BENEFICIAL EFFECT OF CO-ADMINISTRATION OF COCONUT WATER AND HONEY ON GLUCOSE STORAGE, HEPATO-RENO FUNCTIONS AND OXIDATIVE BALANCE IN SPRAGUE-DAWLEY RATS

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Abstract

Together with effective medical treatment, using recipes of traditional medicine such as coconut water and honey may be of immense benefit in the treatment of several diseases. The current study investigated whether co-administration of coconut water and honey has a synergistic effect on glucose storage, hepato-reno functions and oxidative balance. Four groups of male Sprague rats weighing between 150-200g were treated with CW and HON (10mg/kg of body weight) and (5.0mg/kg body weight of 50%) respectively thus: group I: Normal saline (1ml/kg body weight); group II: CW; Group III: HON; group IV: CW and HON synergistically for 21days. At the end of the experiment, fasting blood glucose (FBG), skeletal and hepatic glycogen contents were determined. Serum cholesterol, triglyceride, LDL and HDL levels were also assessed. Antioxidant enzymes superoxide dismutase (SOD), reduced glutathione (GSH), CAT and lipid peroxidation's marker malonaldehyde (MDA) was assessed. Liver enzymes' alkaline phosphatase (ALT), alanine amino transferase (AST), alkaline phosphatase (ALP), albumin and kidney function enzymes' urea and creatinine was determined. Treatment with CW, HON and both synergistically decreased FBG and significantly increased hepatic and skeletal glycogen contents compared with control ($p<0.05$). Serum triglyceride level significantly decreased in all the treated groups compared with control ($p<0.05$). There was a significant decreased in urea and creatinine levels in all the treated groups compared with control ($p<0.05$). Treatment with CW, HON and both synergistically elicited lower AST, ALT, ALP levels compared with control ($p<0.05$). The result shows a significant increase in albumin level when treated with both CW and HON synergistically compared with control ($p<0.05$). GSH, SOD and CAT showed a significant increase ($p<0.05$) and lipid peroxidation's marker, MDA significantly reduced compared with control ($p<0.05$). The current studies provide substantial evidence that CW and HON improves hepatic glucose storage, possess hypolipidemic and hepato-reno effects. They had synergistic effect on oxidative balance with concomitant reduction of lipid peroxidation.

Keywords*:* Coconut, Honey, Hypoglycemia, Glycogen, Oxidative, Stress

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Introduction:

Nowadays, the orthodox medicine is turning more and more on the medicinal benefits of natural products in the management of several diseases especially metabolic syndrome. Together with effective medical treatment, using recipes of traditional medicine such as coconut water and honey may be of immense benefit in the treatment of several diseases.

Cocos nucifera L., also referred to as "life tree"[1], is a monocotyledon plant that belongs to the Palmae family. Coconut water is consumed as a refreshing[2], tasty and nutritious drink, which can be a reliable source of minerals and is a natural isotonic[3] with similar

composition to that of saline. Its polyphenolic composition has been associated with the inhibition of hypoglycemic and nephroprotective activities in alloxan induced diabetes rats[4]

Honey is a natural substance produced by honey bees, which is widely used both for nutritional and medicinal purpose[5-7]. Research in the last few years provided authentic pieces of evidence in support of the antioxidant, anti-microbial and wound healing properties of honey[8,9]. Previous in vitro studies on antioxidant properties of honey showed that Tualang honey had the highest phenolics and flavonoids contents as well as the best free radical scavenging properties when compared to other Malaysian honey[10,11].There is also, the presence of many phenolic acids, such as gallic, syringic, benzoic, trans-cinnamic, P-coumaric acids and flavonoid compounds like catechin and kaempferol in honey[10]

It is therefore not surprising that, in the recent decade, there has been an increase in the utilization of complementary and alternative medicines in the treatment of several diseases such as diabetes mellitus and oxidative stress[12].In addition to fewer episodes of side effects, most of the complementary, alternative or traditional medicine agents are of natural origin and cheaper though efficacy and safety remain serious concerns. Honey is one of such natural agents with many attributed pleiotropic effects[11]

Despite several experimental evidences on the use of coconut water and honey in the management of several diseases, it remains unclear whether coconut water and honey has a synergistic effect on glucose storage, hepatic functions and oxidative balance in healthy rats. Hence, the current study investigated the effect of coconut water, honey and both synergistically on glucose storage, lipid metabolism, hepatic and renal functions. The current study also investigated the synergistic effect on markers of oxidative balance such as reduced glutathione (GSH), superoxide dismutase (S0D), catalase (CAT) and lipid peroxidation's marker malonaldehyde (MDA).

