

EFFECTS OF THE INTAKE OF NATURAL COCOA POWDER ON SOME BIOCHEMICAL AND HAEMATOLOGICAL INDICES IN THE RAT

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SUMMARY

Background: Natural cocoa and cocoa products are increasingly attracting the attention of many investigators and the general public because of their potential nutritional and medicinal properties and other claims.

Objective: This study sought to evaluate the effect the consumption of natural cocoa powder has on some biochemical and hematological indices in the rat, as a way of establishing the biochemical basis for some of the claims made for the consumption of cocoa and its products.

Methods: Male Wistar albino rats were fed natural cocoa powder in an aqueous suspension for 48 days, Biochemical and haematological indices were then determined from blood samples.

Results: The treatment had no significant effect on ALT, AST, ALP, uric acid, total protein, haemoglobin and haematocrit levels. However, there were significant reductions in the total cholesterol level (2.52 ± 0.07 mmol/L) versus (1.88 ± 0.23 mmol/L), LDL – cholesterol level (1.09 ± 0.03 m mol/L) versus (0.74 ± 0.06 m mol/L), and in triglyceride level (1.28 ± 0.15 m mol/L) versus (1.08 ± 0.04 m mol/L) after treatment ($p < 0.05$). The results further indicated significant increases in white blood cell ($7.53 \pm 0.19 \times 10^3/\text{mm}^3$) versus ($10.40 \pm 1.66 \times 10^3/\text{mm}^3$) and platelet counts ($379 \pm 112.0 \times 10^3/\text{mm}^3$) versus ($583.8 \pm 11.4 \times 10^3/\text{mm}^3$).

Conclusion: The administration of natural cocoa powder to rats caused significant reductions in total serum cholesterol levels, LDL-cholesterol levels and triglycerides with a significant increase in white blood cell counts.

Keywords: Cocoa powder, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lipid profile.

INTRODUCTION

Claims for the medicinal capabilities of cocoa include, treatment of heart pain, shortness of breath, anaemia, burns, snakebite and wounds, angina, lowering of blood pressure, improving the efficiency of insulin action and anti-inflammatory properties amongst oth-

ers.¹ These medicinal properties have long been associated with the polyphenolic compounds which give flavor and color to chocolate.² Cocoa polyphenols (flavanols) have been reported to have a wide range of biological properties including modulating eicosanoid synthesis, increasing nitric oxide synthesis, lowering the rate of LDL-cholesterol oxidation, inhibiting platelets activation, stimulating the production of anti-inflammatory cytokines among others.^{3,4}

By helping to protect tissues against stress, certain polyphenols work as preventive medicines for problems such as cardiovascular diseases, cancer, arthritis and autoimmune disorders.⁵ They act as antioxidants due to their free radical scavenging properties, their ability to reduce the formation of free radicals and their ability to stabilize membrane by decreasing membrane fluidity.^{6,7} Among botanical medicines, cocoa, ginkgo, elderberry and green tea are examples of rich sources of antioxidant polyphenols.⁸ Some polyphenols (such as proanthocyanidins) exert beneficial cardiovascular effects through inhibition of platelet aggregation.⁹

Excess amounts of these polyphenols could theoretically extend blood clotting times.¹⁰ Examples of polyphenolic compounds present in cocoa are the flavan-3-ols or flavanols, which include the monomeric forms, (-) - epicatechin and (-) - catechin, and the oligomeric forms of the monomeric units, the procyanidins.^{11,12}

There have been calls for the use natural cocoa and its products for various benefits. According to Addai,¹³ the regular intake of cocoa and chocolate is a must for healthy living. While the intake of cocoa is not good for people with very low blood pressure, it could reduce the rate of diabetes and hypertension, and could reduce the risk of getting stroke and aneurysm.¹³ The objective of this investigation was to evaluate the effect the consumption of natural cocoa powder has on some biochemical and haematological indices in the rat, as a way of establishing the biochemical basis for some of the claims made for the consumption of cocoa and its products.

Biochemical parameters investigated included lipid profile, which helps to determine risk of coronary heart disease; serum proteins to ascertain how the liver and kidney are functioning, development of infection and fluid collection among others. Uric acid is needed to monitor how quickly cells are broken down in the body or inefficiencies in uric acid metabolism. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) are used to evaluate hepatocellular function, detect and monitor cardiac disease as well as bone integrity.

Haematological indices (full blood count), gives or provides valuable information about the blood and the bone marrow. This information is useful in diagnosing anaemia, infection and other diseases associated with blood cell function.¹⁴

METHOD

Animals and treatment

Male Wistar albino rats (4–5 weeks old) weighing between 71 and 116 grams obtained from the departmental animal house were used. The animals were housed (5 rats to a stainless steel cage) in a well ventilated room and allowed free access to food and water. In addition to their food and water the experimental group was fed 1ml of an aqueous cocoa suspension each day for 48 days by force feeding / oral gavage.

