

Full Length Research Paper

Gene regulating effects of *Cymbopogon citratus* on glucose metabolism of normal albino rats

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***Cymbopogon citratus* has been reported to have hypoglycaemic and antidiabetic activities. This study evaluates the insulinotropic properties of *C. citratus* via gene expression. *C. citratus* was administered to normal rats as pulverized leaves (2, 10 and 30%) mixed with animal feed for one week; as aqueous and ethanol extracts at a dosage of 30 and 100 mg/kg body weight for 30 days; and as isolated saponins, flavonoids and tannins at 30 µg/kg for 7 days. Animals were sacrificed after the treatment protocol and organs of interest removed for analysis. Gene expression analysis of *C. citratus* was based on polymerase chain reaction (PCR) for glucagon like peptide-1 (GLP-1), insulin, glucose transporter 4 (GLUT4) and potassium ion gated channel (KCNJ5), using isolated mRNA. Results showed that the extracts and phytochemicals fractions (particularly flavonoids) of *C. citratus* increased insulin gene expression, whereas only the whole plant feeding of normal rats increased GLP-1 gene expression in a dose-dependent manner. Administration of plant extracts increased GLUT-4 expression while phytochemical fractions of *C. citratus* did not alter the expression of KCNJ5 gene. It can be concluded that the pharmacology of *C. citratus*, especially the whole plant and the aqueous and ethanol extracts, favours the up-regulation of some insulinotropic genes.**

Key word: Insulinotropic properties, *Cymbopogon citratus*, gene expression, insulin, saponins, flavonoids, tannins.

INTRODUCTION

Insulin dysfunction has been described as one of the major contributors to ill health and premature mortality worldwide (WHO, 2019, 2021). This can lead to aberration in glucose metabolism as a result of insulin secretion and action defects (IDF, 2020). Diabetes mellitus is a globally known chronic disease (Al-Lawati, 2017), that occurs either when the pancreas does not

produce enough insulin or when the body cannot effectively use the insulin it produces (WHO, 2020). Studies have shown the existence of a complex interaction between inflammation, endoplasmic reticulum stress, oxidative stress, mitochondrial dysfunction and autophagy dysregulation and diabetes mellitus which play important roles in insulin resistance (Cho et al., 2018).

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Insulin controls both the metabolism of carbohydrates and fats by facilitating the absorption of glucose from the blood to skeletal muscles and fat tissue and by promoting fat storage than its usage (Zheng et al., 2018).

It is well known that insulin resistance plays an important role in the onset of diabetes mellitus and that failure of pancreatic β -cells to produce and release insulin is instrumental to the development of hyperglycaemic condition (Einarson et al., 2018). The major role of islets of Langerhans of the pancreatic β -cells is to secrete insulin to control blood glucose homeostasis. Insulin secretion is subject to control by nutrients and hormonal, neural, and pharmacological factors (Di Meo et al., 2016). The alteration of these factors contributes to the pathogenesis of type 1 and type 2 diabetes (Di Meo et al., 2018).

The major gut insulinotropic hormones or incretins is glucagon-like peptide-1 (GLP-1) (Zhang et al., 2017). GLP-1 is produced mainly by the enteroendocrine L cells of the distal intestine in response to nutrient ingestion (Baggio et al., 2018). The mechanism of action of GLP-1 involves its binding to its receptor, which in turn affects blood glucose levels by stimulating insulin secretion, thus inhibiting glucagon secretion, inhibiting gastric emptying and reducing food intake (Burmeister et al., 2017). Its physiological functions range from hypoglycaemic effect, anti-inflammatory, antioxidative, neurogenerative, as well as vascular protective effects in various cells and tissues including the kidney, lung, heart, hypothalamus, endothelial cells, neurons, astrocytes, microglia, and pancreatic beta cells (Drucker et al., 2017; Hou et al., 2016).

Report by Klip et al. (2019) showed that the level of glucose transporter 4 (GLUT-4) expression determines the maximal effect of insulin on glucose transport. Thus, diabetes leads to suppression at a pre-translational step resulting in a marked decrease in GLUT-4 expression in skeletal muscle and adipose tissue (Lauritano and Ianora, 2016). Potassium Voltage-Gated Channel Subfamily J Member 5 (KCNJ5) is an example of ATP-sensitive K^+ gated channels, which plays an important role in electrogenic events within the cell (Wang et al., 2021). KCNJ5 is sensitive to metabolic changes in the pancreatic β -cell, coupling cellular metabolism to electrical activity and to insulin secretion (Wang et al., 2021). Invariably, when K^+ channels channels are opened, β -cell repolarise and insulin secretion is suppressed, leading to diabetic state, and when it is closed, β -cell depolarise and insulin secretion is activated (Wu et al., 2019).