Materials and methods:

Animals

Twenty-four healthy male adult Sprague-Dawley rats were obtained from animal house unit, College of Medicine of the University of Lagos, CMUL, Nigeria. The rats were allowed to acclimatize for 14 days. They were housed separately in plastic cages in an animal room. The animal house was well ventilated and had a temperature of 26 ± 2 °C with a 12-hour light/dark cycle. The rats were given normal rat chow and drinking water *ad libitum*. The study protocol was in accordance with guidelines of the Animal care and Research Ethics Committee of College of Medicine of the University of Lagos[12]

Collection of coconut water

Matured coconuts (Cocosnucifera) were obtained from a fruits seller in Itori-Ewekoro, Ogun state, Nigeria. The coconuts were carefully broken and the coconut water (CW) was collected. It was diluted with distilled water (1:1) and used for this experiment.

Honey

The honey was obtained from a bee farm in Abeokuta, Ogun State, Nigeria. The honey had a NAFDAC (National Agency for Food and Drug Administration Control) number. The honey was bought from a farm registered with NAFDAC to ensure the honey used in the study was original and not adulterated. The honey was dissolved in distilled water (1:1) before oral administration

Experimental design

After acclimatization the rats were divided into 4 groups of 6 rats each, and treated thus for 21 days:

Group I: Normal saline

Group II: Coconut water (CW)

Group III: Honey (HON)

Group IV: CW+HON

Dosage

Normal saline (1ml/100g body weight)

CW: 10mg/kg of body weight[11]
HON: 5g/kg body weight daily of 50% honey[11]

Fasting blood glucose

After overnight fasting for 12 h, initial fasting blood glucose levels were estimated. Blood glucose concentrations were determined from the tail vein with an Acu-check glucometer (Roche) and glucose strip[13]

Collection of blood sample

Five (5ml) of blood sample was taken by retro-orbital puncture. Blood was allowed to clot for 1 hour at 4°C, then centrifuged at 3,000 rpm for 10 minutes and the serum samples were kept at -20◦c until assayed[13]

Blood lipids

Plasma lipid levels of triglyceride (TG), cholesterol (CHOL), low density lipoprotein (LDL), and high density lipoprotein (HDL) after treatment were determined by automatic biochemistry analyzer (BT, 2000 Plus, Germany) using diagnostic kits for each, purchased from BioSystems® (S.A Costa Brava of Barcelona, Spain).

Liver functions

Albumin, alkaline phosphatase (ALP), alkaline amino transferase (ALT), aspartate amino transferase (AST) and Albumin were determined using both serum samples by an automated analyzer (Mindray BS-120, ChemaDiagnostica, Italy)

Kidney Functions

Urea and Creatinine were determined using both serum samples by an automated analyzer (Mindray BS-120, Chema Diagnostica, Italy)

Tissue isolation

After the last glucose level was determined, the animals were fasted overnight and then sacrificed by cervical dislocation. Tissue samples of the liver and gastrocnemius muscle were carefully dissected over ice, rinsed with 1.15 % KCl, blotted and weighed.

Muscle and liver glycogen

Glycogen contents in hepatocytes and myocytes were determined as described by Morakinyo *et al.,* (2018). By this method, liver and gastrocnemius muscles of experimental animals were harvested and cleaned immediately before known weight were homogenized in ice-cold trichloroacetic acid (deproteinizing) solution and incubated for 15 min in water-bath. After discarding the precipitate, the supernatant was mixed with sulphuric acid and heated for 5 min and the absorbance read with ELISA reader (BiobaseBioindustry Co. Ltd., Shandong, China) at 520 nm wavelength. A standard glycogen (Sigma; St. Louis, MO, USA) was also prepared and employed for the standard curve.

