The Protective Effects of a Phenolic Clove Extract on Mitochondria: An Animal Study

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Abstract— **Background: Plants represent a treasure chest with many unrevealed medical applications that have made them traditionally used for thousands of years to treat different diseases. One of these applications is the hypoglycemic and antioxidant roles of some plants. A pathological condition characterized by hyperglycemia, insulin resistance, and dyslipidemia known as metabolic syndrome.**

Objectives: This work focuses on examining the hypoglycemic effect of Syzygium aromaticum on a metabolic syndrome-induced animal model and studying the role of the extracted phenolic compounds on different mitochondrial function genomic parameters.

Methods: Sánchez-Rangel et al. (2013) described the extraction and quantification of phenolic components from Syzygium aromaticum using the Folin-Ciocalteu reagent. In this work, forty albino rats were involved. They were split up into the following four groups, each consisting of five animals: Syzygium aromaticum treatment for metabolic syndrome, non-metabolic syndrome animals treated with Syzygium aromaticum, and negative control (normal), positive control (metabolic syndrome produced with fructose without treatment). Serum glucose, pyruvate, lactate, lipid profile, liver function tests, and molar lactate/pyruvate ratio. Green and red mitotrackers are used to measure the mass and action potential of mitochondria.

Results: According to the study's findings, clove extract dramatically lowers blood sugar levels in rats with metabolic syndrome. The plants return serum lactate, pyruvate, and molar lactate: pyruvate ratio to normal control negative values. Mitochondrial mass and action potential significantly improve after treatment. In comparison to metabolic syndrome animals, the mitochondrial copy number of peripheral blood cells significantly increases after treatment with the plant extract.

Conclusion: Metabolic syndrome significantly reduces mitochondrial genome copies number. The application of the phenolic extract of Syzygium aromaticum.

Keywords: **phenolic compounds, glycemic profile, mitochondrial copies number, action potential, lactate: pyruvate ratio.**

Introduction

Metabolic syndrome (MtS) refers to a group of risk-factors for cardiovascular disease that includes central- obesity, hyperglycemia, dyslipidemia, and hypertension (Frazier-wood & Wang, 2016). Multiple organisations have formulated their own interpretations of MtS. Subsequently, these organisations amalgamated and formulated a novel concept called "harmonised criteria," encompassing central obesity, hypertension, elevated serum triglyceride levels, reduced serum HDL cholesterol, the existence of abnormally high serum glucose and/or insulin resistance, and hyperuricemia (Prakaschandra & Naidoo, 2022). Metabolic syndrome diagnosed when a patient has at least three of the conditions listed above. High-carbohydrate especially Fructose and/ or high-fat diets, as well as sedentary lifestyles, all contribute to the development of MtS (Vatashchuk et al., 2022).

In our previous works, induction of MTS by high fructose diet caused significant elevation in oxidative stress; induce apoptosis and /or necrosis in time dependent manner, in addition to the deteriorating effect on mitochondrial function and genome (Taqa et al., 2022). In some works, insulin resistance was correlate to abnormal mitochondrial activity (Sergi et al., 2019), (Patti & Corvera, 2010), while others works demonstrated The mechanism by which decreased mitochondrial function can contribute to the development of insulin resistance (Heo et al., 2021) (Ruegsegger et al., 2018). Sebastián et al. (2012) presented a detailed analysis of the particular coordination role of mitofusion proteins in the operation of mitochondria and endoplasmic reticulum. This coordination has an impact on insulin signalling and the regulation of glucose levels in the liver, as demonstrated in their study on liver-specific Mfn-2 KO-mice (Sebastián et al., 2012).

This work focuses on examining the hypoglycemic effect of three plants on induced-metabolic syndrome- rattus model and studying the role of the extracted phenolic compounds from Clove on different mitochondrial function and genomic parameters.

