positive control)	205.70 ± 14.79[a]	167.00 ± 16.61[a]	751.14 ±23.50[a]
Group 3 (Met)	113.80 ± 11.19[a]	64.40 ± 11.08[a]	470.07 ± 29.09[a]
Group 4 (Low dose Clove)	74.20 ± 6.65[a]	55.80 ± 5.68[a]	356.20 ± 22.98[a]
Group 5 (Met + Low dose Clove)	56.00 ± 7.11[a]	42.00 ± 4.30[a]	258.80 ± 18.84[a]
Group 6 (High dose Clove	96.00 ± 10.7[a]	104.60 ± 15.47[a]	364.60 ± 27.31[a]
Group 7 (Met + high dose Clove)	70.50±8.43[a]	49.75 ± 5.56[a]	381.30 ± 27.91[a]
p-value	<0.001	<0.001	<0.001
f-value	81.55	63.05	66.93
Summary	S	S	S

S – Significant, a – Significant difference versus positive control (post hoc)

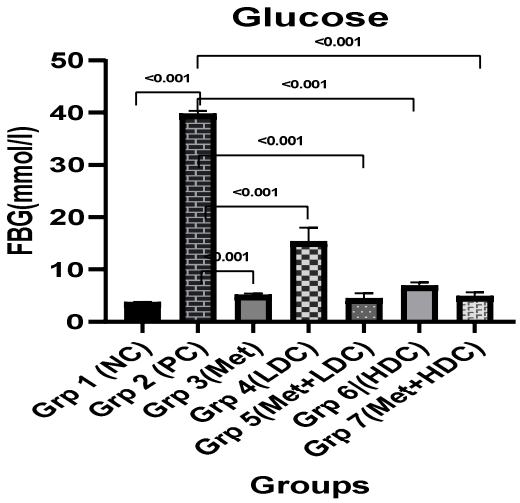

Fig. 1. Graphical comparison of the effect of clove treatment on glucose across the groups

In this work also, the mean total cholesterol, triglyceride and low density lipoprotein cholesterol (Figs. 2-5) levels among the treatment group (3-7) showed significant decrease in levels of these lipid parameters when compared to the positive control group 2 that was elevated at (P > 0.001) whereas HDL-C showed no significant difference across the treatment groups when compared to the positive control group 2 at P = 0.0627. The reason for the increase in the diabetic group 2 Triglycerides level compared to the treatment groups could be as a result of beta cells destruction thereby not yielding to the production of insulin to carry the glucose from the peripheral blood for utilization by other organs and tissues, a decrease or lack of insulin affects the activity of lipoprotein lipase (LPL) hence hypertriglyceridemia results due to inability of Lipoprotein lipase to incorporate triglycerides to be taken up by peripheral tissues after being broken down to fatty tissues.

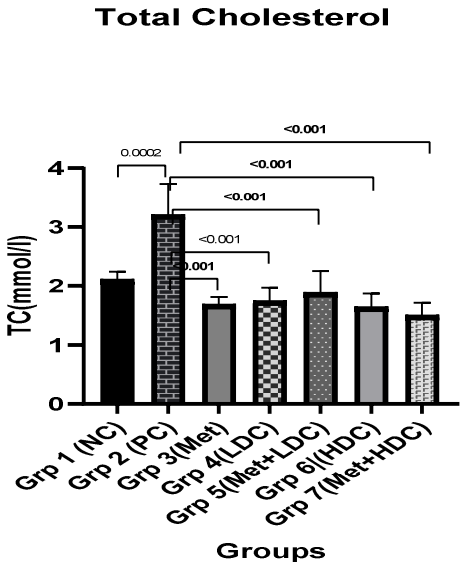

Fig. 2. Graphical comparison of the effect of clove treatment on Total Cholesterol across the groups

That is there is an inhibiting function of LPL leading to accumulation of triglycerides due to inability of LPL to breakdown triglycerides to form free fatty acids and glycerol, hence the abnormality in lipid parameters in hyperglycermia but treatment with clove extract due to its constituents i.e. active ingredient e.g. Eugenol and β-carophyllene acts by increasing insulin secretions from β-cells of pancreas hence restoring the enzyme lipo-protein lipase activities. Also the photochemical constituents of clove alters enzymes activities with regards to metabolism of $C_6H_{12}O_6$ Eugenol also acts like insulin in most cases.