Superoxide dismutase (SOD)

Briefly; SOD activity was measured by the inhibition autoxidative capacity of pyrogallol. The SOD activity was evaluated using a spectrophotometer at 420 nm. A calibration curve was constructed using SOD as standard. A 50% inhibition of autoxidation of pyrogallol was defined as one SOD unit[14]

Reduced glutathione (GSH)

The protein content of the samples was initially precipitated by metaphosphoric acid (MPA) at the ratio of 1:1 (homogenate/MPA). The samples were centrifuged at 3000rpm for 10 minutes. The supernatant was collected and mixed with sodium phosphate buffer (0.1M, pH 7.4), containing EDTA (5mM) and ortho-phthaldialdehyde (1 mg/mL in methanol). The mixture was incubated in the dark at room temperature for 15 min and fluorescence was measured at 350 nm (excitation) and 420 nm (emission). A standard curve of GSH (0.001–0.1 mM) was used for linear regression[14]

Catalase (CAT)

Briefly, sample (1ml) was mixed with 49 ml of distilled water to give a 1 in 50 dilution of the sample. The assay mixture contained 4ml of H_2O_2 solution (800 µmoles) and 5ml of Phosphate buffer in a 10ml flat bottom flask. 1ml of properly diluted enzymes preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run

at room temperature. A 1ml portion of the reaction mixture was blown into 2ml of dichromate acetic acid reagent at 60s intervals**.** Catalase (CAT) activity was determined by measuring the exponential disappearance of H_2O_2 at 240nm and expressed in units/mg of protein[15]

Malonaldehyde (MDA)

Briefly, the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems, MDA was estimated with the method of[16] which is based on its interaction with thiobarbituric acid (TBA) to form pink complex with absorption at 535nm. Absorbance was read using Microlab 300 recording spectrophotometer (UV 160) in all measurements.

Statistical analysis

All results are presented as the mean standard error of mean (SEM). Statistical analyses were conducted using Graph Pad Prism Software (GraphPad, Inc., La Jolla, CA, USA). Data analyses were performed by one-way analysis of variance (ANOVA) with post hoc Tukey's multiple comparison test. Statistical significance was set at $p < 0.05$.

Results:

Synergistic effects of CW and HON on FBG, Hepatic and Skeletal glycogen

We examined the effect of CW, HON and both synergistically on FBG. Figure 1 shows significant decrease ($p<0.05$) in FBG level in all the treated groups compared with CONT. FBG significantly increased in the groups treated with HON and CW+HON compared with compared with the group treated with CW only ($p<0.05$). Treatment with CW+HON significantly increased compared with treatment with HON only ($P<0.05$).

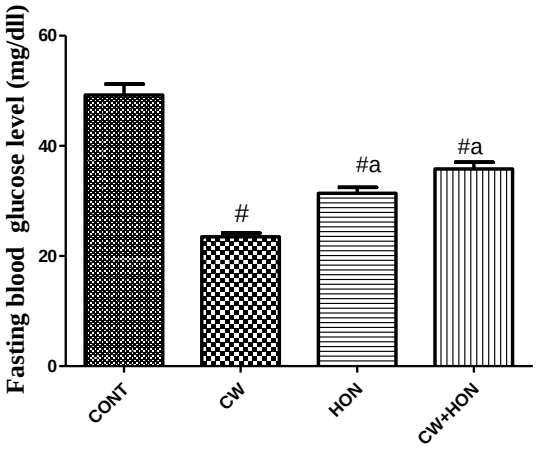

Figure 1: FBG level in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6. Significant ($^{\#}p<0.05$ vs. CONT, $^{a}p<0.05$ vs CW

Synergistic effects of CW and HON on Hepatic and Skeletal glycogen

We assessed the effect of CW, HON and both synergistically on hepatic and skeletal glycogen contents Treatment with CW, HON and both synergistically significantly ($p<0.05$) increased hepatic and skeletal glycogen contents compared with CONT. Treatment with HON and CW+HON significantly decreased and increased respectively compared with treatment with CW and HON only ($p<0.05$). Figure 2 and 3.