Materials and Methods

The phenolic components of Syzygium aromaticum were extracted and measured using the Folin–Ciocalteu reagent, following the methodology published by Sánchez-Rangel et al. (Sánchez-Rangel et al., 2013). The study used a total of twenty

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male albino rats. The creatures were categorised into four categories, with each group consisting of five animals, as follows: Normal control, Experimental control (metabolic syndrome induced with fructose with no treatment), and metabolic syndrome treatment with Syzygium aromaticum. Non-metabolic syndrome animals treated with Syzygium aromaticum.

Serum glucose, lipid profile, and liver function tests assessed by Fully-auto Biochemistry Analyzer, BA-A-160 (Bioevopeak). The serum levels of lactate and pyruvate, which are indirect indicators of mitochondrial function, were measured using fluorescence-based methods provided by Cayman Chemicals (700510 and 700470 respectively) and molar lactate/pyruvate ratio. Mitochondrial copy number was assayed using qPCR to detect the changes in copies number of mt-ND1 gene with Forward sequence AGGACCATTCGCCCTATTCT and reverse sequence GGGTAGGATGCTCGGATTCA (Ahmed, 2018) and GAPDH as housekeeping gene with Forward sequence ACATGCACAGGGTACTTCGA and reverse sequence TTACCCCAGCCTTCTCCATG. The data was analysed using Eco system software. The data is presented as the mean value plus or minus the standard deviation, ANOVA-test was used to detect the degree of significance at $p<0.05$ and less. SPSS software used in this work.

RESULTS

According to our theory biochemical parameters corrected after treatment with phenolic-extract of the clove. Serum glucose significantly elevated in positive group (145±12.6) in comparison to negative control group (75±5.4) at $p<0.001$. Metabolic syndrome rate treated with the extract (112±7) shows significant reduction in comparison to positive group at $p<0.001$. Rats with no metabolic syndrome and treated with the extract (94±6) shows significant reduction in comparison to positive group at $p<0.001$.

The lipid profile parameters indicate a significant rise in serum cholesterol levels in the positive control group (163±36) compared to the negative control group (65±15) with a P-value of less than 0.0001. The serum cholesterol levels of the Mt-S treated with the extract (136±36) and the rats without Mt-S + treated with the extract both exhibited a substantial reduction in comparison to the positive control group at a significance level of $p<0.05$. (figure 1) .

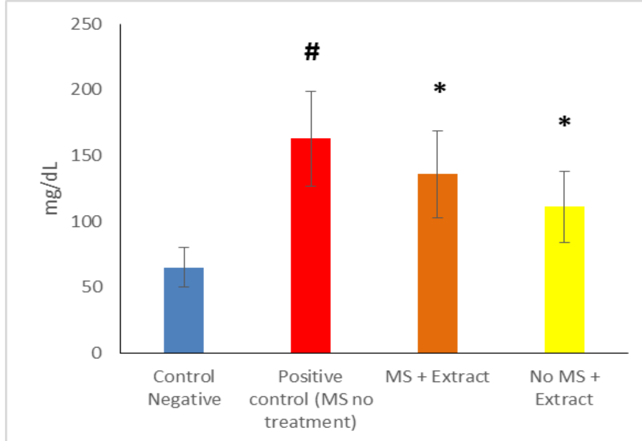

Figure 1. The serum cholesterol level (measured in mg/dL) for the four groups in the study. The symbol "#" indicates a substantial change when compared to the negative control, while the symbol "*" indicates a significant change when compared to the positive control.

The positive control group exhibited a substantial increase in serum triglycerides (257±39) compared to the negative control group (97±10) at a significance level of $p<0.001$. The results indicate a substantial decrease in triglyceride levels in both the group of individuals with Mt-S who were treated with the extract (220±41) and the group of individuals without Mt-S who were treated with the extract (118±38), compared to the positive control group, with a significance level of $p<0.05$ (figure 2).

