Antigenotoxicity Screening of Coffee (*Coffea arabica* Linn) and Cacao (*Theobroma cacao* Linn.)

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Abstract - *This study evaluated the potential antigenotoxicity of coffee and cacao against tetracycline-induced genotoxicity in Swiss albino mice. Laid out on a Completely Randomized Design, eight treatments replicated three times were randomly distributed to 24 caged mice. The experimental treatments were as follows: Treatment 0, Pure distilled water (Negative control); Treatment 1, Roasted coffee plus tetracycline; Treatment 2, Unroasted coffee plus tetracycline; Treatment 3, Instant coffee plus tetracycline; Treatment 4, Roasted cacao plus tetracycline; Treatment 5, Unroasted cacao plus tetracycline; Treatment 6, Instant cacao plus tetracycline; Treatment 7, Tetracycline (Positive control).*

Micronucleus test was used to assess the inhibitory effect of coffee and cacao prepared extracts. DMRT was used to assess the significant differences among treatment means while Dunnett's test was used to compare experimental treatments against positive and negative controls.

ANOVA showed significant differences among treatments at 5% level of significance. Unroasted coffee possessed the highest antigenotoxic potential while instant coffee had the least among the coffee extracts. In contrast, instant cocoa had the highest antigenotoxic potential while unroasted cocoa had the least among the cocoa extracts. Both coffee and cacao extracts are effective in significantly reducing ($p<0.05$) the formation of micronuclei in polychromatic erythrocytes of the tetracycline-treated albino mice.

Keywords-*Antigenotoxicity, tetracycline, mutagenic, genotoxins*

INTRODUCTION

It is indeed a particular concern of many biological scientists/researchers and medical practitioners to lessen the risk of suffering from DNA damage, since DNA is susceptible to various types of mutagenic and carcinogenic substances present in the environment. If the structural alterations in the DNA of somatic cells are not repaired, cancer would be induced. Although this damage is not transmissible, it may be cumulative and produce severe changes that may result to the death of the individual, depending on the extent of toxicity and the time at which the consequences occur [4].

Somatic mutation, chromosomal aberrations and tumor cells are administered by several kinds of toxic chemicals that attack cells. These are genotoxins, substances that alter the structure of DNA, the genetic substance of the living cell. Mis-pair, loss or damage of the coded information in the DNA is dangerous for all cells. Genotoxins such as benzo(a)pyrene, dimethylnitrosamine, urethane, methylnitronitrosoguanidine, ethyl methanesulfonate and tetracycline are group of well-established mutagenic/carcinogenic chemicals that usually trigger genotoxicity in genes [1]. These fragmented the chromatin material of bone marrow cells, thus, inducing the formation of micro nucleated polychromatic erythrocytes [12].

Antigenotoxins are substances that can reduce and suppress the activity of genotoxins. Genotoxicity and antigenotoxicity can be determined in in vivo using the Micronucleus Test. The in vivo mammalian micronucleus test [10], which detects the damage of chromosomes or mitotic apparatus caused by chemical, is used to examine the chromosome-damaging effect of the test agent.

Coffee consumption has been proven to reduce the risk of liver cirrhosis by 80%, to help manage asthma and even control attacks when medication is unavailable, and in moderation to decrease the risk of developing acute coronary disease [3]. Cacao on the other hand, reported to be antiseptic, diuretic, ecbolic, emmenagogue, and parasiticide, is a folk remedy for alopecia, burns, cough, dry lips, fever, listlessness, malaria, nephrosis, parturition, pregnancy, rheumatism, snake bite, and wounds [5]. These plants

may not only be beneficial for their economic value and medicinal contribution, but may also be utilized as antigenotoxic agents [1]. This study may contribute in generating a possible protective agent that may help lessen the genotoxic activity of certain mutagenic and carcinogenic substances present in human environment known to alter the structure of DNA.

This research was confined on screening the potential antigenotoxic activity of coffee and cacao extracts against tetracycline-treated albino mice.

MATERIALS AND METHODS

A. Experimental Design

This experiment was conducted in a Completely Randomized Design (CRD). Twenty four (24) Swiss albino mice were used and distributed to 24 individual cages. Eight treatments replicated 3 times were randomly assigned to the caged mice each representing a replicate. All data gathered were subjected to Analysis of Variance (ANOVA). Differences among treatment means were compared using Duncan's Multiple Range Test (DMRT) while Dunnett's test was used to compare experimental treatments against positive and negative controls.

B. Experimental treatments

Seven experimental treatments were used in the study. They are the following: Treatment 0, Pure distilled water (Negative control); Treatment 1, Roasted coffee (10g/90ml) plus tetracycline; Treatment 2, Unroasted coffee (10g/90ml) + tetracycline; Treatment 3, Instant coffee (10g/90ml) plus tetracycline; Treatment 4, Roasted cacao (10g/90ml) plus tetracycline; Treatment 5, Unroasted cacao (10g/90ml) plus tetracycline; Treatment 6, Instant cacao (10g/90ml) plus tetracycline; Treatment 7, Tetracycline (Positive control). Roasted, unroasted and instant extracts of coffee and cocoa were homogenized at uniform concentration (10g of extract per 90ml of distilled water). Tetracycline (2000mg/kg body wt.) alone diluted in distilled water was the positive control while pure distilled drinking water served as negative control. Experimental animals were fed ad libitum with mice pellets. Treatment solutions were administered orally 24-hour apart over a period of 23 days based on the mouse daily average water intake. After this period, the experimental mice, except those in the negative control, were administered twice with tetracycline by oral gavage 24-hour apart for two consecutive days.