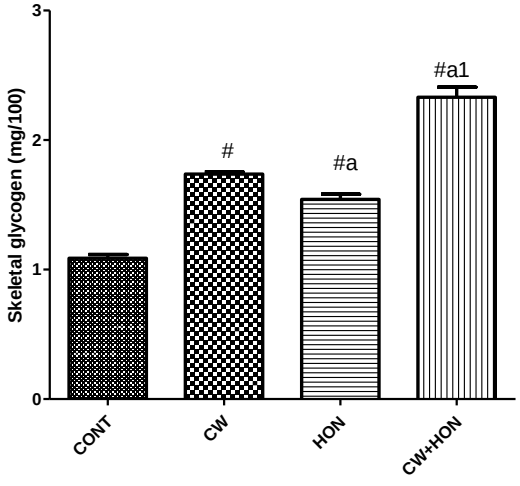

Figure 2: Skeletal glycogen in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6. Significant ($^{\#}$p<0.05 vs. CONT, ^{a}P<0.05 vs CW, ^{1}p<0.05 vs HON)

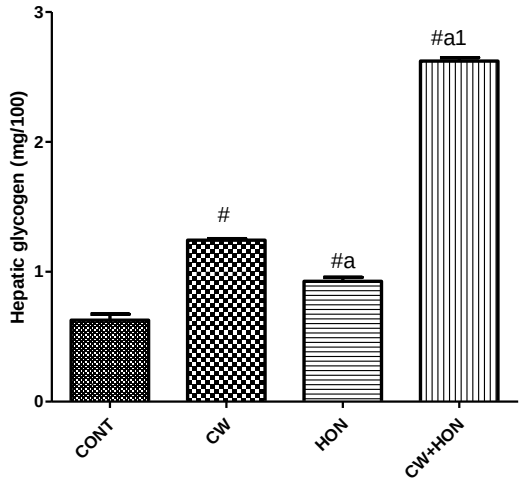

Figure 3: Hepatic glycogen in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6. Significant ($^{\#}$p<0.05 vs. CONT, ^{a}p<0.05 vs CW, ^{1}p<0.05 vs HON)

Synergistic effects of CW and HON on CHOL, TG, HDL and LDL

Table 1 shows no significant difference in CHOL, HDL and LDL in all the groups treated with CW, HON and both compared with CONT (p>0.05) with the exception of LDL which significantly increased in the group treated only with CW compared with CONT (p<0.05). TG level significantly decreased in all the groups treated with CW, HON and both synergistically compared with CONT (p<0.05).

Table 1: CHOL, TG, HDL and LDL in Sprague-Dawley rats treated with CW, HON and CW+HON

Parameters (mmol/l)	CONT	CW	HON	CW+HON
CHOL	2.00±0.06	2.01±0.05	1.98±0.09	1.93±0.05
TG	0.63±0.005	0.47±0.009$^{\#}$	0.47±0.008$^{\#}$	0.49±0.006$^{\#}$
LDL	0.72±0.04	0.92±0.04$^{\#}$	0.80±0.04	0.85±0.03
HDL	0.98±0.04	0.92±0.02	0.95±0.03	0.85±0.03

Values represent Mean ± SEM, n=6. Significant ($^{\#}$p<0.05 vs. CONT)

Synergistic effects of CW and HON on creatinine and urea levels

The results are illustrated in figure 4a and b. Creatinine and urea levels significantly decreased in all the groups treated with CW, HON and both synergistically compared with CONT (p<0.05). However, treatment with HON only significantly decreased while treatment with CW+HON significantly increased compared with treatment with CW only (p<0.05). CW+HON treatment produced a significant increase compared with treatment with HON only (p<0.05).