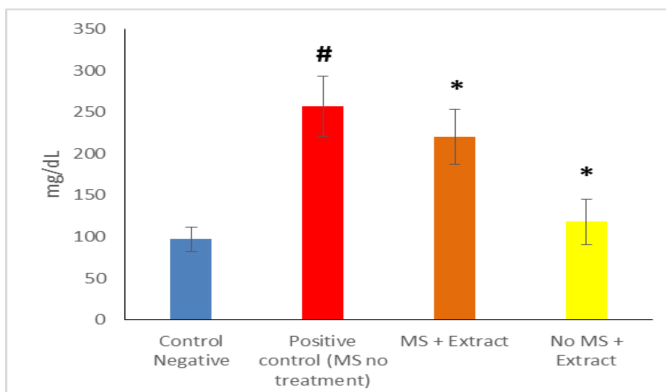

Figure 2. Serum triglycerides level (mg/dL) for the four study groups. # mean significant change in comparison to negative control. * Mean significant change in comparison to positive control.

Serum HDL significantly decline after induction of metabolic syndrome in the positive control group (27±6) in comparison to negative control group (45±10) at $p<0.001$. Serum HDL-C level significantly elevated after treatment with extracts in comparison to positive control group at $p<0.01$ (figure 3).

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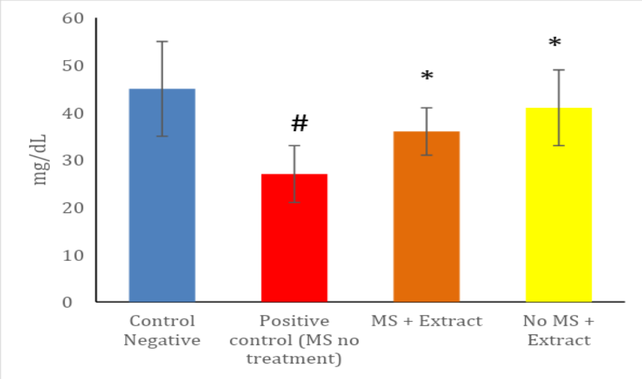

Figure 3. Serum HDL-C level (mg/dL) for the four study groups. # mean significant change in comparison to negative control. * Mean significant change in comparison to positive control.

The liver enzyme GOT, which is a measure of liver function, exhibited a substantial increase in the positive control group (131±30) compared to the negative control group (77±10) with a p-value of less than 0.001. The S.GOT levels in the Mt-S group (108±27) were considerably lower compared to the positive control group at a significance level of $p<0.05$. The non-Mt-S rats administered with the extract (92±17) exhibited a substantial decrease in S.GOT activity compared to the positive control group with a p-value of less than 0.001.
In the case of S.GPT, there was a notable increase in enzyme activity in the positive control group (57±12) compared to the negative control group (42±8), with a statistically significant difference at $p<0.001$. The treatment with the extract did not result in any significant changes in S.GPT activity in Mt-S rats (55±11) compared to the positive control group at $p>0.05$. Non-Mt-S rats administered with the extract (48±7) exhibited a significant reduction compared to the positive control group at a significance level of $p<0.05$.
The alkaline phosphatase enzyme exhibited a substantial increase in the positive control group (374±59) compared to the negative control group (175±9) at a significance level of $p<0.001$. Both the Mt-S rats treated with the extract (206±25) and the Non-Mt-S rats treated with the extract (192±18) exhibit a substantial decrease in activity compared to the positive control group, with a p-value of less than 0.001.
The findings of this study demonstrated a substantial elevation in blood lactate levels in rats subjected to positive control, with an increase of 186% compared to the negative control group. This difference was statistically significant at a p-value of 0.01, as indicated in the results Table -1.

TABLE 1. SERUM LACTATE, PYRUVATE AND MOLAR LACTATE: PYRUVATE RATIO AS INDIRECT MARKER FOR MITOCHONDRIAL FUNCTION.