Cymbopogon citratus is a well-known medicinal herb in tropical and subtropical countries, used as a medicine because of its wide-ranging therapeutic properties (Coelho et al., 2016; Lawal et al., 2017). Studies have shown that *C. citratus* has antibacterial, antifungal, anti-inflammatory, antimalarial, anti-protozoan, and ascaricidal effects (Bello et al., 2019). This medicinal plant has also

been reported to have hypocholesterolaemic, hypoglycaemic, hypolipidaemic, anticancer, anti-hypertensive, free radical scavenging and antioxidant properties (Ajayi et al., 2016; Coelho et al., 2016). *C. citratus* have been reported to reduce the fasting and postprandial blood sugar levels, bringing them towards normal and healthy state (Ajayi et al., 2016). The plant have been said to have hypoglycaemic property via insulin synthesis and secretion or increased peripheral glucose utilization (Lawal et al., 2017).

Certain plants phytochemicals such as saponins, tannins, flavonoids, and others, have been shown to possess medicinal properties for instance, certain bioactive compounds of *C. citratus* displayed over 60% inhibition of reactive oxidative species (ROS) generation with increased glucose metabolism and utilization (Ajayi et al., 2016; Coelho et al., 2016). To better understand the effect of *C. citratus* on glucose metabolism, especially in relation to stimulation of insulin production, this study assessed its effect on some genes with insulinotropic effects.

MATERIALS AND METHODS

Plant collection and preparation

C. citratus was harvested from a farm in Akure and authenticated in the Department of Plant Science and Biotechnology, University of Benin. A herbarium specimen with voucher number UBH-C451, was deposited in the University of Benin herbarium. *C. citratus* leaves were washed in clean water three times, sliced and spread on a clean surface at room temperature for two weeks. The dried leaves were milled and sieved into a powdered sample.

Extraction of *C. citratus*

A slight modification of Onoagbe and Esekheigbe (1999) method was used to prepare the extract. The powdered leaves of the plant were used. Two different weights of 1 kg each were soaked in distilled water (aqueous extract) and absolute ethanol (ethanol extract) with continuous stirring for 72 h in a glass container and covered with cheesecloth. At the end of the third day, they were filtered through two layers of cheesecloth and later with a filter paper to completely remove residues. The filtrate was concentrated by a rotary evaporator, and then evaporated to dryness in a freeze dryer. The dried extract was weighed and stored in an air-tight container and kept in the freezer until use.

Isolation of phytochemicals

Solvent extraction of dried powder (500 g) of *C. citratus* was done each for flavonoids by the method described by Subramanian and Nagarjan (1969); saponins by Woo et al. (1980); and tannins by Wall et al. (1996).

Animal ethical clearance

Treatment of the animals was in accordance with the Principle of Laboratory Animal Care. The Local ethical approval was received

from the Ethics Committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, with reference number EC/FC/FP/020/020.

Experimental design

Effect of *C. citratus* leaves on normal rats

Sixteen Wistar rats in four groups of four rats each were given *C. citratus* leaves mixed with their feed for one week using the experimental design: Normal control received 100% normal rat chow, other groups received 2, 10 and 30% *C. citratus* in rat chows.

Effect of aqueous and ethanol extracts of *C. citratus* on normal rats

Twenty Wistar rats in five groups of four rats each were given *C. citratus* extracts administered for thirty days using the experimental design: Normal control received 100% normal rat chow, other groups received 30 mg/kg body weight (bw) and 100 mg/kg bw of aqueous extracts of *C. citratus* and 30 and 100 mg/kg bw ethanol extracts of *C. citratus*

Effect of saponins, flavonoids and tannins extracted from *C. citratus* on normal rats

Sixteen Wistar rats in four groups of four rats each were given *C. citratus* isolated photochemical for one week using the experimental design: Normal control received 100% normal rat chow, 30 µg/kg bw saponins, 30 µg/kg bw flavonoids, and 30 µg/kg bw tannins isolated from *C. citratus*

Gene expression

After the final treatment, the animals were sacrificed and mRNA was isolated from the rat liver, ileum, kidney and pancreas, with TRIzol Reagent (Thermo Fisher Scientific) converted to cDNA using Proto Script First Strand cDNA Synthesis Kit (NEB). PCR amplification was done using OneTaq® 2X Master Mix (NEB) using the following primer set:

ACCGTTTACATCGTGGCTGG;	GLP-1 (Forward	5'-3'
CCCTGTGAATGGCGTTTGTG);	Reverse	5'-3'
GAGGCTCTGTACCTGGTGTG;	Insulin (Forward	5'-3'
ACTCCAGTGCCAAGGTTT);	Reverse	5'-3'
TCTCCGGTTCCTTGGGTTGT;	GLUT4 (Forward	5'-3'
TTCCCCATCTTCAGAGCCGAT);	Reverse	5'-3'
ACACTTCTACAATGAGCTGCG;	β-actin (Forward	5'-3'
CCAGAGGCATACAGGACAAC);	Reverse	5'-3'
CGACCAAGAGTGGATTCTT;	KCNJ5 (Forward	5'-3'
AGGGTCTCCGCTCTCTTCTT).	Reverse	5'-3'

Gene expression study data analysis

The intensities of the bands from agarose gel electrophoresis were quantified densitometrically using ImageJ software. Pymol was used to visualize the mRNA.

RESULTS

The inclusion of *C. citratus* powder in rat's diet, administration of its aqueous and ethanol extracts as well

as saponins, flavonoids and tannins fractions caused significant alteration in insulinotropic gene expression of normal rats. Thus, the potential of *C. citratus* to activate the release of GLP-1 and other target genes to enhance insulin release as observed in this study implies the ability of this plant to produce insulinotropic effects. The release of insulin from the pancreatic β cells through GLP-1 gene modulation by *C. citratus* powder was seen to be dose dependent (Figure 1). Most of the extracts administered did not alter the GLP-1 gene expression (with the exception of aqueous extract at 100 mg/kg body weight) (Figure 2), whereas saponins, flavonoids, and tannins fractions administration caused repression of GLP-1 gene expression in rat ileum (Figure 3). This report is complemented by the activation of insulin gene expression by the aqueous and ethanol extracts (Figures 4 and 5) as well as the flavonoids of *C. citratus*.

From this study, ethanol extract of *C. citratus* caused up-regulation in GLUT-4 expression in the liver cells after 4 weeks of administration (Figure 6). From this present study, administration of saponins, flavonoids, and tannins fractions from *C. citratus* did not alter the expression of KCNJ5 gene (Figure 7) implying that up-regulation of KCNJ5 gene may not be a major pathway for the isolated phytochemicals of *C. citratus* to exert its insulinotropic effect.

Feeding normal rats at different concentrations of *C. citratus* infused feed caused a dose dependent increase in GLP-1 gene expression in the ileum of the rats. The 30% feed formulation caused a nearly three-fold increase (1.2 ± 0.01) compared to control (0.39 ± 0.05).

Aqueous extract at 30 mg/kg bw (0.69 ± 0.02) and ethanol extract at 30 mg/kg bw (0.77 ± 0.03) and 100 mg (0.68 ± 0.02) treated rats showed GLP-1 levels that were mostly comparable with control (0.65 ± 0.05). Meanwhile, aqueous extract at 100 mg/kg bw (0.21 ± 0.01), showed a significant reduction in GLP-1 gene expression in the ileum of rats when compared with control.

Saponins (0.29 ± 0.05), flavonoids (0.43 ± 0.03) and tannins (0.035 ± 0.001) fractions of *C. citratus* at 30 µg/kg bw repressed GLP-1 gene with tannin having most repression and flavonoids the least, when compared with control (0.58 ± 0.04).

All doses of aqueous 30 mg/kg (1.13 ± 0.03) and 100 mg/kg bw (1.14 ± 0.05) and ethanol extracts- 30 mg/kg bw (1.05 ± 0.03) and 100 mg/kg bw (1.18 ± 0.03) of *C. citratus* significantly ($p < 0.05$) increased the expression of the insulin gene in the pancreas of normal rats compared to control (0.68 ± 0.04).

Flavonoids (1.80 ± 0.05) fraction of *C. citratus* at 30 µg/kg caused a significant increase in insulin gene expression when compared with control; meanwhile, saponins (1.21 ± 0.05) and tannins (1.00 ± 0.02) fractions at 30 µg/kg bw showed no significant differences in insulin gene expression when compared with control (1.29 ± 0.03).

