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ANTICLASTOGENIC PROPERTIES OF METHANOLIC EXTRACT OF *Cnestis ferruginea* LEAVES

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ABSTRACT

The inhibitory activity of methanolic extract of Cnestis ferruginea was investigated in bone marrow cells of mice using micronucleus assay. The mice were fed with the extracts at two different concentrations (100 and 200mg/kg body weight) while control mice were fed with corn oil for six days ad libitum. On the seventh day the mice were administered a single dose of sodium arsenite (2.5mg/kg) and sacrificed after 24hrs. Bone marrow smear were prepared for observation of clastogenic effect.

Results show micronuclei formation in the polychromatic erythrocyte (PCEs) in the Sodium arsenite treated mice. Clastogenecity induced by sodium arsenite was significantly reduced in mice pretreated with the extract at 100mg/kg body weight while more significant reduction was noted at 200mg/kg body weight. The result indicates a dose dependent relationship of the inhibitory activity of the extract against sodium arsenite induced clastogenecity.

Key words: *Cnestis ferruginea*, micronucleus assay, sodium arsenite, clastogenic.

INTRODUCTION

Cnestic ferruginea is a 6-meter high decidouous forest and secondary shrub common in Senegal, West Cameroon and in other parts of tropical Africa. Different parts of the plant are put to use in folk medicine in different parts of Africa.

It is employed as a remedy for treating skin infections in Nigeria and report documented in literature indicated its effectiveness against *Sarcina lutea* and *Staphylococcus aureus*, but no action against gram-negative organism or fungi[1]

Root decoction is taken by draught in Ivory Coast as an aphrodiasiac and by enema for gynecological troubles, dysentery and urethral discharge[1]. The roots are held to be a remedy against snakebite in Senegal[2]. It is common knowledge that dietary inhibitors of mutagenesis are of particular importance because they may be useful for prevention of human cancer[3]. Recently a great interest has been generated in the desmutagenic properties of higher plants for their potential antitoxic properties have been a relatively neglected field[4]. It is noteworthy to state that there is dearth of information on the toxicity and clastogenic properties of *Cnestis ferruginea* leaf. More importantly, the presence of any parts of the plant and arsenic at the same time in the human body is not impossible.

Arsenic is a genotoxic agent that induces chromosornal aberrations, micronuclei and sister chromatid exchange in mammalian cells[5]. The arsenic content in tube tubewell water has been fonud to range between 0.20 and 6.4mg kg even at depth of 200-300 feet below the surface[4].

The present work was undertaken to study the degree of protection provided by *Cnestis ferruginea* leaf at two different dose levels against clastogenic potential of sodium arsenate.

MATERIALS AND MEHTODS

Experimental Animal

Male swiss albino mice were used for this study. The mice were bred in the Biochemistry departmental animal house at the University of Ibadan and were 8-10 weeks old with average weight of 19.5g. They were kept and were fed pellets and water *ad libitum*. The animals were maintained under a 12hr light and 12 hr dark photo period at

temperature of 28 ± 2°C and relative humidity of 60 ± 5%.

Plant material

Cnestis ferruginea leaves were air dried for three to four weeks until it is crispy and then blended into powder. 200g part of it were suspended in 2 liters of methanol and left for 2 weeks. The resulting solution was sieved by filtration. The filtrate was concentrated by rotary evaporator under reduced pressure at a temperature 40° C.

Experimental protocol

Mice were divided into six groups of 4 animals per group. Group F served as the negative control in which the mice were fed with corn oil being a vehicle for methanolic extract of *Cnestis ferrugenea* leaves. Groups A and B were fed with 100mg/kg body weight extract while Groups C and D were fed with 200mg/kg body weight. On the 7th day each mouse in Groups B, D and E were fed with 0.25mg/kg body weight sodium arsenite. Twenty-four hours after exposure to arsenite all the mice were sacrificed by cervical dislocation. Bone marrow cells from the femurs were flushed out and slides, prepared following the procedure of Heddle and Salamone[6]. This was followed by fixation in glacial acetic acid-ethanol (1:3 v/v) and air-drying[7] The slides were pretreated in undiluted and diluted May-Gruenowald solutions for 3 mins and 2 mins respectively. They were then stained in Giemsa solution. The stained slides were coded and scored under a direct light compound microscope [leitz wetzlar model].

Clastogen

Sodium arsenite (Na As O_2; mol. Wt 129.9. As 57.6%; (Cas No 7784-46-5) from Sigma Chemical Co, St. louis Mo was dissolved in glass distilled water. A dose of 2.5mg/kg, corresponding to 1/10th of the oral LD_{50} dose, was administered by gavage.