Parameters	Negative control	Positive control	Mt-S treated	No Mt-S+ no treatment
Serum Lactate mmol/L	1.31±0.14	2.44±0.34#	1.54± 0.12*	1.58± 0.13*
Serum Pyruvate mmol/L	0.078±0.005	0.098± 0.031#	0.084±0.009	0.098±0.007
Molar lactate/ pyruvate ratio	16.8±0.25	24.9±0.36#	18.4±0.80*	16.2±0.74*

The rats with metabolic syndrome who received phenolic extract experienced a substantial reduction in serum lactate levels ($p<0.01$) compared to the positive group. Similarly, the animals without metabolic syndrome who were treated with the extract also exhibited a significant decrease in lactate levels ($p<0.01$) compared to the positive group.

The results indicate a significant increase in serum pyruvate levels in rats from the positive control group compared to the negative control group, with a statistical significance of $p<0.01$. There were no notable alterations in the pyruvate concentration seen between the rats administered with Mt-S extract and the rats without metabolic syndrome that received the extract. The results were compared to the positive group and no statistically significant difference was seen ($p>0.05$).
The molar lactate: pyruvate ratio, which serves as an indirect measure of mitochondrial function, was assessed in this work. The comparison of molar ratios of lactate to pyruvate in rat serum revealed a significant rise in both the positive and negative groups, with a p-value of less than 0.01. The rats with metabolic syndrome that were treated with the extract exhibited a substantial decrease in comparison to the positive control group, with a statistical significance of $p< 0.001$
For rats without Mt-S and dosed with extract showed significant reduction in comparison to the positive control group at $P<0.001$.
The above data indicated significant effect for the extract on mitochondrial function and this leads us to study the changes in the mt-DNA changes that reflected by changes in ND1 gene.
Mitochondrial genome copies number recruited here to explain the changes in mt-DNA the different groups in this work. The results shows that the positive control group with the metabolic syndrome showed a significant increase in the number of copies mt-DNA that reflect in ΔΔ CT value (12.7± 2.95%) compared to the negative control group (-0.87±0.26) at a significant level (0.01) as showed in figure 4.

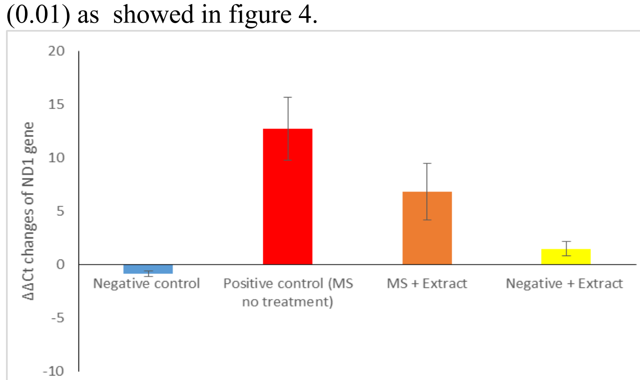

Figure 4. Delta-Delta Ct value changes of ND1 gene in the studied groups.

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The investigation demonstrated a notable reduction in the number of mt-DNA copies (6.8±2.65) in animals treated with extract compared to the positive control group, indicating a meaningful effect of Mt-S treatment at a significance level of p<0.05. The negative control group, which was treated with ethanolic extract, exhibited a significant decrease in the number of mt-DNA copies (1.5±0.65) compared to the positive control group, with a p-value of less than 0.01.

Discussion

According to our theory biochemical parameters corrected after treatment with phenolic-extract of the clove. a

Serum glucose significantly elevated in positive group (145±12.6) in comparison to negative control group (75±5.4) at p<0.001. Metabolic syndrome rate treated with the extract (112±7) shows significant reduction in comparison to positive group at p<0.001. Rats with no metabolic syndrome and treated with the extract (94±6) shows significant reduction in comparison to positive group at p<0.001.

The anti-diabetic properties of clove extract have been extensively documented. Based on assumptions, it is believed that clove extract imitates the impact of insulin on the activity of the genes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. Additionally, both clove and insulin have a similar effect on the activity of multiple genes (Milind and Deepa, 2011). A study conducted by Adefegha et al. (2014) discovered that rats with induced type 2 diabetes, who were fed diets containing clove, had lower blood glucose levels compared to diabetic rats who were fed diets without clove.