The ethanol extract of *C. citratus* at 100 mg/kg bw (0.594 ± 0.08) caused a significant increase in GLUT4

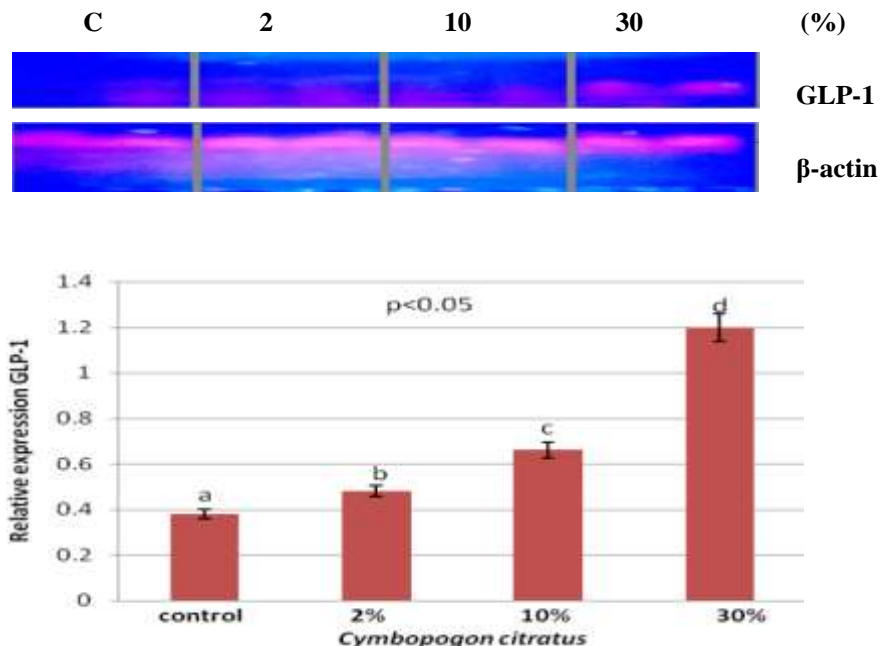


Figure 1. Qualitative-PCR analysis of GLP-1 expression in the ileum of rats fed leaves of *C. citratus* (n= 4) for 7 days at different concentrations. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on GLP-1 gene followed by densitometric analysis. Values carrying different levels are significantly different at $p < 0.05$. Source: Kendall and Michael (2010).

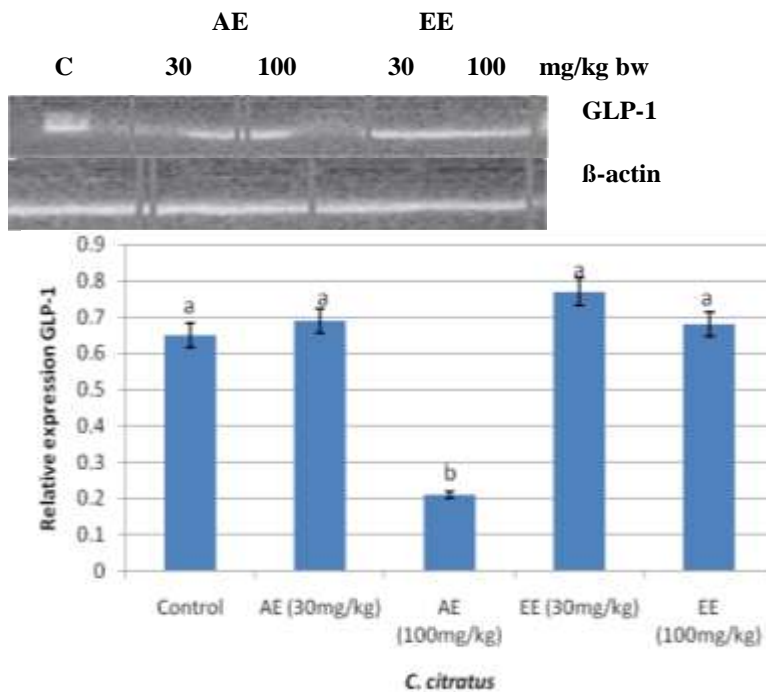


Figure 2. Qualitative-PCR analysis of GLP-1 expression in ileum of rats given aqueous and ethanol extracts of *C. citratus* (n = 4) for 30 days. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on GLP-1 gene followed by densitometric analysis. Values carrying different levels are significantly different at $p < 0.05$. C= Control; AE= Aqueous extract; EE= Ethanol extract. Source: Kendall and Michael (2010).