The capability of this plant extracts due to its active ingredient helps restore pancreatic beta cells function thereby making a rise in insulin dependent processes this gives clove its antidiabetic potential, this also agrees with the work done by [28]. These hypolipidemic effects of clove is due to the mechanism of action of its active ingredient eugenol and β-carophyllene which acts as a transcription co-activator by inducing PGC1α expression thereby leading to the activation of PPAR pathways and improvement mitochondrial spare respiratory capacity. It also activates energy sensing molecules including AMPK and SIRTI metabolism as well as mitochondria function in muscle cell thereby in turn regulates glucose and fatty acid metabolism as illustrated or showed by the work of [29].

Diabetic groups treated with different doses of clove extract showed an improvement in the levels of glucose, total cholesterol ,triglycerides HDL-C, LDL-C, urea and creatinine when compared to the untreated diabetic group at ($p > 0.001$) with group 5 (250mg/kg clove extract + 65mg/kg metformin) showing the most significant improvement at ($p < 0.001$) previous studies have found that clove extracts and eugenol, the major bioactive component of clove, significantly reduce blood glucose level in animal models and inhibit PEPCK and G6 Pase gene expression in hepatocytes. Another study explored the potential mechanism of *syzygiumaromaticum* on metabolism in comparison to metformin and insulin. It has been reported that metformin increases insulin sensitivity, enhances peripheral glucose uptake and increases fatty acid oxidation [30].

It was also found in another study that clove extract was very useful in dyslipidemia, hepatic steatosis and fasting hyperglycemia because of the PPARY against action of eugenol present in clove oil. [31] PPAR –y (Peroxisome proliferator – activated receptor) Y ligard-binding activity. This study also showed a significance difference in urea and creatinine, kidney parameters in the treatment group (3-7), as compared to the positive diabetic control with group 5 being the most significantly reduced hence yielded a desired effect when compared to other groups P < 0.001). Similar to other studies by [25], and Shukri et al. 2010. Moreover, STZ stimulates

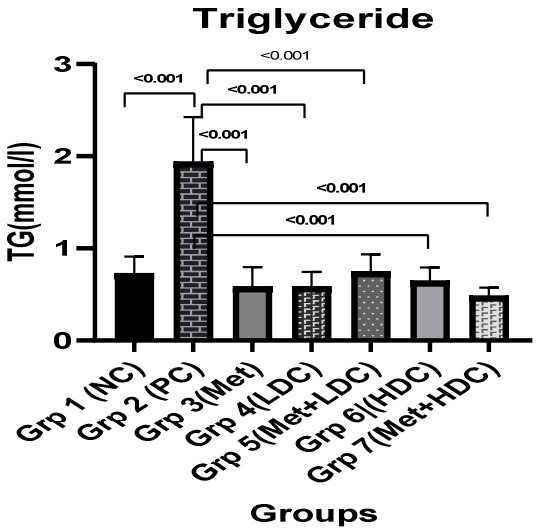

Fig. 3. Graphical comparison of the effect of clove treatment on Triglyceride across the groups

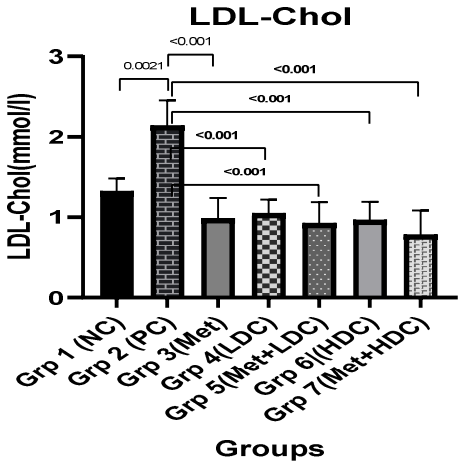

Fig. 4. Graphical comparison of the effect of clove treatment on LDL-Chol across the groups

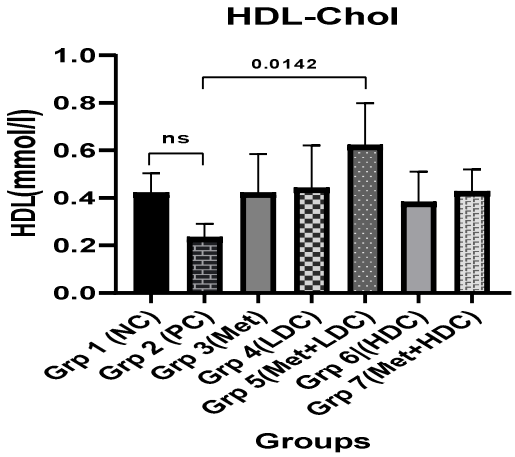

Fig. 5. Graphical comparison of the effect of clove treatment on HDL-Chol across the groups