A recent study examined the effects of clove-supplemented diet on blood sugar and lipid peroxidation in diabetic rats. The study found that the diet led to an increase in blood sugar and lipid peroxidation in vivo. Additionally, the levels of antioxidant enzymes were restored.

The aqueous extract of Syzygium increased insulin receptor (IR and IRS-1) activity and reduced obstruction of the insulin signalling pathway in induced diabetic rats, consequently mitigating hepatic insulin resistance (Konda et al., 2021).

In addition, when Syzygium remedies were administered to rats with type-2 diabetes, the levels of IR mRNA restored to the normal level, suggesting an enhancement in insulin secretion (Sampath et al., 2013).

Studies conducted on live mice shown that administration of extracts derived from Syzygium aromaticum resulted in enhanced blood sugar levels (Kuroda et al., 2012; Sanae et al., 2014).

Lipid profile parameters shows that serum cholesterol significantly increase in positive control group (163±36) in comparison to negative control group (65±15) at P<0.0001. Serum cholesterol of Mt-S that treated with the extract (136±36) and the rats with no Mt-S + treated with the extract both shows significant reduction significantly reduced in comparison to positive control group at p<0.05 as shows in figure-1.

Serum triglycerides shows significant elevation in positive control group (257±39) in comparison to negative control group (97±10) at p<0.001. results shows there were significant reduction in triglycerides in both Mt-S treated with extract (220±41) and Non Mt-S treated with the extract(118±38) in comparison to positive control group at p<0.05 as shows in figure-2.63

Serum HDL significantly decline after induction of metabolic syndrome in the positive control group (27±6) in coparison to negative control group(45±10) at p<0.001. Serum HDL-C level significantly elevated after treatment with exteact in comparison to positive control group at p<0.01 as showed in figire-3.

By lowering serum triglyceride and cholesterol levels, the in vitro and in vivo studies have demonstrated the antiobesity effectiveness of Syzygium aromaticum extracts (Przygodzka *et al.*, 2016).

(Abd El-Rahman, 2015) reports that the dietary inclusion of diabetic rats induced by Syzygium aromaticum resulted in a significant reduction in serum glucose, total cholesterol, LDL-C, VLDL-C, and triglyceride levels.

Liver function parameters, liver enzyme GOT, shows significant elevation in positive control group ((131±30) in comparison to negative control group (77±10) at p<0.001. S.GOT significantly reduced in Mt-S group (108±27) in comparison to positive control group at p<0.05. In the non-Mt-S rats treated with the extract (92±17) there was significant reduction in S.GOT activity in comparison to positive control group at p<0.001.

For S.GPT, enzyme activity significantly significant elevation in positive control group (57±12) in comparison to negative control group (42±8) at p<0.001. No Significant changes in S.GPT activity was noticed in Mt-S rats after treatment with the extract (55±11) in comparison to positive control group at p>0.05). While in non Mt-S rats that treated with the extract (48±7) shows significant reduction in comparison to positive control group at p<0.05.

Alkaline phosphatase enzyme shows significant elevation in positive control group (374±59) in comparison to negative control group (175±9) at p<0.001. While both Mt-S rats treated with the extract (206±25) and Non Mt-S treated with the extract (192±18) shows significant reduction in the activities in comparison to positive control group at p<0.001.

(Abd El-Rahman, 2015) reports that dietary inclusion of diabetic rats induced by Syzygium aromaticum resulted in a significant reduction in liver function parameters, including GOT, GPT, and Alkaline phosphatase.

Clove intake has been shown to improve GPT and GOT levels in hyperlipidemic rats when compared to normal values (Shyamala, Venukumar and Latha, 2003)

The group of rats with metabolic syndrome treated with ethanolic extract showed significant decrease in the level of serum lactate p<0.01 compared to the positive group and for the animals without the metabolic syndrome and treated with

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the extract, there was a significant decrease in the lactate level at p<0.01, compared with the positive group.