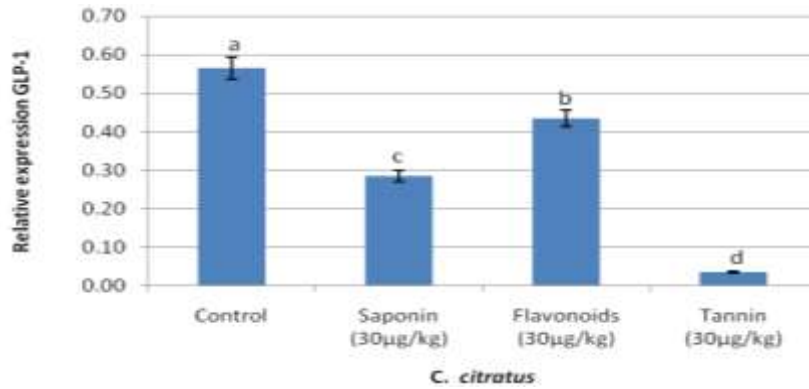
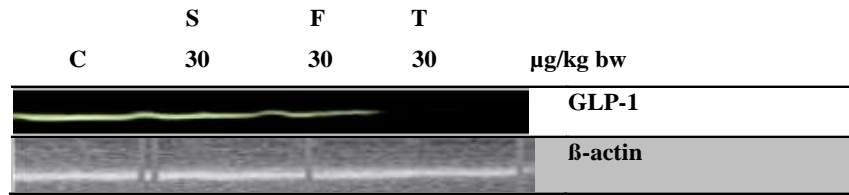


Figure 3. Qualitative-PCR analysis of GLP-1 expression in ileum of rats given saponins, flavonoids and tannins fractions of *C. citratus* (n= 4) for 7 days. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on GLP-1 gene followed by densitometric analysis. Values carrying different levels are significantly different at p<0.05. C= Control; S= Saponins; F= Flavonoids; T = Tannins. Source: Kendall and Michael (2010).

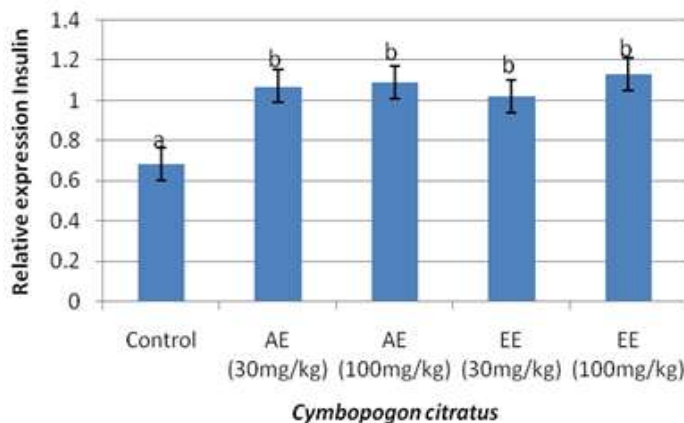
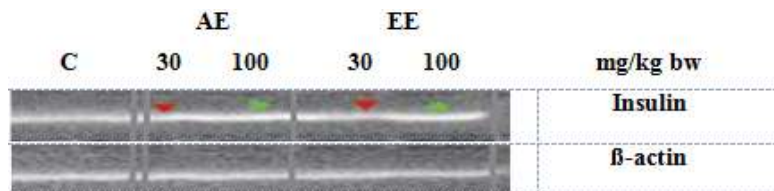


Figure 4. Qualitative-PCR analysis of insulin expression in the pancreas of rats given aqueous and ethanol extracts of *C. citratus* (n= 4) for 30 days. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on insulin gene followed by densitometric analysis. Values carrying different levels are significantly different at p<0.05. C= Control; AE= Aqueous extract; EE= Ethanol extract. Source: Kendall and Michael (2010).

