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Research Article

Corn silk (*Stigma maydis*) aqueous extract attenuates high-salt induced glucose dysregulation and cardiac dyslipidemia: Involvement of phosphoinositide 3-kinase activities

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Keywords:
Cardiac dysmetabolism, Corn silk, Glucose dysregulation, High-salt diet, Phosphoinositide 3-kinase.

ABSTRACT

Background: Corn silk (*Stigma maydis*) is the long silky tuft of hairs from the female inflorescence of maize (Zea mays L) with rich antioxidant activity and free radicals scavenging capacity. High-salt diet on the other hand, has been shown to alter vascular and alter metabolic disorders. However, the exact ameliorating mechanism of corn silk (CS) effect is still being widely studied. This study examined the effect of aqueous corn silk extract on high salt-induced cardiac glucose and lipid dysmetabolism. **Methods:** Twenty male Sprague-Dawley rats (100-110g) were randomly selected into four groups (n=5) after a week of acclimatization and fed with rat chow (CTR), corn silk extract (CS; 500 mg/kg), high salt diet (HSD; 8%) and corn silk extract plus high salt feed (HSD; 8% + CS; 500 mg/kg) respectively for six weeks. At the end of the experimental procedure, each animal was anesthetized by exposure to chloroform vapor and blood samples collected by cardiac puncture. Data were analyzed and expressed as mean ± SEM and p-values < 0.05 were accepted as significant. **Results:** Corn silk extract resulted in attenuated plasma and cardiac glycogen production, triglycerides, free fatty acids, and total cholesterol associated with high-salt diet. However, the plasma level of Phosphoinositide 3-kinase (PI3K), and nitric oxide was significantly elevated in CS groups compared with control. Corn silk extract also decreased fasting blood glucose, insulin, and glycogen synthase activity (P<0.05) in HSD-fed rats. **Conclusion:** It is noteworthy from our data that corn silk possesses antilipidemic and glucoregulatory properties associated with enhanced phosphoinositide-3-kinases (PI3K) activity, an insulin dependent signaling pathway and may form an important component of nutritional candidate for ameliorating cardiometabolic diseases.

INTRODUCTION

Corn silk is a waste material from maize grass family Graminae (Zea mays L) with potential therapeutic applications for health care practices. Corn silk has been used as therapy for conditions like depression, fatigue, and diabetes. In a study by (Oyabambi et al., 2020) corn silk extract was shown to reduce inflammation by inhibiting cytokines release in the peripheral thigh of male wounded rats. Maysin, a corn silk flavonoid, is believed to be responsible for some of the unique therapeutic effects of corn silk and is known to activate the receptors for the binding of human peroxisome proliferator activators via increasing insulin level as well as recovering injured beta cells (Wei et al., 2018). It has been previously established that corn silk reversed high salt-induced endothelial dysfunction and oxidative damage via uric acid and vascular cell adhesion molecule-1-dependent mechanisms (Hasanudin et al., 2012; Oyabambi et al., 2020).

High salt intake on the other hand has adverse effects beyond those involving the cardiovascular system such as endothelial dysfunction, insulin resistance, diabetes (Radzeviciene and Ostrauskas, 2017). Therefore, there is a renewed interest in the relationships between high

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salt intake and other related diseases (Francesco et al., 2013).

Phosphoinositide-3-kinases (PI3Ks) are a family of lipid and protein kinases that primarily function by catalyzing the phosphorylation of D3 position of the inositol ring of phosphatidylinositol (Ptdlns) (Alessandra and Mingchuan, 2015). PI3K has been considered as therapeutic targets for cardiovascular diseases. Phosphoinositide-3-kinases pathway connected to the production of lipid second messenger is potentially involved in metabolic regulation especially impaired insulin-mediated glucose disposal in insulin resistant, diabetic or hypertensive conditions. Therefore, outside the rich involvement of its potent antioxidants, we sought to determine the involvement of a signaling molecule in the ameliorative effect of corn silk on high salt-induced impaired glucoregulation and cardiac dyslipidemia.

