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HEPATOPROTECTIVE POTENTAL OF HONEY, COFFEE AND VITAMIN E IN MALE WISTAR RATS

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ABSTRACT
This research was carried out to determine the potential hepatoprotective effect of honey, coffee and vitamin E on normal male wistar rats. A total of 30 healthy male wistar rats were randomly divided into six (6) groups with five (5) rats in each. Treatments include vitamin E 0.15ml, honey 2ml, coffee 1.6ml; honey 2ml co-administered coffee 1.6ml, honey 2ml co-administered vitamin E 0.15ml. The experimental period lasted for 56 days. Liver function markers assayed for include; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (AP), bilirubin, total bilirubin and conjugated bilirubin. From the outcome of this research, honey significantly reduced liver enzyme markers, bilirubin, conjugated and total bilirubin but significantly increased total proteins compared to both control and vitamin E treatment. Coffee increased the blood level of liver enzymes, bilirubin, conjugated and total bilirubin but significantly reduced total proteins compared to both control and vitamin E treatment. Vitamin E and honey co-administered vitamin E significantly increased the total proteins in blood, but significantly reduced liver enzymes, bilirubin, conjugated and total bilirubin compared to control. Honey and vitamin E or co-administration of both may possess therapeutic organic and inorganic constituents that have hepatoprotective function. Coffee, except co-administered honey, may have an adverse effect on liver function.

KEYWORDS: Liver function markers, Honey, Coffee, Vitamin E, Hepatoprotective.

INTRODUCTION
Our daily exposure to both endogenous and exogenous toxins exposes our vital organs to physiologic changes and pathologic complications.[1] The liver has metabolic[2], protective[3] and homeostatic functions[2], that species survival without it is impossible. What humans ingest is usually metabolized by the liver[1], and some of these ingested agents may as well affect liver function.[5][6] Liver function test can be used to determine the functional state of the liver in both physiologic and pathologic conditions.[8] Honey may have organic therapeutic agents[12], but its effect on the liver is still uncertain. Coffee may have controversial effect on blood glucose regulation[18] and liver function[17] [19], but the underlying mechanism behind such controversy is still unknown. Vitamin E, a known antioxidant[20], may have the ability to mop up reactive oxygen species[21], but if this mopping action is beneficial to the liver is yet to be known.

MATERIALS AND METHODS
Purchase of treatment agents
Fresh honey was purchased from University of Port Harcourt food and drugs facility. The purity of the honey was authenticated in the laboratory for Microbiological sciences, Department of Microbiology, Faculty of Natural Sciences; University of Port Harcourt. Coffee was purchased from an online retail outlet. Vitamin E was purchased from Ebus Pharmacy in Port Harcourt.

Experimental animals and protocols
Thirty (30) adult male wistar rats weighing 200 to 220 grams were obtained from the Experimental animal unit, Department of Human Physiology, University of Port Harcourt. Using the standard protocols[26], all experimental animals were properly screened and confirmed by a Vertinarian in the institution to be physically healthy. With simple random technique of sampling, the animals were divided into four (6) groups containing six (5) rats per group. The animals were allowed to acclimatize for 2 weeks before the start of the

experiment which lasted for 56 days. All animals had access to food and water *ad libitium*. The cages were properly cleaned twice daily to avoid coprophagy.

Table 1; Study design.

Groups	Treatments	Dose	Days	Experimental periods
1	Normal saline	*Ad libitium*	0	before onset of treatment
2	Vitamin E	0.15ml	18	18th day after onset of treatment
3	Honey	2ml	37	37th day after onset of treatment
4	Coffee	1.6ml	56	56th day after onset of treatment
5	Honey + Coffee	2ml+1.6ml		
6	Honey + Vitamin E	0.15ml+1.6ml		

The normal rat feed from Anifeed® industries, containing carbohydrate, proteins and fat, in a balanced ratio, was used as feed throughout this research.