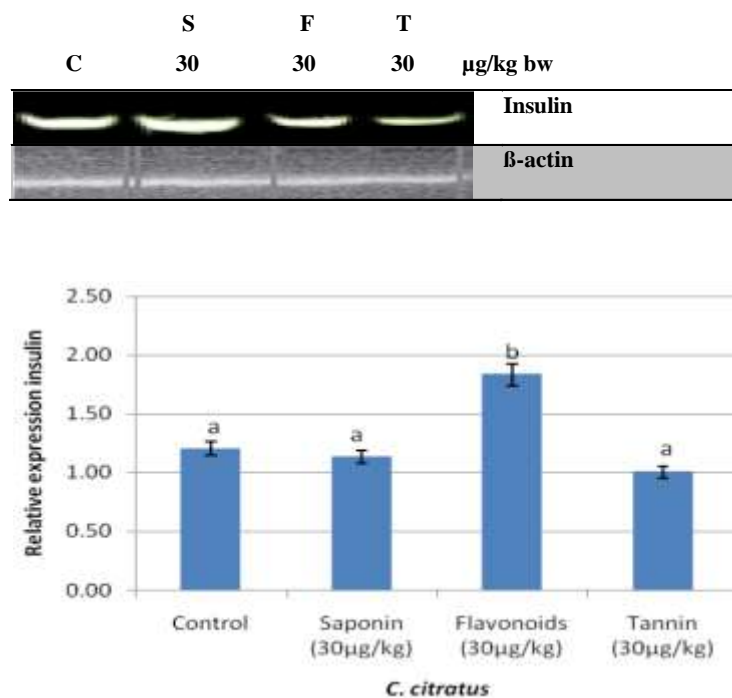


Figure 5. Qualitative-PCR analysis of insulin expression in pancreas of rats given saponins, flavonoids and tannins fractions of *C. citratus* (n= 4) for 7 days. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on insulin gene followed by densitometric analysis. Values carrying different levels are significantly different at $p<0.05$. C = Control; S = Saponins; F = Flavonoids; T = Tannins. Source: Kendall and Michael, 2010.

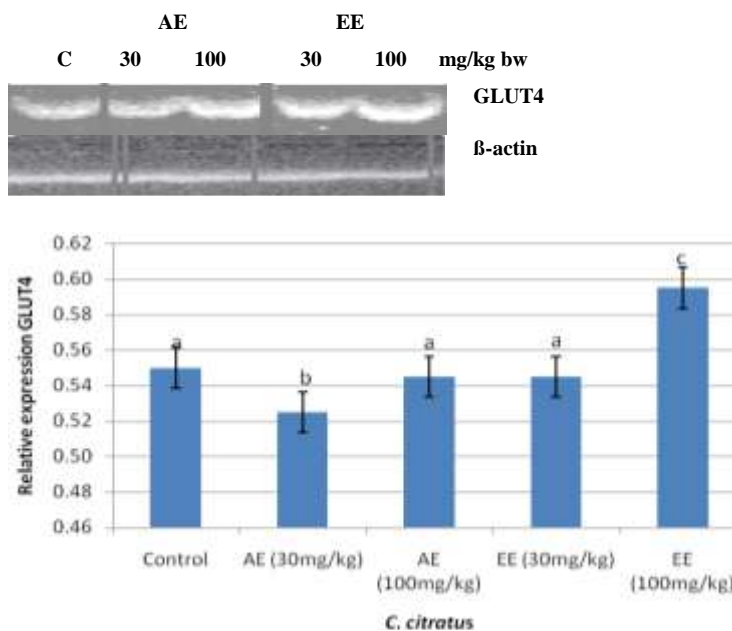


Figure 6. Qualitative-PCR analysis of GLUT4 expression in liver of rats given aqueous and ethanol extracts of *C. citratus* (n= 4) for 30 days. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on GLUT4 gene followed by densitometric analysis. Values carrying different levels are significantly different at $p<0.05$. C = Control; AE = Aqueous extract; EE= Ethanol extract. Source: Kendall and Michael (2010).