H_2O_2 generation which causes DNA fragmentation and pancreas Beta cells [32]. Owing to the fact that lipid peroxidation process contains a series of free mediated chain reaction process and is also associated with several types of biological damages to ease or reduce the impact of these damages on cells and tissues. *Syzygium aromaticum* active constituents induce the expression of SIRTI and PGC1α causes this two exerts broad influences on energy metabolism. Clove extract aids the increase expression of SIRT 1 thereby increasing fatty acid oxidation also increasing the ratio of NAD^+/NADH which triggers SIRT1 activation. [33], hence the decrease in the levels of lipid parameters cholesterol and triglycerides after treatment with clove extract.

As a transcription coactivator, PGC1α plays a critical role in mitochondrial function; clove induced PGC1α expression potentially gives rise to the activation of PPAR pathways and improvement of mitochondria function thereby reducing oxidative stress that may have resulted from lipid peroxidation cells/tissues of liver and kidney. Hence, clove extract enhances peripheral glucose uptake and increases fatty acid oxidation, this effect is often decreased by SIRT1 inhibitor nicotinamide stimulate their maintenance and survival through constitutive antioxidants defense systems or signaling pathways activation that up regulate antioxidant proteins resulting in an adoptive stress response but in hyperglycemic subjects, the role of oxidative damage overwhelms repair capacity

and the cells of the pancreas (Beta cells) is being destroyed but the action of the active ingredient of clove, eugenol which acts like insulin enhances insulin sensitivity, improvement and regulation of pancreas beta cells. Absorption of $C_6H_{12}O_6$ reduces its contents in the gut; enhancing enzymes activities with regards to metabolism of $C_6H_{12}O_6$ thereby bringing about hyperglycemic effects as experienced among the experiment groups. *Syzygium aromaticum* also possess alpha glucosidase inhibiting properties [34].

From this work, the effect of treatment of different doses of *Syzygium aromaticum* and metformin showed a significant decrease in the levels of urea and creatinine (Figs. 6 and 7) among the treatment groups 4–7, except groups 3 that showed a significance difference from both positive and negative controls. Hyperglycemia gives rise to oxidative stress which damages renal tissue and promotes inflammation leading to further tissue injury with accumulation of impaired biomacromolecules. Following injury, the epithelial cells undergo structural changes or cell death triggering endothelial activation and infiltration of cells releasing inflammatory mediators [35]. This explains the increase in the levels of urea and creatinine in the positive control group 2. Hyperglycemia results to impaired liver and tissue utilization of glucose and hepatic glycogenolysis and gluconeogenesis from protein and fatty acid carbon residues are increased.

The increase blood glucose levels lead to increased glomerular filtration of glucose and if the renal threshold is exceeded glucosuria result. Increased blood glucose also leads to increased extracellular osmolality which leads to intracellular dehydration thereby resulting to oxidative stress. The significance decrease in renal parameter, urea and creatinine was brought about by treatment with clove extract which inhibit free radical production due to the antioxidant contents of the active ingredient eugenol. This active ingredient eugenol and β-caryphyllene also possess a scavenging function initiating radicals, chelating the transition metal catalyst and breaking chain reactions that may results in oxidative stress [36].

In addition [37] reported that diabetic rats treated with clove had significantly reduced necrotic cells compared to diabetic rats by 21%. Eugenol has the capacity to sustain liver, kidney function to near normal by suppressing lipid peroxidation and the release of cytokines. The mechanism of eugenol protection might result from the diminished generator of ROS and reduction in inflammatory cells infiltration and generation of cytokines from the liver and kidneys [38].

The study demonstrated that the administration of clove at a low dose of 250mg/kg together with a known anti-diabetic drugs metformin at 65mg/kg yielded a better therapeutic result when compared to other diabetic treatment groups, hence a good adjuvant for the management and treatment of diabetes mellitus.