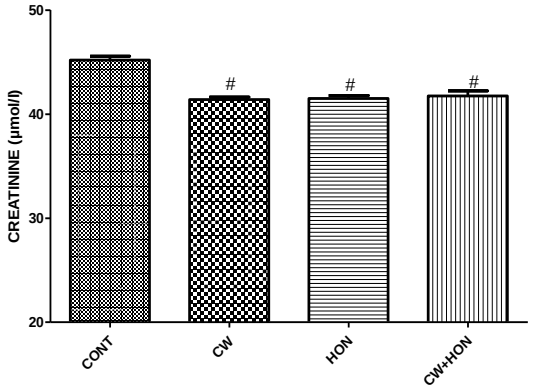

Figure 4a: Creatinine level in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6

Significant ($^{\#}$p<0.05 vs. CONT)

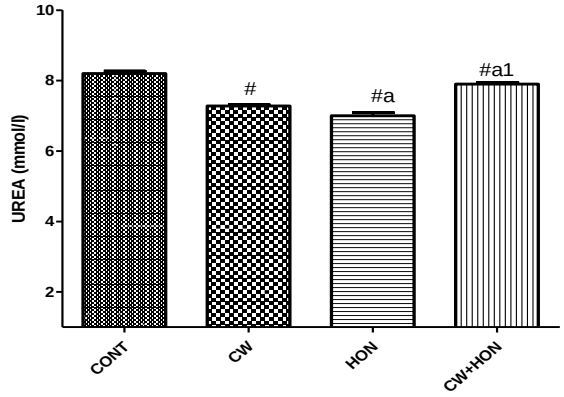

Figure 4b: Urea level in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6

Significant ($^{\#}$p<0.05 vs. CONT, ^{a}p<0.05 vs CW, ^{1}p<0.05 vs HON)

Synergistic effects of CW and HON on albumin, AST, ALT and ALP levels

There was no significant difference(p>0.05) in albumin levels in the groups treated with CW and HON but treatment with both synergistically significantly (p<0.05) increased albumin level compared with CONT. AST level significantly decreased in all the treated groups compared with CONT (p<0.05). ALT and ALP levels significantly (p<0.05) decreased only in the groups treated with HON and CW+HON synergistically compared with CONT. However, treatment with CW+HON significantly decreased AST, ALT and ALP compared with treatment with CW and HON only (p<0.05). Table 2.

Table 2: Albumin, AST, ALT and ALP levels in Sprague-Dawley rats treated with CW, HON, and CW+HON

Parameters	CONT	CW	HON	CW+HON
Albumin (g/l)	32.24±0.70	33.46±0.29	34.10±0.58	34.88±0.38$^{\#}$
AST (u/l)	242.3±4.47	209.9±1.45$^{\#}$	230.0±2.04$^{\#a}$	189.8±2.06$^{\#a1}$
ALT (u/l)	70.20±0.68	73.56±1.01$^{\#}$	62.40±0.74$^{\#a}$	61.87±0.64$^{\#a}$
ALP(u/l)	260.6±4.51	272.6±2.56	251.09±1.65	216.9±2.08$^{\#a1}$

Values represent Mean ± SEM, **n=6**

Significant ($^{\#}$p<0.05 vs. CONT, ^{a}p<0.05 vs CW, ^{1}p<0.05 vs HON)

Synergistic effects of CW and HON on GSH, SOD, CAT activities

We investigated the effect of CW, HON and both synergistically on GSH, SOD and CAT activities. GSH and SOD significantly increased (p<0.05) in all the groups treated with CW, HON

and both synergistically compared with CONT. Treatment with CW+HON produced a significant increase in GSH activity compared with treatment with CW and HON only (p<0.05). CAT remained unchanged when treated with CW and HON. A significant increase (p<0.05) was observed in the group treated synergistically with CW and HON. Figures 5a, b, c and d.

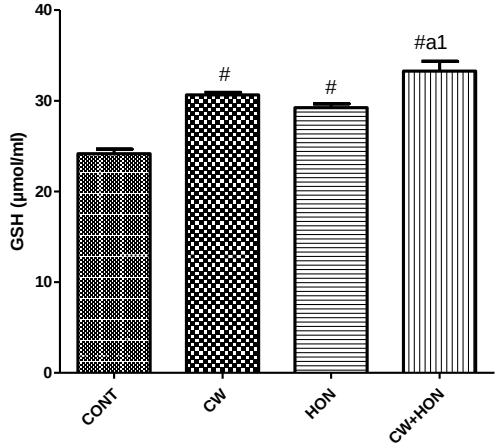

Figure 5a: activity in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6. Significant ($^{\#}$p<0.05 vs. CONT, ^{a}P<0.05 vs CW, ^{1}p<0.05 vs HON)