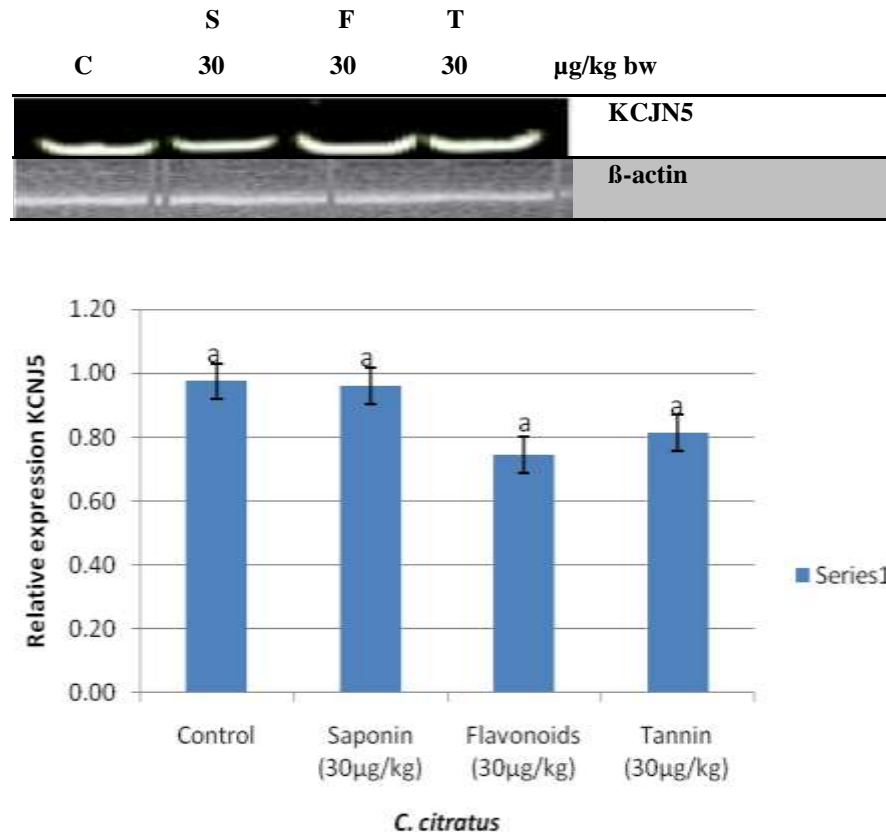


Figure 7. Qualitative-PCR analysis of KCJN5 expression in pancreas of rats given saponins, flavonoids and tannins fractions of *C. citratus* (n= 4) for 7 days. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on KCJN5 gene followed by densitometric analysis. Values carrying different levels are significantly different at $p < 0.05$. C = Control; S = Saponins; F = Flavonoids; T = Tannins. Source: Kendall and Michael, 2010.

gene expression when compared with control; meanwhile, the aqueous extract at 30 mg/kg (0.525 ± 0.05) caused a slight repression in GLUT4 gene expression in the liver of rats, whereas aqueous extract at 100 mg/kg (0.545 ± 0.05) as well as the ethanol extract at 30 mg/kg bw (0.545 ± 0.05) showed no significant difference when compared with control (0.55 ± 0.02).

Saponins (0.95 ± 0.05), flavonoids (0.75 ± 0.03), and tannins (0.81 ± 0.02) fractions of *C. citratus* at 30 µg/kg bw levels did not significantly alter KCJN5 gene expression as the levels observed were statistically similar to control (0.97 ± 0.05).

DISCUSSION

Insulin affects fuel metabolism by the stimulation of glycolysis, glycogenesis, and lipogenesis as well as inhibition of gluconeogenesis, glycogenolysis, and lipolysis (Zhao et al., 2017). The mechanism of insulin action on carbohydrate metabolism occurs when glucose

concentration is higher, such as after eating (Najjar and Perdomo, 2019). Insulin secreted by β -cells into the blood stream promotes glycolysis to lower elevated glucose levels by removal of glucose from blood stream to most body cells causing its storage, and used by almost all tissues of the body directly (Giugliano et al., 2019). Alteration or reduction in the activities of insulin termed insulin resistance often results to type 2 diabetes (Demircik et al., 2019), and has been linked with both genetic and environmental factors (Giugliano et al., 2019). This may lead to blockage in the biochemical pathways, by which glucose uptake and metabolism by the cells occurs (Kuzulugil et al., 2019; Athyros et al., 2020).

The relationship between insulin and glucagon like-peptide-1 (GLP-1) has been established and explained through scientific research-based evidences. GLP-1, as an incretin, functions by potentiating the secretion of insulin after meal ingestion in a glucose-dependent manner, exerting its insulinotropic actions through distinct G-protein-coupled receptors that is highly expressed on

islet β -cells (Nauck and Meier, 2019). The increase in GLP-1 gene expression by *C. citratus* powder administered rat complied with the reports of Steinert et al. (2017) and Reimann and Gribble (2016), who explained the secretory controls and physiological roles of GLP-1 in eating and glycemia in health, obesity.