METHODS

Experimental animal and study design

Twenty weanling male Sprague-Dawley rats acquired from the Animal Holding unit of the Department of Physiology, University of Lagos, Lagos State, Nigeria with mean weight between 100±10g. The animals were housed in the animal house of the University of Ilorin, Ilorin, Kwara State in clean cages placed in well-ventilated housing conditions. Acclimatization period lasted for seven (7) days during which they were fed *ad libitum* with pellet form of grower feed (Vital poultry feed) and had access to drinking water. They were thereafter divided into four groups; group 1 was fed with normal rat chow (CTR), group 2 on high salt diet (HSD), group 3 on corn silk (CS) and group 4 on corn silk and high salt diet (CS + HSD).

Ethics statement

The animal handling and management procedure were as prescribed in the guidelines for the care and use of laboratory animals of University of Ilorin and the study protocols were approved by University of Ilorin Ethical Review Committee (UERC/ASN/2018/357).

Plant material

Fresh maize (*Zea mays* L.) of the cultivar 'Oba Super9', belonging to the family *Gramineae*, was obtained from Oke Oyi Farm in Ilorin-East LGA, Ilorin, Nigeria. The husks were removed and fresh corn silk (*Stigma maydis*) were collected, and authenticated by Mr. Bolus Ajayi of Department of Plant Biology, Faculty of Life Science, University of Ilorin, Ilorin Nigeria. A voucher specimen with voucher number UILH/001/1219 was deposited in the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria for reference.

Collection of Blood Sample

After six weeks of treatment, the rats were anesthetized with 0.3ml of ketamine hydrochloride. Blood was collected by cardiac puncture into plain bottles and was left to clot. It was then centrifuged at 3000rpm at room temperature for 5 minutes to separate. The collected serum was stored frozen for biochemical assay.

Phytochemical Analysis

Phytochemical screening (PS) of the local CS was done by standard photochemical screening according to the methods of George, 2016 and the proximate compositions of the extract obtained. PS of the CS reflected that alkaloid contain the highest % by weight/kg (115.69mg/log) followed by terpenoids (90.36mg/log).

Corn silk extracts preparation

Freshly collected *stigma maydis* ~50 g was thoroughly washed with distilled water, cut into shreds and air dried at room temperature for 7 days and then dissolved in 200ml of 100% methanol (MeOH) in a conical flask according to the methods of (Larson et al., 2016; Oyabambi et al., 2021). This was then placed in a shaker for approximately 120 hours at 28± 2^{O}C. The resulting extracts were then transferred to clean vessels, evaporated to dryness. After pulverization, 100g of the extract was soaked in 1 liter of distilled water for 72 hours, and then concentrated using a water bath. This was done repeatedly until the required stock solution was obtained. Extracts were administered at a dose of 0.5g/kg body weight of rats in distilled water p.o. (Ha et al., 2018).

Determination of sodium ion content in Feed

The sodium ion content of the normal rat chow was determined to be 0.3% atomic emission spectroscopy method. While the high salt diet was prepared to contain 8% of high salt in accordance with (Sofola et al., 2002).

Preparation of cardiac tissue homogenates

After dissection, the heart was excised, cleared of adhering connective tissues, blotted, and weighed. After weighing, 100 mg of tissue was carefully removed, homogenized in phosphate-buffered solution (PBS) with a glass homogenizer, and centrifuged at 10,000 rpm for 10 min at 4°C.