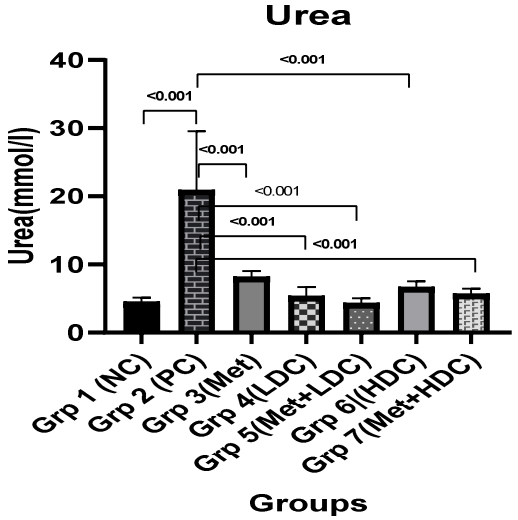

Fig. 6. Graphical comparison of the effect of clove treatment on Urea across the groups

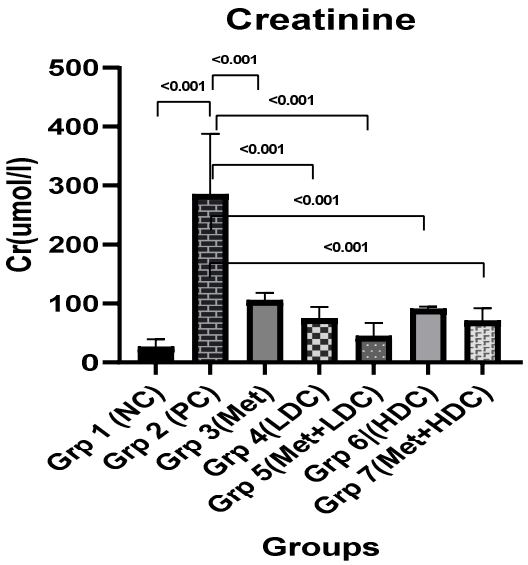

Fig. 7. Graphical comparison of the effect of clove treatment on Creatinine across the groups

4. CONCLUSION

This study together with existing reports demonstrates that the administration of cloves *Syzygum aromaticum* has hepatoprotective and antidiabetic effect. Therefore, low dose clove supplementation with a known anti-diabetic drug (eg. metformin) could be an excellent adjuvant support in the therapy of diabetes mellitus and its complications.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. Before the commencement of treatments. Threats had free access to standard rat feed and clean water. Handling of animals with relevant institutional and ethical guidelines as approved for scientific study.

ACKNOWLEDGEMENTS

The authors acknowledge the efforts of Dr. Femi and Dr. Holy Brown for their efforts in securing the laboratory animals and statistical analysis respectively, and Mr/Mrs Sammy Jacobs for funding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hana Y, Adma D. Diabetes and other endocrine metabolic abnormalities. J Clin Diab Endo. 2017;3:3-10.
2. Finkel T, Chu-Xia D, Raul M. Biology and physiology of sirtuins. J Nat. 2009; 460(7255):587–91.
3. Oputa RN, Chinenye S. Diabetes in Nigeria—a translational medicine approach. African J Diab Med. 2015;23(1): 7-10.
4. Uloko AE, Musa BM, Ramalan MA, Gezawa ID, Puepet FH, Uloko AT, Sada KB. Prevalence and risk factors for diabetes mellitus in Nigeria: A systematic review and meta-analysis. Dia Ther. 2018;9(3):1307-16.
5. Berg P, Fang X, Palanivel R, Zhou X, Liu Y. Hyperglycermia and Hyperinsulinemia-induced alteration of adiponectin receptor expression. J Mol Endo. 1997;35(3):465-76.
6. Al-Attar A. Physiological effects of some plant oils supplementation on