The result of the work indicates a significant increase in the level of serum pyruvate in rats with positive control group in comparison to the negative control group at p<0.01.

In the animals group with Mt-S treated extract and rats without metabolic syndrome dosed with extract, there was no significant changes in the pyruvate level in comparison to the positive group at p>0.05.

The molar lactate: pyruvate ratio – as indirect mitochondrial function index – in this study indicated that; in comparison between the molar ratios of lactate to pyruvate in rat serum showed a significant increase in the positive group and the negative group at a p< 0.01. Metabolic syndrome animals treated with extract shows significant reduction comparison to the positive control group at p< 0.001. For animals without Mt-S and dosed with extract showed significant reduction in comparison to the positive control group at P<0.001.

The above data indicated significant effect for the extract on mitochondrial function and this will lead us to study the changes in the mt-DNA changes that reflected by changes in ND1 gene.

Mitochondrial genome copies number recruited here to explain the changes in mt-DNA the different groups in this work. The results shows that the positive control group with the metabolic syndrome showed a significant increase in the number of copies mt-DNA that reflect in ΔΔ CT value (12.7± 2.95%) compared to the negative control group (-0.87±0.26) at a significant level (0.01).

Lactate / pyruvate ratio used as indirect mitochondrial function index significantly reduced blow 20 after treatment with phenolic extract.

This is the first study that showed significant reduction in mt-DNA copy number after treatment with phenolic extract in metabolic syndrome rats.

In conclusion, metabolic syndrome significantly reduce mitochondrial genome copies number that can be antagonized by application of the ethanolic extract of the clove.

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Reference

Abd El-Rahman, A.N. (2015) 'Anti-Diabetic and Hepatoprotective Effect of Clove (Syzygium Aromaticum Linn) on Rats Induced by Alloxan', J. Home Econ, 25(3), pp. 133–150.

Adefegha, S.A. et al. (2014) 'Antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative effects of dietary clove (Szyzgium aromaticum) bud powder in a high-fat diet/streptozotocin-induced diabetes rat model', Journal of the Science of Food and Agriculture, 94(13), pp. 2726–2737.

Koh, T. et al. (2013) 'Re-evaluation of anti-inflammatory potential of eugenol in IL-1β-stimulated gingival fibroblast and pulp cells', in vivo, 27(2), pp. 269–273.

Konda, P.Y. et al. (2021) 'Ethno-pharmacological insulin signaling induction of aqueous extract of Syzygium paniculatum fruits in a high-fat diet induced hepatic insulin resistance', Journal of ethnopharmacology, 268, p. 113576.

Kuroda, M. et al. (2012) 'Hypoglycemic effects of clove (Syzygium aromaticum flower buds) on genetically diabetic KK-A y mice and identification of the active ingredients', Journal of natural medicines, 66, pp. 394–399.

Milind, P. and Deepa, K. (2011) 'Clove: a champion spice', Int J Res Ayurveda Pharm, 2(1), pp. 47–54.

Przygodzka, M. et al. (2016) 'Effect of selected spices on chemical and sensory markers in fortified rye-buckwheat cakes', Food Science & Nutrition, 4(4), pp. 651–660.

Sampath, S. et al. (2013) 'Effect of homeopathic preparations of Syzygium jambolanum and Cephalandra indica on gastrocnemius muscle of high fat and high fructose-induced type-2 diabetic rats', Homeopathy, 102(03), pp. 160–171.

Sanae, F. et al. (2014) 'Effects of eugenol-reduced clove extract on glycogen phosphorylase b and the development of diabetes in db/db mice', Food & function, 5(2), pp. 214–219.

Shyamala, M.P., Venukumar, M.R. and Latha, M.S. (2003) 'Antioxidant potential of the Syzygium aromaticum (Gaertn.) Linn.(cloves) in rats fed with high fat diet', Indian Journal of pharmacology, 35(2), pp. 99–103.

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