Biochemical Assay

Plasma insulin was determined using ELISA kit from Ray Biotechnology, Inc. (Georgia, USA). Insulin resistance *(IR)* was determined using the homeostatic model assessment for insulin resistance (HOMA-IR; (fasting insulin* fasting glucose)/22.5). Plasma and

cardiac nitric oxide were measured by quantification of nitrite using Griess Reagent. Plasma and cardiac TG, Cholesterol, high density lipoprotein cholesterol, and free fatty acid were measured by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Antrim, UK). Cardiac glycogen was estimated by a standard spectrophotometric method. Phosphotylinositol 3 Kinase (also known as PI3K), assay was done using the Sandwich-ELISA kit purchased from Elabscience® with Cat.NoE0438Ra

Statistical Analysis

The data obtained from blood samples were analyzed and expressed as mean ± standard error of mean (mean ± SEM). Means were compared by analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test. $P<0.05$ was taken as statistically significant.

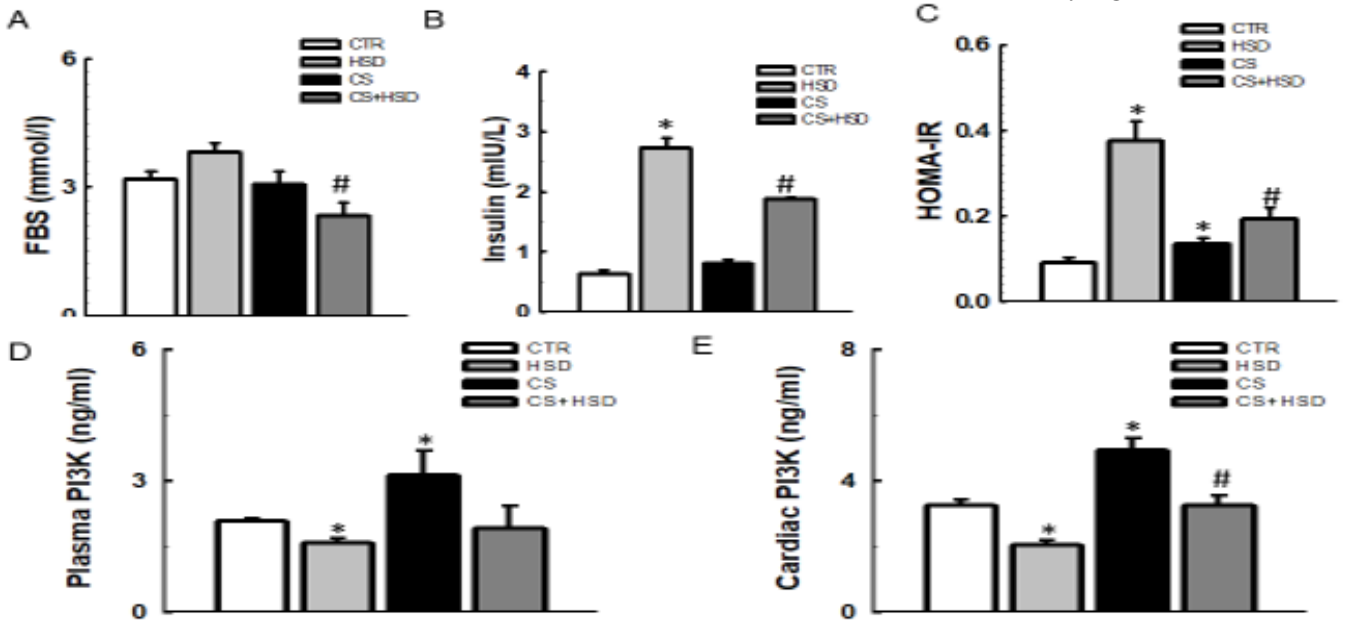

Fig. 1. Effect of corn silk on plasma fasting blood glucose (A), insulin (B), HOMA-IR (C): Homeostasis assessment of insulin resistance, and PI3K (D): Phosphotylinositol 3 Kinase in male Sprague-Dawley rats fed a high salt diet. Data were analyzed by one-way ANOVA followed by Bonferroni's *Post hoc* test. Values are expressed as mean ± SEM of 5 rats per group and $P<0.05$ was taken as statistically significant. *$p<0.05$vs control; #$p<0.05$ vs HSD

RESULTS

Effect of corn silk on fasting blood sugar, insulin, HOMA-IR and PI3K in male Sprague-Dawley rats fed high salt diet.

