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Research Article

EFFECTS OF THE CONSUMPTION OF SMOKELESS TOBACCO (CHEWING TOBACCO) PRODUCTS ON CERTAIN HAEMATOLOGICAL PARAMETERS IN ALBINO RATS

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ABSTRACT
The tobacco plant (*Nicotiana tabacum)* has been used globally for several years as chewing tobacco. Snuff is a form of tobacco that is processed to fine grains and packaged either in cans or pouches. Gutkha is chewing tobacco that is large-grain tobacco leaves that are twisted or shredded and come loose in paper packets or small cans. Mostly used forms of smokeless tobacco are gutkha and snuff. This study was carried out to investigate the effects of snuff and gutkha tobacco on some haematological parameters of albino rats. Albino rats with weights between 100-150 g were used for the study. The oral LD50 for the tobacco concentration was determined as 3 mg per 100 g rat body weight. The control group A was untreated group and group B was treated with snuff mg per body weight and group C was treated with gutkha tobacco respectively, through an oral route with the gavage tube daily up to the period of 28 days. Blood samples were collected under chloroform anaesthesia and analysed for Total Erythrocyte count, Total Leucocyte count, Haemoglobin concentration, Packed Cell Volume, Erythrocyte Sedimentation Rate and Absolute Values. Smokeless tobacco may possibly affect all the Haematological parameters.

Keywords: *Smokeless tobacco Snuff, Gutkha and Haematological parameters*

INTRODUCTION

Tobacco is the dried and processed leaves of the plant Nicotiana tabaccum, a perennial herbaceous plant and it is most commonly grown of all plants in 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 colourless, odourless liquid with an oily consistency, but when exposed to light or air. It acquires a brown colour and gives off a strong odour of tobacco. Smokeless tobacco used in various forms such as Paan masala or chewing tobacco or Gutkha, Snuff, Naswat, Snus, Zarda, Chaw, Supari, Iq'mik, Ariva, Mawa, Shammah, Mishri, Toombak, Qiwam or Kima and Chimo. Areca nut, Ashana and Lime are some of the main ingredients used in these mixtures (Vidhubala *et al.,* 2016). Mostly used forms of smokeless tobacco is snuff and gutkha.

Snuff is a fine-grain tobacco that sometimes comes in pouches that look like teabags. It is user takes a "pinch", "dip" or "quid", and places it between the lower lip or cheek and gum and suck on it (Mesembe, *et al.,* 2008). Gutkha is chewing tobacco that is large-grain tobacco leaves that are twisted or shredded and come loose in paper packets or small cans. Gutkha is consumed by placing a pinch of it between the gum and cheek and gently sucking and chewing, similar to chewing tobacco. Both types of smokeless tobacco are very addictive and can cause serious health problems. These smokeless tobacco are a mixture of tobacco-nicotine, sugar, salt, slacked lime, spices and flavorings. They may release hundreds of chemicals and poisons. 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 bacicity) 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 be entered through the blood where it alters the heamatological values which are widely used to determine systemic relationship and physiological adaptations including the assessment of general health conditions of living beings. In the present study, the effect of tobacco snuff and Gutkha on haematological parameters viz. Total Erythrocyte count (TEC), Total Leucocyte Count (TLC), Haemoglobin concentration, Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR), Absolute values (MCV, MCH, MCHC) are the indicators affected by inflammation and oxidative stress. Therefore, the present study is intended to examine the impact of smokeless tobacco products consumption on hematological 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 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±5°C, relative humidity 55±5% and photoperiod of 12 hours/day cycle. The cages were made of galvanized steel mesh and cleaned regularly to remove excreta and to avoid any undesirable odour in the laboratory. Each cage was equipped with a metallic food plate and water bottle. The albino rats were fed daily a standard laboratory pelleted feed and water was given ad libitum. The albino rats were acclimatized for or week before commencement of the experiment.

ETHICAL APPROVAL- The research was approved by the ethical committee of the institution. The standard rules and regulations of use of animal for research purposes was strictly adhered to as approved by the committee.

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.

