



## Impact of Smokeless Tobacco Product Consumption on Certain Haematological Parameters in Albino Rats

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### Abstract

The tobacco plant (*Nicotiana tabacum*) has historically been utilized as chewing tobacco globally. Snuff is a form of tobacco processed into small particles and packaged in cans or pouches. This study aimed to investigate the impact of snuff tobacco on certain hematological indicators in albino rats. Albino rats with a weight range of 100 to 150 grams were utilized in the study. The oral LD<sub>50</sub> for tobacco snuff was established at 3 mg per 100 g of rat body mass. Group A served as the control and received no treatment, whereas group B was administered snuff in milligrams per body weight orally using a gavage tube daily for 28 days. Blood samples were collected under chloroform anesthesia and analyzed for total erythrocyte count, total leucocyte count, hemoglobin concentration, packed cell volume, erythrocyte sedimentation rate, and absolute values. Total Erythrocyte count (TEC) was significantly decreased in group B ( $5.41 \pm 0.04$  million/mm<sup>3</sup>) as compared to the control ( $6.33 \pm 0.3$  million/mm<sup>3</sup>). Total Leucocyte Count was significantly increased in group B ( $9.0 \pm 0.21 \times 10^9/L$ ) respectively as compared to the control group A ( $7.2 \pm 0.28 \times 10^9/L$ ). Haemoglobin concentration was significantly decreased. Smokeless tobacco may possibly affect all the Haematological parameters.

**Keywords:** Smokeless tobacco Snuff and Haematological parameters.

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### Introduction

Tobacco is derived from the desiccated and processed foliage of the perennial herbaceous plant *Nicotiana tabacum*, the most extensively cultivated species of the *Nicotiana* genus. This is widely cultivated and commercially grown in many countries of the world (Ukoha, *et al.*, 2012). It is mostly consumed in the form of smoking, chewing, snuffing or dipping tobacco (Gilman and Xun, 2004). Tobacco is a colorless, odorless liquid with an oily consistency, but it changes upon exposure to light or air. It attains a brown hue and has a potent aroma of tobacco. Smokeless tobacco is utilized in numerous forms, including Paan masala, chewing tobacco, Gutkha, snuff, Naswat, snus, Zarda, Chaw, Supari, Iq'mik, Ariva, Mawa, Shammah, Mishri, Toombak, Qiwam, Kima, and Chimo. Areca nut, Ashana, and Lime are primary components utilized in these combinations (Vidhubala *et al.*, 2016). The most commonly utilized form of smokeless tobacco is snuff.

Snuff is a finely ground tobacco that is occasionally packaged in packets like teabags. The user takes a "pinch," "dip," or "quid," inserts it between the lower lip or cheek and gum, and sucks on it (Mesembe, *et al.*, 2008). Another method of utilizing snuff, however uncommon, is through nasal inhalation. Both varieties of smokeless tobacco are highly addictive and can lead to significant health issues. These smokeless tobacco products have a blend of tobacco-nicotine, sugar, salt, slaked lime, spices, and flavorings. They may emit numerous chemicals and toxins. Among these chemicals many are dangerous health risk agents. Nicotine stays in the blood longer for users of smokeless tobacco than for smokers (Ukoha, *et al.*, 2012). The level of nicotine in the blood depends on the amount of nicotine in the smokeless tobacco product; the tobacco cut size, the product's pH (a measure of its acidity or basicity) and other factors (Richter *et al.*, 2008). Chronic use of smokeless tobacco may alter the status of hematological parameters and further define the impacts of tobacco use to health, in light of the diverse pharmacological activities of nicotine and additives and the widespread usage in many areas and nation (Kathuria *et al.*, 2022). Due to addictive properties, using smokeless tobacco can develop into lifetime habit that had detrimental impacts on one's health over time (Kumar *et al.*, 2024).

The extract of smokeless tobacco can enter the bloodstream, where it alters hematological values that are commonly utilized to measure systemic relationships and physiological adaptations, including the evaluation of the overall health status of organisms. This study examines the impact of tobacco snuff and Gutkha on haematological parameters, including Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), haemoglobin concentration, Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR), and absolute values (MCV, MCH, MCHC), which are influenced by inflammation and oxidative stress. Therefore, the present study is intended to examine the impact of smokeless tobacco products consumption on haematological parameters of albino rats, *Rattus norvegicus* (Berkenhout).

Albino rats were selected for the present study as an experimental animal is mainly due to its easy availability, easy adaptability in laboratory conditions and physiological similar to the human beings.