streptozotocin-induced diabetic rats. Res J Med Medi Sci. 2010;5(1):55-71.
7. Shabana T, Devarajan A, Kumpatla V. The socioeconomic of diabetes form a developing country. Diab Res Clin Pract. 2009;89(3):334–40.
8. Grover JK, Yadav S, Vats V. Medicinal Plants of India with antidiabetic potential. J of Ethno Pharm. 2002;81:81–100.
9. World Health Organization. WHO Traditional Medicine Strategy: WHO Pub. 2002;1–6.
10. Owen DL, Eliane de pedicepuis, Armel G, Mohammed N. Efficacy of clove oil and benzocaine as anesthetics for protopterus annecters. J Fish. 2019;8:203–09.
11. Trinder P. Determination of blood glucose using 4-aminophenazone as oxygen acceptor. J Clin Path. 1969;22(246): 1-6.
12. Allain CC, Poon LS, Chan CS, Richmond WFPC, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20(4):470-75.
13. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem. 1983;*29*(3):538-42.
14. Burstein MSHR, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lip Res. 1970;11(6):583-95.
15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6): 499-502.
16. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Path. 1957; 28(1):56-63.
17. Rec GSCC. Colorimetric method for serum alkaline phosphatase determination. J Clin Biochem. 1972;10(20):182-4.
18. Jendrassik L, Grof P. Colorimetric method of determination of bilirubin. Biochem. 1938;297:81-2.
19. Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Ana Chem. 1967;39(8): 971-4.
20. Bartels H, Bohmer M. Kinetic determination of creatinine concentration. Clin Chim Acta. 1972;*37*;193-7.
21. Schmitz R, Perreira L, Stefanello N, Mazzantic, Spanevello R, Gutierres J, Bagatini M, Martins C Abdalla F, Daci da Silva Series J, Zanini D, Vieira J, Cardoso A, Schetinger M, Morsh V. Effects of resveratol on biomarkers of oxidative stress and on the acitivity of delta aminolevulnic acid of delta aminolevulinic acid dehydrate in liver and kidney of streptozocin induced diabetic rats. Biochem. 2012;94(2):374–83.
22. Adefegha S, Oboh G, Adefegha O, Boligon A, Athayde, M. Antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative effects of dietary clove (*Szyzgium aromaticum*) bud powder in a highfatdiet/streptozotocin-induced diabetes rat model. J Sc Agric. 2014;94(13):2726-37.
23. Mansour H, Newairy A, Yousef M, Sheweita S. Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Tox. 2002;170(3): 221-8.
24. Al-Attar N, Zari T. Modulatory Effects of ginger and Clove oils on Physiological responses in streptozotocin induced diabetic rats Int'l J of Pharm. 2007;3(1): 34-40.
25. Hassanen N. Protective effct of cinnamon, clove and ginger spicces or their essential oils on oxidative stress of streptozotocininduced diavetic rats. Arab Universities Journal of Agricultural Science. 2010;18(1):137-54.
26. Han J, Cheng G, Zhou P, Guo L. *In Vitro* antimutagenic antioxidant activities and total phenolics of clove Pakistan. Journal of Pharmaceutical Sciences. 2014;27(4): 1-6.
27. Abozid M, EL-Sayed S. Antioxidant and protective effect of clove extracts and clove essential oil on hydrogen peroxide treated rats. International Journal of Chemical Technology Research. 2013;5(4):1477-85.
28. Narasimhulu G, Mohamed J, Reddy K. Antihyperglycemic effect of pimpinella tirupatiensis leaves in streptozotocin-induced diabetic rats. Bulletin of Environ. Pharmacology Life Science. 2014;3(2): 5-13.
29. Zheng TU, Tijuana Moss-Pierce Paulford, Alan TJ. Regulation of Energy metabolism in myocytes by *Syzygium aromaticum*. Journal of Medicinal Food. 2012;17(9):1-5.
30. Gabe E, Jung-Nien L, Chien-Tung W, Chen–Chang Y. Metformin treatment on

diabetes, a deceased risk of Non proliferative Diabetic retinopathy in type 2 Diabetes. Journal of Diabetes Research. 2020;1-6.
31. Adeniyi J, Doharty P, Oladipo O, Bolaji O. Challenges and opportunities in development of PPAR agonists. Journal of Molecular Endocrinology. 2014;4:1-7.
32. Bolkent S, Sacan O, Karatug A, Yanardag R. The effects of vitamin B6 on the liver of diabetic rats: A morphological and biochemical study. Journal of Biology. 2008;67(1):1-7.
33. Jung H, kim Y, Noh J, Gang G, Kim K. The protective role of NAD (P) H: Quinone oxidoreductase 1 on acetaminophen induced liver injury. Achieves of Toxicology. 2015;89:2159-66.
34. Khan A, Qadir S, Nawaz K, Anderson R. Clove effect on glucose, cholesteriol and triglycerides of people with type 2 diabetes mellitus. Experimental Biology. 2006;4: 640-50.
35. Sharfuddin AA. Moderate reduction in Kidney perfusion. National Review of Nephrology Advance Online Publication. 2011;10:1038-43.
36. Maritim A, Sanders R, Watkins J. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Tox. 2003;17(1):24-38.
37. Shukri R, Mohamed S, Mustapha N. Cloves protect the heart, liver and lens of diabetic rats. F Chem. 2010;122(4): 1116-21.
38. Yogalakshmi B, Viswanathan P, Anuradha C. Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. Tox. 2010; 268(3):204-12.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61685