

## CYTOTOXICITY OF AQUEOUS EXTRACT OF ARECANUT AND TOBACCO ON HUMAN ERYTHROCYTES (RED BLOOD CORPUSCLES)

\* A.S Kolhe, \*\* Nanda Patil

Samples of arecanut and tobacco were used for preparing aqueous saline (0.9% NaCl) extract. Saline suspension of RBC were prepared from blood samples collected. When RBC suspension was exposed to different concentrations (5-100 mg/ml) of arecanut/tobacco for 16 hours, a concentration dependent increase in percent hemolysis was recorded. Tobacco extract was comparatively more potent than arecanut.

Oral cancer is the common form of cancer among the Indian males. A positive correlation between oral cancer and the habit of consuming tobacco and betelnut, in various ways, has been reported. [1,2] Sinor and his associates [3] have reported positive correlation between chewing of mava (a preparation containing thin shaving of arecanut with addition of some tobacco and lime, common among young males of Bhavanager), and occurrence of 'submucous fibrosis' (SMF), a precancerous condition rendering the mucosa more vulnerable to the action of carcinogens. An aqueous as well as dimethyl sulphoxide (DMSO) extract of betelnut have been shown to produce tumors in experimental animals [4,5] In the present communication, we report cytotoxic effect of aqueous extract of tobacco and betelnut on Human erythrocytes (RBC) which may be responsible for toxicity or oral mucosa too.

**MATERIALS AND METHODS**—To prepare aqueous extracts of arecanut and tobacco, samples were air dried in the shade and ground in a mortar and pestle. Five hundred mg of powder was suspended in 100 ml of double distilled water and soxholated for two hours. The cooled extract was filtered (Whatman filter paper No.1) and the residue extracted 3-4 times in 100 ml of double distilled water. The filtrates were pooled, evaporated to dryness on a waterbath and the powder was used for making up the required dose by dissolving it in saline (0.9% NaCl) Human Venous blood was collected directly into EDTA bulbs. After dilution with saline, the samples were centrifuged at 1000 rpm for 10 min. Supernatants were discarded and the RBC pellet was further washed twice with saline by centrifugation Final RBC suspension was prepared in saline to have  $2 \times 10^4$  cells/ml. [6] For examining effects of aqueous extract of arecanut tobacco on RBC, two sets of tubes were prepared as follows: (a) control

tubes containing 1.0ml RBC suspension and (b) treated tubes containing 1.0 ml RBC suspension and aqueous extract (5 to 100 mg/ml). Total volume of each tube was made to 2.0 ml by adding saline (0.9% NaCl). All the tubes were incubated at 37°C for 16 hrs with intermittent shaking. Morphological alterations in RBC was observed after staining RBC smear with Leishman's stain. Tubes were centrifuged at 1000 rpm for 10 min and color density of supernatant was measured spectrophotometrically at 540 nm [7] percent hemolysis was calculated by the formula:

$$\text{Percent hemolysis} = \frac{\text{Absorbance of individual tubes}}{\text{Absorbance with 100\% hemolysis}} \times 100$$

Student 't' test was used for statistical analysis of the data.

**RESULTS AND DISCUSSION**—Control:- The normal human red blood cells appear as flattened, indented spheres or biconcave discs. The unucleated red blood cells may be looked upon as a membrane enclosing proteins, electrolytes and other component of energy system. This cell exhibit remarkable plasticity. In the incubation tube, the cells remained settled at the bottom with almost clear ambient saline. Morphological aberration and cytotoxicity :-Effect of various concentrations of aqueous extract (Arecanut and Tobacco) on RBC suspension are shown in Table. Morphological observation revealed concentration dependent swelling of RBC. Hemolysis was recorded with 5 ug/ml concentration or above. A concentration dependent increase in hemolysis was noted between 5 ug/ml -100ug/ml. The maximum hemolysis occurred at 100ug/ml concentration of aqueous extract of both, arecanut and tobacco. The amount of pellet at the bottom of the tube decreased accompanied with appearance of red color in the ambient solution. Aqueous extract of tobacco was significantly more toxic than arecanut. Present experiment clearly indicates that aqueous extracts of both arecanut and tobacco are cytotoxic which may be responsible for toxicity on oral mucosa. If absorbed in gastrointestinal tract, cytotoxicity on RBC and other cell type cannot be ruled out. The factors responsible for such

\* Department of zoology, Art & Science College, Bhalod Dist., Jalgaon (M.S.) (India) 425304

\*\* Department of zoology, G.G.Khadse Arts and Science College Muktainagar

cytotoxic action is not clearly known. It has been reported that alkaloides present in arecanut (major arecoline) and tobacco (nicotine, nornicotine and anatabine) cause sister chromatid exchange (SCE) frequency and genotoxicity. [8-12]. Many people have the habit of chewing arecanut/mixtures and Tobacco throughout the day allowing it to remain in mouth for comparatively longer period. Some have the habit of keeping them overnight even while sleeping Thus

fungi find [13] an opportunity to establish their pathological effects. It is also now proved the *Aspergillus flavus* and some other *Aspergilli* produce thermostable aflatoxins in the substrate they grow on [14]. Cytotoxic effects of aflatoxin on cells in culture [15-16], and on RBC [6] are established. It is well known carcinogenic, mutagenic and teratogenic agent [17-20]. Thus aflatoxin contamination of arecanut and tobacco might be responsible for induced cytotoxicity and cancer.

**Table Effect of aqueous extracts of Arecanut & Tobacco on percent hemolysis in vitro**

Aqueous extract (µg/ml)	percent Hemolysis	
	Arecanut extract	Tobacco extract
0 (control)	1.14 ± 0.42	1.14 ± 0.42
5	18.36 ± 0.50*	33.67 ± 1.45*
10	36.20 ± 0.77*	42.31 ± 1.30*
20	42.04 ± 0.86*	48.07 ± 1.16*
30	47.57 ± 0.88*	50.26 ± 2.22*
50	56.84 ± 0.84*	61.97 ± 1.92*
100	64.42 ± 0.50*	72.27 ± 2.28*

N = 10, (Number of Samples), values are mean SE ±, Values for the same parameter in the same row with superscript significantly differ at the level : \* P < 0.001

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