### Materials and Methods

**Maintenance and Experimental Animal** - Healthy and adult albino rats, weighing from 100-150 gm were kept in the polypropylene cages, measuring 45x27x15 cm at the temperature  $25 \pm 5^\circ\text{C}$ , relative humidity  $55 \pm 5\%$  and photoperiod of 12 hours/day cycle. The cages were constructed from galvanized steel mesh and were routinely cleaned to eliminate excrement and prevent any unpleasant odors in the laboratory. Each cage was furnished with a metallic food dish and a water dispenser. The albino rats were provided with a regular laboratory pelleted diet daily and water was available ad libitum. The albino rats were acclimatized for one week prior to the initiation of the experiment.

**Ethical Approval**- The ethical committee of the institution sanctioned the research. The committee rigorously complied with the established guidelines governing the utilization of animals for scientific reasons.

**Dose of experimental compound Snuff tobacco** - Snuff (Chaini Khaini) was used as experimental chemical. 15 mg compound was prepared in 100 ml distilled water and given to rats orally by gavage tube. The dose of snuff was given to rats 2mg/rat/b.wt/day for 28 days.

### Snuff Tobacco

Snuff of chaini khaini brand manufactured by Harsh international, companies. A company under MAHAK group It contains Nitrosamines, of are organic compounds of the chemical structure  $R_2N-N=O$ , where R is usually an alkyl group.

Formula	:	$H_2N_2O$
Molecular weight	:	46.029 g/mol
Melting point $25^\circ\text{C}$	:	Boiling point: $154^\circ\text{C}$
Density	:	1.005g/ml

**Collection of Blood** - Albino rats of control set (A), and experimental set (B) were sacrificed under light anaesthesia (diethyl ether). The blood sample were collected directly by cardiac puncture of the dissected albino rats with the help of 5ml sterilized disposal syringe fitted with the hypodermic needle and then blood transferred immediately directly into the vials each vials coated with an anticoagulant of 0.9 mg/ml EDTA. The blood samples were analyzed individually for each animal.

**Calculation of Result** - The mean (X), standard deviation (S.D), standard error of mean (S.E.m) and test of significance 't' test, were calculated by a statistical software stat pac version 3.0

Parameters	Result	Snuff group
TEC (million/mm <sup>3</sup> )	6.33±0.331	5.41±0.044
TLC (x10 <sup>9</sup> /L)	7.2±0.28	9.0±0.21
HC (g/dl)	15.89±0.26	13.08±0.33
PCV (%)	44.34±0.69	31.18±0.23
ESR (mm/hr)	15±0.70	11.6±0.54
MCV (fl)	63.60±0.95	85.69±0.31
MCH (pg)	20.18±0.72	33.44±0.23
MCHC (g/dl)	31.73±0.82	35.76±0.35

### Discussion

Nicotine and nitrosamine are the most often utilized components of smokeless tobacco in the creation of snuff and gutkha, respectively. The assessment of haematological parameters is crucial for identifying the toxicity of foreign substances within the body. The toxic effect of a chemical is generally shown by alterations in blood values, reflecting either an excess or deficiency in blood cell production, or an imbalance between blood cell formation and destruction. The use of smokeless tobacco products may have little physiological consequences; however, it is detrimental to the respiratory tract and causes discomfort during the initiation phase. Upon intake, it is promptly absorbed into the bloodstream, resulting in the animal displaying signs of distress, discomfort, and respiratory depression as a consequence. The use of numerous chemical agents can interact with certain tissues in the body, leading to alterations in haematological parameters. The metrics encompass total erythrocyte count (TEC), total leucocyte count (TLC), hemoglobin concentration, packed cell volume (PCV), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Diseases linked to smokeless tobacco may come from bone marrow depression, characterized by a reduction in the production and maturation of erythrocyte progenitor cells. Moreover, smokeless tobacco products, including snuff and gutkha, may contribute to an elevation in the degradation of red blood cells. The current study observed a decrease in Total Erythrocyte Count (TEC), hemoglobin concentration, Packed Cell Volume (PCV), and Erythrocyte

Sedimentation Rate (ESR), alongside an increase in Total Leucocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) following the consumption of smokeless tobacco products (snuff and gutkha) in albino rats.

### Conclusion

TEC, HC PCV, and ESR decreased in albino rats administered sub-lethal doses of tobacco snuff, while TLC, MCV, and MCHC rose. This conclusion can be derived from the supplied data. These alterations may render the body more vulnerable to adverse health conditions, including inflammatory illnesses, bleeding tendencies, infections, and restricted blood circulation.

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