High salt diet led to significant increase in serum insulin and HOMA-IR (Fig. 1B and C), accompanied by significant decrease in cardiac PI3K when compared with control (Fig.1D and E). Interestingly, corn silk decreased serum insulin and HOMA-IR in high salt-fed rats while it reduced cardiac PI3K significantly when compared with high salt diet alone (fig 1B, C and D).

Effect of corn silk extract on plasma and cardiac nitric oxide and glycogen in male Sprague-Dawley rat fed high salt diet

Nitric oxide was significantly reduced in the plasma and cardiac tissue of high salt-fed rats when compared with control but led to elevated plasma and cardiac glycogen seen with HSD when compared with control (Fig. 2A, B, C, and D). However, corn silk (CS) increased plasma and cardiac nitric oxide and reduced cardiac glycogen compared with control. Furthermore, CS increased the plasma and cardiac nitric oxide in HSD-exposed group and decreased plasma and cardiac glycogen when compared with HSD only.

Effect of corn silk plasma and cardiac lipid metabolism in male Sprague-Dawley rats fed with a high salt diet

HSD led to increased plasma and cardiac TG and FFA (Fig. 3A and B) but did not significantly affect plasma and cardiac cholesterol (Fig. 4A and B) when compared with control. There was however a significant reduction of plasma and cardiac HDL-c in HSD-fed rats when compared with control (Fig. 3C and D). Reduction in plasma and cardiac TG and FFA alongside increased plasma and cardiac HDL-c was observed in HSD-fed rats fed with corn silk when compared with control. Interestingly, combination of corn silk and HSD led to reduced plasma and cardiac TG, and FFA as well as increased plasma and cardiac HDL-c when compared with HSD group.