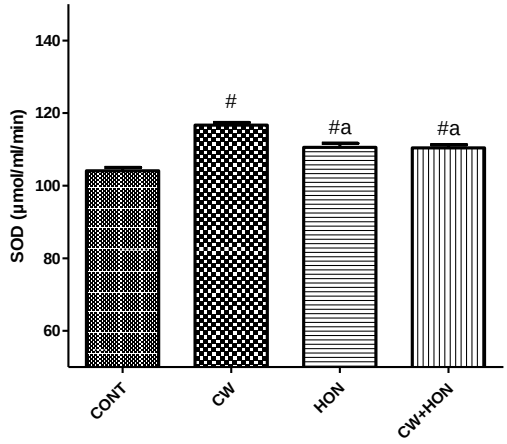

Figure 5b: SOD activity in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6. Significant ($^{\#}$p<0.05 vs. CONT, ^{a}p<0.05 vs CW)

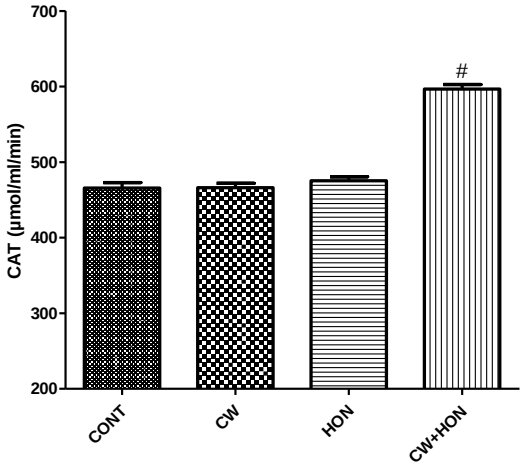

Figure 5c: CAT activity in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, **n=6. Significant ($^{\#}$p<0.05 vs. CONT)**

Synergistic effects of CW and HON on MDA level

We investigated the synergistic effect of CW and HON on MDA. Figure 6 shows a significant decrease(p<0.05) in all the treated groups with CW, HON and both synergistically compared with CONT.

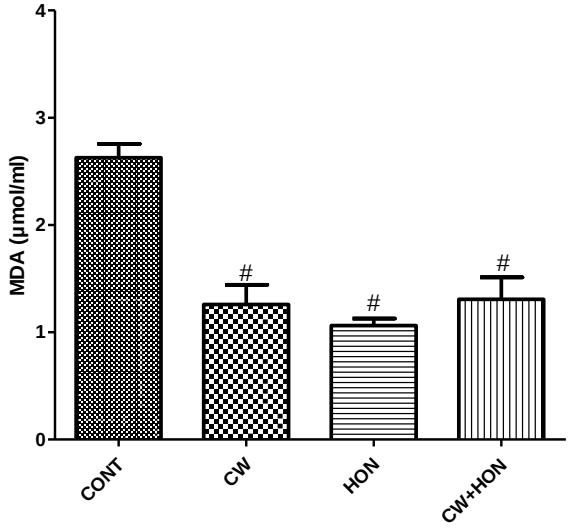

Figure 6: CAT activity in Sprague-Dawley rats treated with CW, HON and CW+HON

Values represent Mean ± SEM, n=6. Significant ($^{\#}p<0.05$ vs. CONT)

DISCUSSION:

Together with effective medical treatment, using recipes of traditional medicine, including the use of coconut water and apicultural products (i.e., honey), humans can maintain the normal level of blood glucose and also their overall health condition.

Treatment with CW, HON and both synergistically demonstrated decreased FBG which is an indication of hypoglycaemia. The current study agrees with hypoglycaemia effect of coconut water in diabetic rats[12]. Honey was demonstrated to have hypoglycemic effect in healthy animals[9]. Previous studies have demonstrated that fructose content of honey varies from 21 to 43% and the fructose/glucose ratio from 0.4 to 1.6 or even higher[17]. Although fructose is the sweetest naturally occurring sweetener, it has a glycemic index of 19, compared to glucose which has 100 or sucrose (refined sugar) with 60. It was suggested that fructose, selective mineral ions (selenium, zinc, copper, and vanadium), phenolic acids, and flavonoids might have a role in the process[9].