The cocoa suspension was prepared by dissolving about four teaspoonfuls of Venaco natural cocoa powder in 1L of water and mixed thoroughly. The resulting suspension was kept in a refrigerator and various portions were taken daily, brought to room temperature and given to the animals as described earlier. The control animals were given the same volume of water over the same period by the same method.

Blood sampling

Approximately 1mL of blood was drawn from the tail vein of the rats under ether anaesthesia. Blood was allowed to clot and centrifuged to obtain haemolysis-free serum which was used for biochemical analyses which required serum. About 0.5mL blood was also collected into vials containing EDTA and used as specimen for haematological analyses which required whole blood.

Biochemical analyses

Biochemical parameters were investigated at the University of Cape Coast Hospital Laboratory, using a Biolabo Diagnostic Auto Analyzer (Biolabo Diagnostics) from Kenza Biochemistry, France. The haematological indices were evaluated using a fully automated ABX, PENTRA equipment from Horiba Abx Diagnostics also at the University of Cape Coast Hospital Laboratory. Baseline determinations were made on the blood samples from a random selection of animals before they were put into the respective treatment groups.

Data analysis

All the data for the biochemical parameters and haematological indices were compared for differences or otherwise between experimental and control groups after the 48 day period, using ANOVA. Results presented are the means \pm standard error of the mean ($n = 6$ in all cases).

RESULTS

Table 1, which presents data on the biochemical investigations, shows a significant 25.4% decrease in the total cholesterol of the experimental as compared to the control, a 32.1% decrease in LDL-cholesterol of experimental as against the control, and a 15.7% decrease in the triglycerides of experimental with respect to the control. Differences observed in other parameters were not significant.

Table 1 Values obtained for the biochemical parameters

Parameter	Baseline	Control	Experimental
Total cholesterol (mmol/L)	2.19 \pm 0.19	2.52 \pm 0.07	1.88 \pm 0.23*
Uric acid (mg/L)	2.90 \pm 1.24	15.0 \pm 2.77	18.27 \pm 2.88
Total protein (g/dL)	7.08 \pm 0.41	7.60 \pm 0.44	7.45 \pm 0.25
HDL-cholesterol (mmol/L)	1.27 \pm 0.08	1.19 \pm 0.03	0.92 \pm 0.16
LDL-cholesterol (mmol/L)	0.90 \pm 0.10	1.09 \pm 0.03	0.74 \pm 0.06*
Triglycerides (mmol/L)	1.30 \pm 0.18	1.28 \pm 0.15	1.08 \pm 0.04*
Alanine aminotransferase (U/L)	75.67 \pm 5.86	71.83 \pm 5.44	74.50 \pm 6.39
Aspartate aminotransferase (U/L)	219.83 \pm 3.86	215.17 \pm 3.57	223.17 \pm 4.68
Alkaline Phosphatase (U/L)	798.70 \pm 72.20	761.50 \pm 61.60	747.30 \pm 66.50

*(significant. $p < 0.05$)

Table 2: Values obtained for haematological parameters.

Parameters	Baseline	Control	Experimental
Red blood cell count ($10^6/\text{mm}^3$)	8.17 ± 0.13	8.10 ± 0.21	$7.39 \pm 0.11^*$
White blood cell count ($10^3/\text{mm}^3$)	9.13 ± 1.01	7.53 ± 0.19	$10.40 \pm 1.66^*$
Platelets ($10^3/\text{mm}^3$)	475.3 ± 68.4	379 ± 112.0	$583.8 \pm 11.4^*$
Haemoglobin (g/dL)	14.38 ± 0.39	14.6 ± 0.18	13.75 ± 0.27
Mean corpuscular volume (μ/m^3)	52.25 ± 2.06	53.50 ± 1.32	54.50 ± 0.65
Mean corpuscular haemoglobin (pg)	17.60 ± 0.69	18.20 ± 0.53	18.55 ± 0.29
Mean corpuscular hemoglobin concentration (g/dL)	33.83 ± 0.15	33.98 ± 0.25	34.00 ± 0.27
Haematocrit (%)	42.45 ± 1.07	35.53 ± 7.65	40.33 ± 0.79

*(significant: $p < 0.05$)

Table 2 has data on the haematological determinations made, and indicates a significant 27.6% increase in white blood cells of experimental over the control, a 35.1% increase in platelets of experimental over control, and a further 8.76% decrease in the red blood cells of experimental compared to the control rats. Differences in the other parameters were not significant.