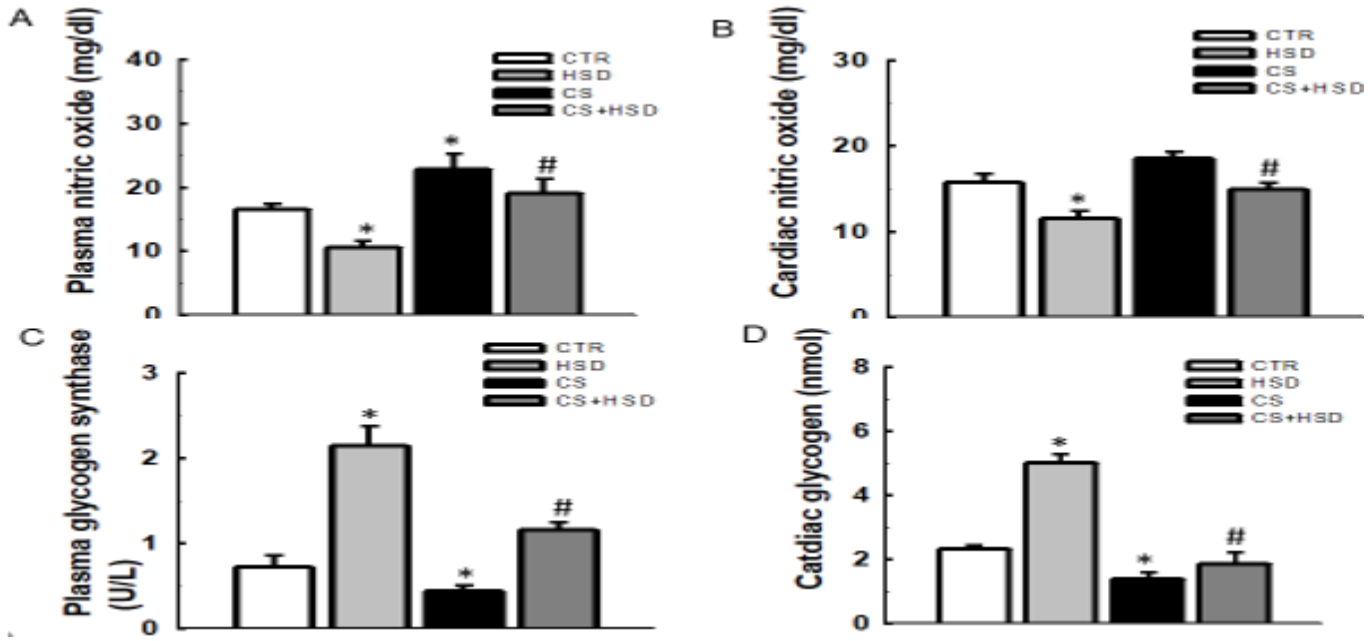

Fig. 2. Effect of corn silk on plasma nitric oxide (A), cardiac nitric oxide (B), plasma glycogen synthase (C), cardiac glycogen (D) in male Sprague-Dawley rats fed a high salt diet; Data were analyzed by one-way ANOVA followed by Bonferroni's *Post hoc* test. Values are expressed as mean ± SEM of 5 rats per group and P<0.05 was taken as statistically significant. *p<0.05vs control; #p<0.05 vs HSD *p<0.05vs control; #p<0.05 vs HSD