Treatment method

All treatment agents were administered via orogastric route using cannulated tube, following standard laboratory procedures.[15]

Collection of blood samples

Blood was collected four (4) times in four (4) different days of equal interval. The collection of blood samples include;

LIVER FUNCTION TESTS

Experiment to determine the liver function biomarkers were carried out using the standard laboratory procedures.[14] The biomarkers tested for include; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total proteins (TP), bilirubin and albumin.[19] This test was carried out on blood samples collected on day 56 of the experimental period.

Test for alanine transaminase (ALT) and aspartate transaminase (AST)

Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the reitman and frankel calorimetric method[17] in which 0.2 ml of serum reacted with 1ml of substrate (Aspartate and α-ketoglutarate for AST, while alanine and α-ketoglutarate for ALT, in phosphate buffer pH 7.4) and was incubated for an hour in the case of AST and 30 minutes for ALT. then 1ml of DNPH (Dinitrophenyl-hydrazine) solution was added to arrest the reaction and kept for 20 minutes in room temperature. After incubation, 1 ml of 0.4 N NaOH was added and absorbance was read at wavelength of 540nm.

Test for alkaline phosphatase (ALP)

Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified condition.

The principle in the test includes;

ALP

ρ- Nitro phenyl Phosphate + H_20 ----------> ρ-Nitro phenol + H_3PO_4

P-Nitro phenyl Phosphate is hydrolyzed to ρ-Nitro phenol and inorganic phosphate. The rate at which the ρ-Nitro phenol Phosphate is hydrolyzed, measured at 405nm, is directly proportional to the alkaline phosphatase activity.

Test for total protein (TP)

The assay is based on a polypeptide chelation of cupric ion (colored chelate) in strong alkali. In general, biuret assays are useful for samples containing -1 to 10 mg protein/ml, which is diluted - 5-fold by the added reagent to give a concentration of 0.2 to 2 mg/ml final assay volume (F.A.V.). Most proteins produce a deep purple color, with a maximum absorbance (λmax) at about 550nm.

Test for bilirubin

Method of estimation of bilirubin in serum was based on an indirect reaction method of Van den Berg: the bilirubin in serum reacted with a freshly prepared solution of Van den Berg's diazotized sulphonilic acid (0.5 ml). Afterwards, purple colored azobilirubin compound was formed which was measured at a wavelength of 540nm. This color was observed after the addition of methanol and serum was diluted with distilled water, (0.2 ml + 1.8 ml distilled water) (Klot, 2005).

Test for albumin

A bromocresol green (BCG) dye binding procedure was first proposed in 1964.[16] This procedure exhibited greater sensitivity and much lower susceptibility to interfering substances.[15] Albumin is bound by the BCG dye to produce an increase in the blue-green color measured at 630nm. The color increase is proportional to the concentration of albumin present.

Ethical Approval

This study was approved by Madonna University Research Ethics Committee. All experimental procedures were done strictly following the guidelines provided by the research ethics committee. The animals were sacrificed after exposure to diethyl ether according to EC directives 86/609/EEC. In addition, the laid down standards according to the 1964 declaration of Helsinki were strictly adhered to.

Statistical Analysis

Experimental data are presented in Mean±SEM.

Percentage (%) change was also calculated to make the data well translated. SPSS 20.0 was used for all calculations and statistical analysis such as One-way analysis of variance (ANOVA). Values are significant at $p \leq 0.05$ or at confidence interval of 95%.

RESULTS

Table 2: Effect of honey, coffee and vitamin E on liver enzymes.

Treatments	AST (IU/L)	%	ALT (IU/L)	%	ALP (IU/L)	%
Control	42.8±0.37	-	35.8±0.58	-	34.6±1.40	-
Vit E	33.4±0.75[a]	-22	31.6±0.68[a]	-11.7	26.0±1.41[a]	-25
Honey	25.6±1.54[ab]	-40.1	28.6±0.68[ab]	-20.1	23.0±0.71[ab]	-34
Coffee	46.4±1.03[a]	8.41	41.8±1.32[ab]	17	42.6±±1.47[a]	23.1
Honey+Cof	36.2±0.37[a,b]	-15.4	29.6±2.25[a,b]	-17.3	32.2±1.11[b]	-7
Honey+Vit E	24.6±0.87[ab]	-42.5	22.6±0.81[ab]	-37	18.8±0.66[ab]	-46

N=5

a,b $p<0.05$were considered significant compared with control and vitamin E group respectively

Table 3: Effect of honey, coffee and vitamin E on liver biomarkers.