DISCUSSION

The present investigation has provided information on some biochemical and haematological effects that have resulted from the administration of natural cocoa powder to rats. The highlights include the significant reductions in total cholesterol, LDL-cholesterol and triglycerides. Further, there were significant increases in white blood cell and platelet counts.

The significant decrease in the total cholesterol levels of the experimental rats (Table 1) may have resulted from the antioxidant properties of the polyphenols in cocoa.¹⁵ These antioxidants have the ability to increase the synthesis of nitric oxide which has the ability to cause vasodilation, resulting in the clearance and prevention of the deposition of excess cholesterol in the blood vessels.¹⁶ The beneficial effects of nitric oxide modulation include the regulation of blood pressure, lowering of NO-affected hypercholesterolemia and monocyte adhesion, all of which are involved in the progression of atherosclerosis.¹⁶

The non-significant difference in total plasma protein indicated that cocoa has little or no effect on total plasma protein concentration. Since plasma proteins are produced in the liver, these results thus indicate that

the administration of cocoa does not affect this aspect of liver function.

Although uric acid levels of both experimental and control animals were elevated above that of the baseline values by the end of the treatment period, the difference between the experimental and the control group was not significant. This suggests that cocoa has no effect on the uric acid turn over.

Although a high level of uric acid is known to cause gout, uric acid or (urate) also plays a beneficial role by acting as a potent antioxidant.¹⁷ Urate is a very efficient scavenger of highly reactive and harmful oxygen species which include hydroxyl radicals, superoxide anion, singlet oxygen and oxygenated heme intermediates in high valence states.¹⁷

The significant decrease in the LDL-cholesterol (Table 1) of the experimental group indicates a possible modulation by cocoa on LDL-cholesterol. Flavanols in cocoa are able to cause the modulation and prevent the oxidation and increase in LDL-cholesterol, which could put a subject at a higher risk of coronary heart disease.^{18,19} This prevention of the oxidation of LDL-cholesterol is related to the mechanism of protecting the heart against heart disease. Since low levels of blood triglycerides help prevent diseases like stroke and hypertension, the reduced blood triglycerides observed, will enhance a healthy living. Stearic acid which is a saturated fatty acid abundant in cocoa is easily converted to oleic acid – a monounsaturated fatty acid, which thus causes no health problem.²⁰

Stearic acid is also reported to cause the reduction of plasma cholesterol by limiting its absorption and enhancing the excretion of endogenous cholesterol.²¹

Over the period of administration of cocoa to the experimental animals, no significant changes were observed in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase are used to identify hepatocellular disease, evaluate hepatocellular and cardiac disease and to detect and monitor diseases of the liver or bone respectively.¹⁵ Injury or disease affecting these vital organs results in the release of these enzymes into the bloodstream, thus elevating their levels. It is seen that the treatment did not affect these serum enzyme levels meaning that the administration of cocoa had no detrimental effect on the functioning of the organs involved.

Although cocoa is known to contain appreciable amounts of iron, there was a significant reduction in the red blood cells of the experimental animals (Table 2). Absorption of iron from cocoa which is a (nonheme iron source) is not high, since the absorption of nonheme iron is less efficient as compared to heme iron sources.²² Also, polyphenols are known to decrease absorption of nonheme iron.²³ The addition of pork or meat to a diet enhances the absorption of nonheme iron.²⁴ The effect on the red blood cells notwithstanding, haemoglobin levels were not significantly affected. Perhaps a more prolonged intake of cocoa could have an effect that may be worth further investigation.

The significant increase in white blood cells of the experimental rats meant that the administration of cocoa could boost the immune system, since these cells are the most important cells responsible for the protection and fighting of infection. This supports and explains an earlier observation by Addai²⁵ that cocoa promotes superlative health by strengthening the immune system. Cocoa powder prevents many diseases particularly viral ailments.²⁵

Polyphenols in cocoa are known to inhibit the activation of platelets.⁹ Increased platelet count (thrombocytosis) may be seen in individuals who may not show any significant medical problems, while others may have a more significant blood problem. Some subjects with an increased number of platelets may have a tendency to bleed due to lack of stickiness between the platelets. In others, the platelets retain their stickiness and, because of their increase in number, tend to stick to each other forming clumps that can block vessels and cause death through thromboembolism.²⁶ In view of this possible danger, the increase in platelet count

observed may need further investigation that may inform any necessary intervention in situations where natural cocoa powder is being consumed.

CONCLUSION

The effect of the administration of natural cocoa powder on some biochemical and haematological indices has been investigated in the rat. Cocoa Powder caused significant reductions in the total cholesterol, LDL-cholesterol, triglycerides, red blood cells and significant increases in white blood cells and platelets of rats.

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