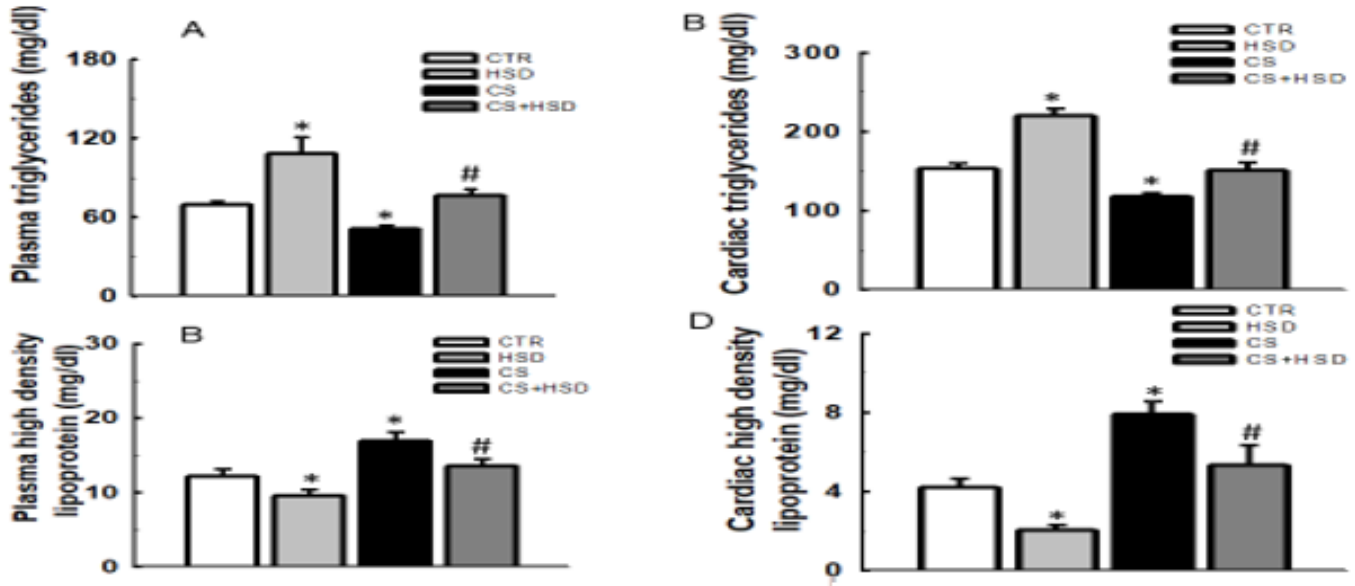

Fig 3: Effect of corn silk on plasma triglyceride (A), cardiac triglyceride (B), plasma HDL-C: high density lipoprotein cholesterol, (C) cardiac HDL-C: high density lipoprotein cholesterol in male in Sprague-Dawley rats fed a high salt diet**;** Data were analyzed by one-way ANOVA followed by Bonferroni's *Post hoc* test. Values are expressed as mean ± SEM of 5 rats per group and P<0.05 was taken as statistically significant. *p<0.05vs control; #p<0.05 vs HSD *p<0.05 vs control; #p<0.05 vs HSD

DISCUSSION

The present study demonstrates that high salt diet (HSD) caused glucose deregulation accompanied by hyperinsulinemia, hyperglycemia, hypertriglyceridemia, which is a component of metabolic syndrome. There was also evidence of lipid accumulation in the heart of HSD-fed animals in this study. These HSD-mediated events can contribute to adverse cardiovascular functions (Graudal et al., 2017). This study showed hopeful findings on the use of corn silk for treatment of metabolic derangement caused by HSD as it averted dyslipidemia, normalized

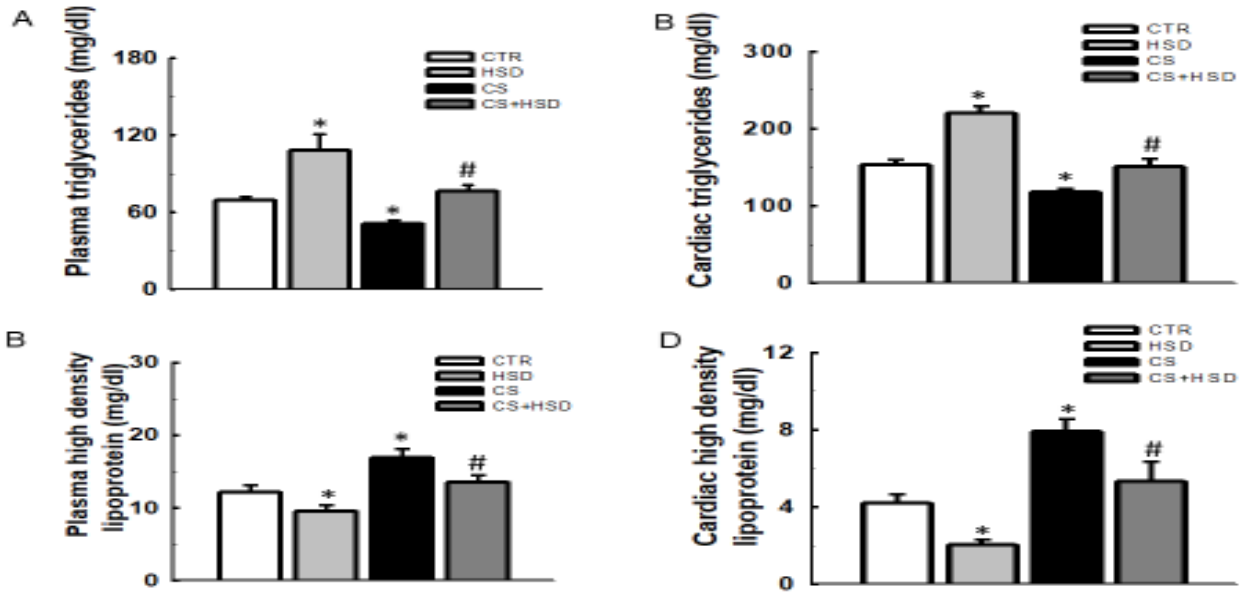

Fig 4: Effect of corn silk on plasma cholesterol (A), cardiac cholesterol (B), plasma free fatty acid, (C) cardiac free fatty in male in Sprague-Dawley rats fed a high salt diet**;** Data were analyzed by one-way ANOVA followed by Bonferroni's *Post hoc* test. Values are expressed as mean ± SEM of 5 rats per group and P<0.05 was taken as statistically significant. *p<0.0 5vs control; #p<0.05 vs HSD *p<0.05 vs control; #p<0.05 vs HSD

glucoregulation and ameliorated cardiac lipid accumulation in high salt diet-exposed animals while increasing circulating levels of PI3K (Fig. 5).