Treatments	Total protein (mg/dL)	%	Bilirubin (mg/dL)	%	Total bilirubin (mg/dL)	%	Conjugated bilirubin (mg/dL)	%
Control	93.8±1.36	-	35.2±0.49	-	12.5±0.16	-	11.2±0.08	-
Vit E	91.0±1.38	-3	30.8±0.37 [a]	-13	10.2±0.20 [a]	-18.4	9.2±0.24[a]	-18
Honey	98.4±0.40[a]	5	26.2±1.53 [ab]	-26	11.1±0.05 [a]	-11.2	7.5±0.28[ab]	-33
Coffee	82.8±0.74[a]	-12	38.0±0.63 [a]	8	13.2±0.54[b]	5.6	12.8±0.15[a]	14.2
Honey+Cof	85.4±1.60[a]	-9	33.2±0.74 [ab]	-6	11.7±0.24 [a,b]	-6.4	9.7±0.49[a,b]	-14
Honey+Vit E	98.2±0.58[a]	5	29.4±0.81 [ab]	-16.4	9.8±0.22 [a]	-21.6	5.7±0.51[ab]	-50

N=5

a,b $p<0.05$were considered significant compared with control and vitamin E group respectively

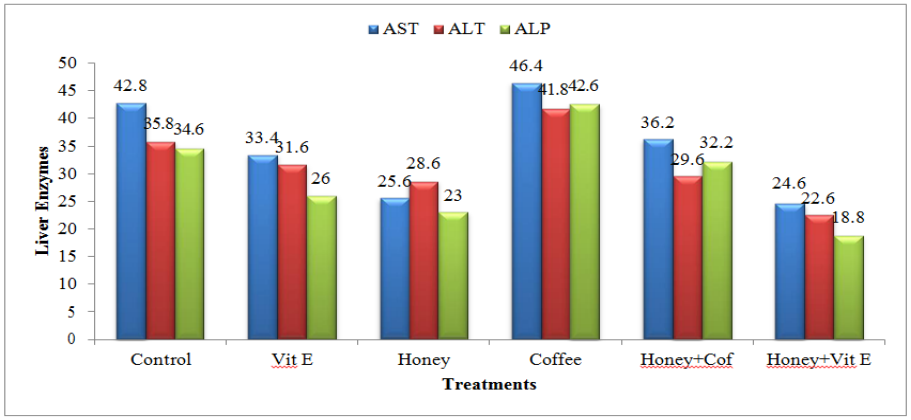

Figure 1: Effect of honey, coffee and Vitamin E on liver enzymes.

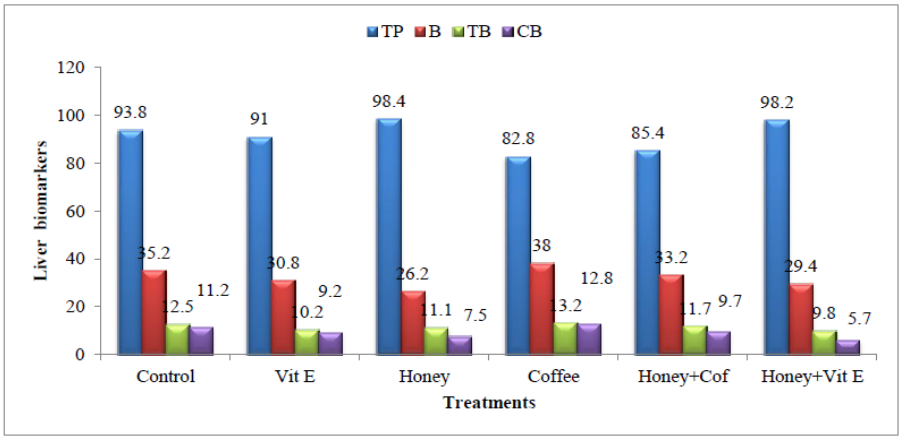

Figure 2: Effect of honey, coffee and Vitamin E on liver biomarkers.