C. Micronucleus Test

Varying treatments of plant extracts were administered orally through manual drinkers for 23 days. Two administration of tetracycline (2000mg/kg BW), a genotoxic substance was done. Six hours after the second administration; the experimental mouse was sacrificed by cervical dislocation. The micronucleus test of Ref [9] was used to assess the micronuclei formation induced by tetracycline and the inhibitory effect of coffee and cacao prepared extracts. After 24 hours, air-dried slides of bone marrow obtained from the sacrificed mouse were stained with Giemsa Stain and were scored for micronucleated polychromatic erythrocytes 1 hour after staining. Smeared slides were scored under oil-immersion objective by counting the number micronucleated polychromatic erythrocytes (MPCE's) per 1000 polychromatic erythrocytes (PCE's).

RESULTS AND DISCUSSIONS

Mice given extracts of unroasted coffee (T2) exhibited the statistically lowest ($p<0.05$) mean micronuclei count (2.40) among the experimental treatments. Those administered roasted coffee (T1) and instant cacao (T6) extracts shared statistically similar effects with mean counts of 3.07 and 3.00 micronuclei count, respectively. Mice given roasted cocoa extracts (T4) had statistically similar micronuclei count with those given roasted coffee (T1), unroasted cocoa (T5) and instant cocoa (T6) extracts. On the other hand, those administered instant coffee extracts (T3) showed the significantly highest micronuclei count (4.33) among the treated experimental units.

Analysis of Variance as shown in Table 1 indicated significant differences among treatment means. Although extract-treated mice were not completely rid of the chromosome breaking effects of the genotoxin as shown by the higher micronucleated polychromatic erythrocytes (MPCE) of T1 to T6 when compared with untreated mice, Dunnett's test revealed that mice treated with coffee and cacao extracts obtained significantly lower micronuclei count that the tetracycline-treated mice. This indicated that the potential micronuclei formation in mice bone marrow cells was significantly inhibited by any of the roasted, unroasted and instant coffee and cacao extracts.

Duncan's Multiple Range Test (DMRT) showed that unroasted coffee had the highest antigenotoxic potential among the coffee extracts which differed significantly from other coffee extracts. The data indicate that extracts from roasted, unroasted and

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instant coffee significantly inhibited the effect of tetracycline to induce an intercalating and hydrogen bonding interaction with base pairs in DNA. This conforms to the observation of Ref [12] in the antigenotoxic effects of akapulko and ampalaya against tetracycline in micronucleus test. Results were also in consonance with the findings of Ref [11] which used the L5178Y mouse lymphoma cell line to assess the modulatory effects of coffee on the genotoxicity of methylnitronitrosoguanidine in vitro and demonstrated significant protective effects of caffeinated instant coffee against methylnitronitrosoguanidine-induced DNA damage in the comet assay and mutation at Tk locus.

On the contrary, the cacao extracts manifested inverse effect as compared to the coffee. The data indicate that instant cocoa significantly lowered the micronucleated polychromatic erythrocytes in mice that roasted and unroasted cocoa. Similar with coffee extracts, results showed that cocoa extracts reduced significantly the tendency of tetracycline to fragment the chromatin material of the bone marrow cells. Chemical constituents of cacao could have significant inhibitory effect on the tetracycline-induced genotoxicity in mice.

Table 1. Varying Extracts of Coffee and Cocoa on Micronuclei Induced by Tetracycline.

Treatment	Mean Micronuclei Count of Male Abino Mice			
	R1	R2	R3	Mean
Untreated (Negative control)	1.6	1.2	1.0	1.27 [a]
Roasted Coffee + tetracycline	3.0	3.0	3.2	3.07 [c]
Unroasted Coffee + tetracycline	2.4	2.2	2.6	2.40 [b]
Instant Coffee + tetracycline	4.2	4.4	4.4	4.33 [e]
Roasted Cocoa + tetracycline	3.4	3.2	3.2	3.27 [cd]
Unroasted Cocoa + tetracycline	3.8	3.8	3.6	3.73 [d]
Instant Cocoa + tetracycline	3.0	3.0	3.0	3.00 [c]
Tetracycline (Positive control)	13.2	12.8	14.2	13.4 [f]

a. Means with the same superscripts are not significantly different.

This is feasible because many plant naturally occurring compounds are known to exhibit discrete mechanism of protection [17]. Plants constituents such as coconut oil [13],[15], akapulko and amplaya drug preparations [12]; lagundi, tsaang gubat, and ulasimang bato [2] and ethanol extract of Mahogany (*Swietenia macrophylla* King) seed [7] had been shown to exhibit potential antigenotoxic effects against dimethyl nitrosamine, benzo(a)pyrene, methyl methanesulfonate and tetracycline, which are known mutacarcinogenic and teratogenic chemicals [12].

SUMMARY AND CONCLUSIONS

Analysis of variance showed significant differences among treatments at 5% level of significance. Unroasted coffee possessed the highest antigenotoxic potential while instant coffee had the least among the coffee extracts. In contrast, instant cocoa had the highest antigenotoxic potential while unroasted cocoa had the least among the cocoa extracts. Both coffee and cacao extracts are effective in significantly reducing ($p<0.05$) the formation of micronuclei in polychromatic erythrocytes of the tetracycline-treated albino mice, reducing the tendency of tetracycline to alter the structure of DNA.

The potential antigenotoxic effects of coffee may be attributed to its chemical components (i. e. alkaloid caffeine, phenolic polymers, polysaccharides, chlorogenic acids and organic acids) and cacao (the alkaloid theobromine, oil of cacao, cacao butter, theine, starch, albuminous matter and ash) which are present in the extracts that possibly inhibited the genotoxic effects of tetracycline to DNA formation.

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