Insulin signaling in adipose tissue plays a key role in the storage of lipid as well as glucose homeostasis regulation. Also, studies in normotensive experimental animals and human subjects have revealed that a key feature of this pressure-independent effect of dietary salt is a profound reduction in vascular nitric oxide (NO) bioavailability that limits endothelium-dependent dilation. This reduction in NO is strongly associated with increased levels of reactive oxygen species (ROS) generated by NAD(P)H oxidase, Xanthine oxidase or uncoupled endothelial NO synthase within the vascular wall, leading not only to scavenging of NO but also to disruption of some signaling pathway that mediate its production (Matthew, 2013). High salt -induced endothelial damage and oxidative stress has been previously reported to be mediated through upregulation of uric acid and vascular cell adhesion molecule-1-dependent pathway (Puddu et al., 2012; Oyabambi et al., 2020). Interestingly, in the same study, it was reported that corn silk extract ameliorated HSD-induced endothelial dysfunction and oxidative stress through the downregulation of uric acid and vascular cell adhesion molecule-1.

In the present study, HSD impaired glucose regulation depicted by insulin resistance (HOMA-IR, increased plasma TG and FFA), hyperinsulinemia, hyperglycemia and elevated plasma and cardiac glycogen synthase activity. Fuenmayor and coworkers reported that high salt intake is been associated with hyperinsulinemia and impaired insulin-mediated glucose uptake. They further extended that the relationship between salt sensitivity and insulin resistance may be mediated by genetics (Fuenmayor et al., 1998). Contrastingly, high salt intake has been reported to ameliorate hyperglycemia and insulin resistance in diabetic rats (Takagi et al., 2018). Also, study from Adeyanju and colleagues demonstrated that high salt diet has no effect on impaired glucose metabolism and dyslipidemia in female Wistar rats (Adeyanju et al., 2017). Ginsberg and Stalenhoef have reported that increased lipolytic activity leads to increased FFAs flux to the liver which causes stimulation of gluconeogenesis as well as depletion of insulin effect on peripheral glucose (Ginsberg and Stalenhoef, 2003). However, aside distorting glucose regulation, the current study revealed that there is an obvious hyperlipidemia in high salt-diet fed group versus the placebo/control group. The obtained data agreed with the findings of (Son *et al*., 2012) who reported high cholesterol as well as triglyceride level in high cholesterol-fed rats (HCD) (Son et al., 2012). Furthermore, (Fruchart *et al*., 1998) demonstrated that

adipose tissue lipid is mostly derived from circulating TG, especially during HSD feeding.

Despite variable results from literature, the present study demonstrated that HSD altered glucose metabolism which might lead to lipid disturbances, hypertension, and diabetes with obesity-related co-morbid derangements (Wooding and Rehman, 2014). These conditions, however, are capable of damaging vital organs like the heart. In the present study, HSD administration induced cardiac lipid dysmetabolism (increased TG, FFA and reduced HDL-c) which can relate with oxidation and glycation of HDL-c and associated inflammatory response. Elevated circulating TG has been established as an independent risk factor for inflammatory disease that triggers coronary heart disease. High plasma TG can lead to ectopic deposition in non-adipose tissues like the heart leading to generation of free radicals and disruption of cardiac glucose metabolism with tendencies of cardiac metabolic inflexibility. High level of free radicals/reactive oxygen species (ROS) has been reported to inflict direct damage to lipids. HSD administration through lipid dysmetabolism in the heart may cause oxidative stress and cellular damage through oxidation of critical cellular components such as membrane lipids, Protein, and DNA (Jain et al., 2010).