From table 2 and 3

Honey significantly (P≤0.05) reduced blood level of liver function enzymes ALT (28.6±0.68[a]), AST (25.6±1.54[a]) and ALP (23.0±0.71[a]) compared to both control and vitamin E. honey also significantly increased blood level of total proteins (98.4±0.40[a]) but significantly reduced blood bilirubin(26.2±1.53 [a]), conjugated(7.5±0.28[a]) and total bilirubin (11.1±0.05[a]).

Coffee significantly (P≤0.05) increased blood level of liver function enzymes ALT (41.8±1.32[a]), AST (46.4±1.03[ab]) and ALP (42.6±±1.47[ab]) compared to both control and vitamin E. Coffee also significantly reduced blood level of total proteins (82.8±0.74[a]) but significantly reduced blood bilirubin(38.0±0.63 [a]), conjugated(12.8±0.15[a]) and total bilirubin (13.2±0.54).

Honey co-administered coffee significantly (P≤0.05) decreased blood level of liver function enzymes ALT (29.6±2.25[a,b]), AST (36.2±0.37[a,b]) and ALP (32.2±1.11[b]) compared to both control and vitamin E. This combined treatment also significantly reduced blood level of total proteins (85.4±1.60[a]), blood bilirubin (33.2±0.74 [b]), conjugated (9.7±0.49[a,b]) and total bilirubin (11.7±0.24a,b).

Honey co-administered vitamin E significantly (P≤0.05) reduced blood level of liver function enzymes ALT (22.6±0.81[ab]), AST (24.6±0.87[ab]) and ALP (18.8±0.66[ab]) compared to both control and vitamin E. This combined treatment also significantly increased blood level of total proteins (98.2±0.58[a]) but significantly reduced blood bilirubin (29.4±0.81 [a]), conjugated (5.7±0.51[a]) and total bilirubin (9.8±0.22 [a]).

DISCUSSIONS

This research studied the potential of honey, coffee and vitamin E to be effective in the management of changes in relation to liver function. From the outcome of this research, honey significantly reduced the blood level of the liver enzymes alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). This may be due to the organic therapeutic biomolecular agents in honey like kaempherol[10], quercetin[10][11], chrysin, luteolin[14], apigenin[7] and vanillic acid[13]. These organic agents have been reported to be of importance in hepatic and biliary medicine.[14] They probably reduce these liver enzymes by maintaining the biomembrane integrity, combating free radical species and regulating the metabolic processes of the liver[13]. Honey also increased total protein (TP) content in blood. This may infer that the synthetic function of the liver in relation to albumin, immunoglobulins and bilirubin was also enhanced. Vitamin E significantly reduced the blood level of liver enzymes. Vitamin E may probably prevent the oxidative modification of the assayed liver enzymes[22] or may have prevented hepatic biomembrane peroxidation[21]. Vitamin E may also be beneficial in regulating bilirubin level in blood. This is in agreement with earlier studies.[23] Coffee alone increased blood level of liver enzymes, total and conjugated bilirubin but when co-administered with honey showed marked decrease in liver enzymes, total and conjugated bilirubin. Coffee may possess adverse hepatic phytoconstituents. Honey co-administered vitamin showed most significant decrease in liver function biomarkers except total protein. This reveals that the active constituents in honey can further potentiate the effect of vitamin E on total proteins. Honey contains vitamin E.[11], which means it may have increased the dose of the antioxidant vitamin administered in this study, and further potentiating the antioxidant protection of total proteins.

CONCLUSION

From this research, honey and vitamin E or co-administration of both may have significant hepatoprotective potential, but coffee, except co-administered with honey, may have adverse effect on liver function.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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