Furthermore, the current study demonstrated improved glucose storage as the treatment with CW, HON and both synergistically increased hepatic and skeletal glycogen content. Fructose stimulates glucokinase in hepatocytes, which plays an important role in the uptake and storage of glucose as glycogen by the liver. Glucose on the other hand, which is present beside fructose in honey, enhances the absorption of fructose and promotes its hepatic actions through its enhanced delivery to the liver[18,19].

The liver is the largest gland and major metabolic organ, and among its several functions, is the production of glucose (via gluconeogenesis and glycogenolysis) and its release into circulation[20].Taken together, it is a principal organ in controlling blood sugar, because an imbalance in glucose release from the liver and uptake from peripheral tissues can lead to the persistent hyperglycaemia; a major contributing factor to the development of diabetes[21]

Treatment with CW, HON and both synergistically decreased CHOL and TG levels suggesting hypocholesterolemia and hypotriglyceridemia. Previous studies have demonstrated that honey provided antihypercholesterolemia and antihpertriglyceridemia in rats[22-24]. Coconut

water has also been reported to possess cholesterol-lowering effect in rats[25]. Phenolic-rich compounds have been shown to inhibit hyperlipidemia[26,27] and previous studies have shown that honey is rich in phenolic acids and flavonoids[28].

Therefore, the effect of honey in suppressing hypercholesterolemia and hypertriglyceridemia may be attributed to honey phenolic and flavonoid content. Honey is enriched in numerous bioactive substances including phytosterols which have been shown to enhance cholesterol metabolism[29]. Epidemiological evidence associates high concentrations of HDL cholesterol with several health beneficial effects including antiatherogenic effect, inhibition of LDL oxidation and healthy endothelial function[29].

Administration of CW, HON and both synergistically decreased creatinine and urea level suggesting effective kidney function. Urea and creatinine are both indices of renal functions. Coconut water and honey has been shown to poses reno-protective effect in diabetic Wistar rats[30] and prevents renal changes in monosodium glutamate-treated Wistar rats[31].
Furthermore, the current studies showed a significant decrease in liver enzymes' albumin, AST, ALT and ALP. Decreased activity of these enzymes is an indication of effective liver functions suggestive of a beneficial effect of CW and HON. In agreement with the current findings, pervious study has demonstrated heptoprotective effect of CW and HON[31].

Oxidative stress is due to the imbalances between antioxidant defense system with free radicals due to the ROS increase and caused hyperglycaemia[32]. Oxidative stress has been recently recognized as a key mechanism in insulin resistance[33]. Some of the mechanisms of reaction which are considered to be involved in oxidative stress Genesis are auto-oxidation glucose, protein glycation, formation of advanced glycation products and *polyol* pathway. It is associated with several diseases including diabetes mellitus and obesity and is considered a potential therapeutic target in these disorders[28]

This research study explains the effects of CW and HON on oxidative balance. There was a significant increase in GSH, CAT, SOD levels ($p<0.05$) and a concomitant significant decrease in lipid peroxidation's index MDA level. This suggest the synergistic effect of both coconut water and honey in preventing lipid peroxidation, an index of oxidative stress development. Previous in vitro studies on antioxidant properties of honey showed that honey possesses phenolics and flavonoids contents as well as the best free radical scavenging properties[10,11]. [12]Earlier reported that coconut water decreased oxidative stress by lowering lipid peroxidation in diabetic rats. Hence, the presence of these compounds may be responsible for the synergistic effect observed in the current study. The current study also agrees with the previous study by[33] on the antioxidant properties of honey.

Conclusion:

In conclusion, coconut water and honey co-administered in synergy exerts profound hypoglycemic effect and increased hepatic glucose storage possibly mediated by the stimulation of glucokinase by fructose in hepatocytes. This may play an important role in the uptake and storage of glucose as glycogen by the liver. Both possess antihypertriglyceridemic effect. Honey and coconut water administered in synergy may be hepato-reno protective. Improved oxidative balance observed is an attestation to the antioxidant properties of coconut water and honey.

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