Statistical analysis

The data obtained were analyzed by one-way analysis of variance (ANOVA)[8] followed by Duncan's multiple range test[9] to compare the significance of differences among the different experimental groups.

RESULTS

The high degree of micornucleated polychromatic erythrocytes (mPCEs) induced in mice bone marrow cells by sodium arsenite was significantly reduced in mice fed with the extract at 100mg/kg body weight and almost completely masked in mice fed with the extract at 200mglkg body weight (Table I). The micronucleated polychromatic erythocyte (mPCEs) in mice fed with extracts alone at two different doses (100 and 200mglkg body weight) compared to control (Group F) did not show any marked difference. The (mPCEs) induced in sodium arsenite dosed mice (Group E) is about 7 folds higher compared to that observed in 100mg/kg body weight fed mice (Group A) and 14 folds higher compared to 200mg/kg body weight fed mice (Group C) as well as that of control. From Table I it appears that *Cnestis ferruginea* methanolic leaf extract has inhibitory activity on sodium arsenite-induced clastogenicity.

Table 1: Mean Number of micronucleated polychromatic erythrocytes (mPCE)/1000. PCEs in Mice bone marrow after administration of Methanolic Extract of *Cnestis ferruginea* leaves.

Group	Treatment	Number of micronucleated PCEs/1000. PCE mean ± S.D
A	Extract at 100mg/kg b.w alone	$1.00^{bc} \pm 1.00$
B	Extract at 100mg/kg b.w.+ Sodium arsenite	$1.67^{bc} \pm 0.57$
C	Extract at 200mg/kg b.w alone	$0.50^{bc} \pm 0.57$
D	Extract at 200mg/kg b.w + Sodium arsenite	$0.33^{bc} \pm 0.57$
E	Sodium arsenite alone	$7.75^{a} \pm 0.96$
F	Corn oil	$0.50^{bc} \pm 0.57$

Values are mean ± S.D (standard deviation); Values with the same superscript are not significant at 5 % level whereas values with different superscript are significant ($p<0.05$).

DISCUSSION

The effectiveness of natural herbs in the treatment of a wide range of ailments is well documented in both folk and orthodox medicine. The use of such natural or synthetic components of herbs to delay, inhibit or reverse the development of cancer in normal or pre neoplastic condition has been hailed by many investigatior to be practically beneficial[10]

Methanolic extract of *Cnestis ferruginea* leaves was used as the test substance because it has been shown to be effective traditionally as laxative bronchitis and as abortifacient[11]. Sodium arsenite is both clastogenic and carcinogen[12] It was used for comparison because co-exposure to *cnestis ferruginea* and sodium arsenite can exist in our environment. Sodium arsenite has been used extensively in our laboratory as positive control substance[13].

In the present study however, a dose of 2.5mg/kg corresponding to $1/10^{th}$ of the oral LD_{50} dose administered to mice in group E markedly induced micronuclei formation in the PCEs (polychromatic erythrocytes) of the mice bone marrow. The frequency of induction is about 11-fold when compared to the micronucleated polychromatic erythrocytes (mPCEs) formed in the bone marrow of negative control mice (group F) fed corn oil.

The degree of induction of micronuclei formation in the mice fed with methanolic extract of *Cnestis ferruginea* is not significantly different at both level of doses (100 and 200mglkg) group A and C when compared to negative control. Interestingly, induction of micronuclei formation in group fed with the extract alone at 200mg/kg body weight is non significantly less than that of negative control.

Co-administration of sodium arsenite and the extract at 100mg/kg-body weight significantly reduced the degree of induction of mPCEs. However, at 200mg/kg dose, the mice in group D (Table 1) showed almost mask clastogenic effect induced by sodium arsenite. The reduction of degree of clastogenecity at 200mg/kg body weight appears to show a kind of dose dependent relationship. These observations indicate that the extract probably has inhibitory capacity for sodium arsenite induced clastogenicity

Crude plant extracts are complex mixtures of various compounds. The antimutagenic and anticlastogenic effects of such mixtures may be the sum of the interactions between the components and the clastogen or mutagen added[14] The inhibitory role displayed by this extract might not be unconnected with the result of several interactions between the variuos constituents of the extract and the clastogen used. Consequently, more work need to be done to ascertain long-term effects of exposure to the extract alone and in combination with sodium arsenite.

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