It has been suggested that flavonoids from corn silk extract possessed protective properties against atherogenesis. The mechanism by which flavonoids extract lowered triglycerides (TG) could be by decreasing very LDL (VLDL) synthesis through increased lipoprotein lipase activity. The observed hypolipidemic effect might be the synergistic action of these compounds by controlling the hydrolysis of lipoprotein and inhibition of cholesterol absorption (Devi and Sharma, 2004).

Here, the findings that corn silk extract possessed hypolipidemic activity as indicated by reduced plasma and cardiac cholesterol, TG as well as increased plasma and cardiac HDL-clevels in HSD-fed rats is noteworthy. This result is in agreement with the findings of Kaup et al., 2011. Weggemans and Trautwein reported that flavonoids intake decreases LDL-c and increased HDL-c that may enhance removal of cholesterol from peripheral tissue to the liver for catabolism and excretion (Weggemans and Trautwein, 2013). Moreover, several studies revealed that isoflavones can decrease cholesterol in serum via elevated LDL-c receptor activity (Kirk et al., 1998; Taku et al., 2007; Ramdath et al 2017). It has been reported that many natural products as well as medicinal plants have lipase inhibitorypotentials. Corn silk extracts decreases serum lipase activity due to its bioactive compounds that have been previously reported to inhibit porcine lipase such as polyphenols, tannins, proanthocyanidin, and flavonoids contents (Keshavarz et al., 2011).

The amelioration of HSD-induced cardiac dyslipidemia by corn silk is mediated via PI3K signaling pathway. Phosphoinositide-3-kinasespathway connected to the production of lipid second messenger is potentially involved in metabolic regulation. It is also a key component of the insulin signaling pathway. Hence, there is great interest in the role of PI3K signaling in diabetes mellitus. PI3K interact with the insulin receptor substrate (IRS) to regulate glucose uptake through series of phosphorylation events (Cammalleri et al., 2003). The present study showed that HSD has overt ameliorative effect on circulating and cardiac PIK3 (Figure 1D) which suggests the plausibility of cardiac insulin resistance and imminent cardiac metabolic inflexibility. Derangement of PIK3 pathway in an insulin resistant milieu will hamper glucose availability and utility but exaggerate insulin-mediated mitogenic (mitogen activated protein kinase; MAPK) pathway which leads to inflammatory responses and eventual worsening of insulin resistance. Therefore, HSD mediates its deleterious cardiac and systemic impact through reduced circulating PI3K level resulting in impaired glucoregulation and dyslipidemia. Interestingly corn silk extract led to elevated circulating levels of PI3K, suggesting that the improved glucoregulation and lipid metabolism noticed in combined corn silk and HSD group may be due to upregulation of PI3K-signaling

CONCLUSION

Taken together, our results demonstrate the protective role corn silk extracts on HSD-induced glucose dysregulation and cardiac lipid dysmetabolism. Corn silk extract mediated its protective effects against HSD-induced dyslipidemia, impaired glucoregulation, impaired nitric oxide biosynthesis through the upregulation of PI3K-dependent pathway. The observed anti-hyperlipidemic and hypoglycemic as well as anti-inflammatory activity of corn silk may also be due to its active phytochemicals. Hence, intake of corn silk may form a common and affordable dietary therapeutic approach to the management of HSD-induced cardiac dyslipidemia and impaired glucoregulation.

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AUTHOR CONTRIBUTIONS

AOO, SSB, OSM, KTO and DOS conceived and designed the research. AOO, AOI, and KTO conducted the experiments. AOO, SSB, and DOS contributed to the new reagents and analytical kits. AOO, SSB, OSM, DOS, AOI, and KTO analyzed and interpreted the data. AOO, OSM, KTO, AOI and DOS drafted the manuscript. All authors read and approved the manuscript, and all data were generated in-house and that no paper mill was used.

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