The mechanism of action of GLP-1, a product of the pre-proglucagon gene, is often based on its released from L cells in the intestine upon food intake and potentially activate the release of insulin from pancreatic β -cells in a glucose-dependent manner in both normal and type 2 diabetic subjects (Modvig et al., 2019). Type 2 diabetes mellitus is usually as a result of defect in insulin secretion and peripheral insulin resistance, thus, therapies based on the incretin, glucagon-like peptide 1 (GLP-1) may be important treatment options for type 2 diabetes (Psichas et al., 2017). This is because they act through a variety of complementary mechanisms, through an increase in the amount of insulin released from beta cells in the pancreas following ingestion of food. Interestingly, a number of additional effects of GLP-1 on the pancreas have been observed to include stimulation of insulin biosynthesis and growth of β -cell mass (Psichas et al., 2017). Thus, GLP-1 may play a vital role in both glycaemic treatment of type 2 diabetes and potentially prevent or delay the progression of diseases.

The current understanding is that GLP-1 stimulates insulin exocytosis. Christiansen et al. (2019) reported that GLP-1 resulted in the increase in immunoreactive insulin content, by lowering postprandial hyperglycemia via three independent mechanisms, which include increased insulin secretion, inhibition of glucagon release, and inhibition of gastrointestinal motility. GLP-1 treatment corrects several of the fundamental defects in diabetic β -cell function through its insulinotropic activities (Holst et al., 2019). It can be said that the activation of GLP-1 genes by *C. citratus* powder and ethanol extracts may produce hypoglycaemic/antidiabetic effects by causing significant improvements in glycemic control through the release of insulin.

The progressive effect of the whole plant, solvent extracts, and phytochemical fractions of *C. citratus* on GLP-1 gene expression implies a progressive decline in the gene activation and activities. In fact, it can be deduced that the bioactive components that promote GLP-1 release reside outside the saponins, flavonoids, and tannins fractions. Several studies have corroborated the finding that specific biological activities of plant extracts are reduced when such extracts are fractionated further (Alema et al., 2020; Meresa et al., 2017; Taye et al., 2020). It can therefore be concluded that the whole plant of *C. citratus* potentiates GLP-1 gene up-regulation better than its extracts or individual phytochemical fractions. The significant increase in the expression of insulin gene occasioned by the administration of aqueous and ethanol extracts of *C. citratus* attested to the insulinotropic effect of this plant. The flavonoid enriched

fractions of this plant also caused significant increase in insulin gene expression; while saponins and tannins did not alter the expression of this gene. This clearly implies that *C. citratus* may initiate and potentiate hypoglycaemia by improving insulin secretion, an effect that is still retained in the flavonoid fractions (Reyes et al., 2017; Leonidas et al., 2017). It is not farfetched to suggest that the antioxidant properties of flavonoids may confer protective effects on pancreatic beta cells and thus optimize its ability to produce enough insulin needed by the body (Abotaleb et al., 2018; Kawser et al., 2016; Tanveer et al., 2017).

This study, also investigated the effect of *C. citratus* on GLUT-4 gene expression in liver cells in order to elucidate the molecular basis of its anti-diabetic potential. The transportation of glucose down its concentration gradient is mediated by members of the facilitative glucose transporter (GLUT) families, which are often expressed on the plasma membrane of all cells (Vargas et al., 2021). Insulin increases glucose uptake rate majorly by stimulating the translocation of the GLUT-4 isoforms from intercellular pools to the surface of cell membrane by increasing rate of glucose transport (Rashmi and Manonmani, 2020). So, it can be said that the effect(s) of *C. citratus* (especially the 100 mg/kg bw ethanol extract) to up regulate GLUT-4 may be useful for treatment of insulin insufficiency or insulin resistance. This up-regulation of the GLUT-4 gene by *C. citratus* ethanol extract leads to increased expression of GLUT-4 gene in treated animals, which provides strong indications that this extract will not only boost insulin level but will provide the channel for efficient tissue assimilation and usage of glucose.

These findings are in agreement with the results of Sleman et al. (2018) and Rashmi and Manonmani (2020), who reported that *Teucrium polium* extracts stimulate GLUT4 translocation to the plasma membrane in rats. Any disorder in KCNJ5 expression results to glucose intolerance or overt diabetes mellitus (Vargas et al., 2021), since the product of this gene increases insulin secretion.

Conclusion

The administration of *C. citratus* potentiated several insulinotropic pathways. The ability of this plant to up-regulate the expression of insulin, GLP-1, and GLUT-4 genes indicates several therapeutic interventions for hyperglycaemia. General comparison of the results obtained from the different administered samples suggests a decline in insulinotropic effects from whole plants to whole solvent extracts to isolated phytochemical. It could therefore be concluded, at least for GLP-1 gene expression, that the entire array of phytochemicals present in the whole plant may produce better insulinotropic effect.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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