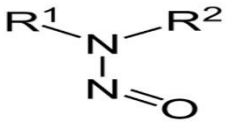
Dose of experimental compound Gutkha tobacco – Gutkha (Gold mohar) was used as experimental chemical. 10 mg compound was prepared in 100 ml distilled water and given to rats orally by gavage tube. The dose of gutkha was given to rats 3mg/rat/b.wt/day for 28 days.

Snuff Tobacco

Snuff of chaini khaini brand manufactured by Harsh international, companies. a company under MAHAK group It contains Nitrosamines, are organic compounds of the chemical structure R2N-N=0, where R is usually an alkyl group.

Formula : H2N2O
Molecular weight : 46.029 g/mol
Melting point 25°C : Boiling point: 154°C

Density : 1.005g/ml

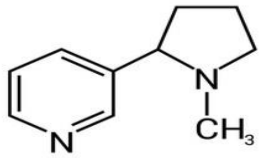


Nitrosamines

Gutkha Tobacco

Gutkha of gold mohar brand manufactured by gold pan masala Pvt. Ltd. It contains Nicotine, is also called 3-(1-methyl-2-pyrrolidinyl) pyridine according to IUPAC nomenclature. It is bicycle compound with pyridine cycle and pyrrolidine cycle. The molecule possesses an asymmetric carbon and so exits in two enantiomeric compounds.

Formula : $C_{10}H_{14}N_2$
Molecular weight : 162,234 g/mol
Melting Point : -79^0C
Density : $d=1,010\ gm/cm^3$



Nicotine

Collection of Blood - Albino rats of control set (A), and experimental set (B) were sacrificed under light anesthesia (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 vails 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.Em) and test of significance 't' test, were calculated by a statistical software stat pac version 3.0

Methodologies

Total Erythrocyte Count (TEC)

Total erythrocyte count was determined by standard improved Neubauer's Haemocytometer (Dacie and Lewis, 1975).

Total Leucocyte Count (TLC)

The total leucocyte was counted with the help of improved standard Neubaur's chamber haemocytometer described by Dacie and Lewis (1975).

Haemoglobin Concentration

The haemoglobin concentration was estimated by the standard Sahli's method (Wintrobe et al, 1981).

Packed Cell Volume (PCV)

The packed cell volume was determined by Wintrobe's method (Wintrobe *et al.,* 1981).

Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate was determined by Wintrobe's method (Wintrobe *et al.,* 1981).

ABSOLUTE VOLUME

Mean Corpuscular Volume (MCV)

The mean corpuscular volume was calculated from the Packed cell volume (PCV) and Total erythrocyte count (TEC) by the following formula (Wintrobe *et al.,* 1981).

Mean Corpuscular Haemoglobin (MCH)

The mean corpuscular haemoglobin values were calculated from the values of haemoglobin concentration (Hb. Conc.) and the total erythrocyte count (TEC) by the following formula (Wintrobe *et al.,* 1981).

Mean Corpuscular Haemoglobin Concentration (MCHC)

The mean corpuscular haemoglobin concentration was calculated from the values of the haemoglobin concentration and packed cell volume with the help of the following formula (Wintrobe *et al.,* 1981).

In studies evaluating hematological parameters, the most commonly used equipment is an automated hematology analyzer, which performs complete blood counts (CBC) and red cell indices like TEC, TLC, Hb, PCV, MCV, MCH, and MCHC.

For your current study, if not already specified, you can mention a standard and widely accepted model used in similar biomedical research. Here is a technically appropriate example:

Hematological Analysis

Hematological parameters, including Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC), were analyzed using an automated hematology analyzer (Sysmex XP-100, Sysmex Corporation, Kobe, Japan). The instrument was calibrated according to the manufacturer's protocol prior to each batch of analysis to ensure accuracy and precision.

RESULTS

Parameters	Control Group	Snuff Group	Gutkha Group
TEC (million/mm^3)	6.33±0.331	5.41±0.044	5.84±0.112
TLC (x10^9/L)	7.2±0.28	9.0±0.21	10.5±0.25
HC (g/dl)	15.89±0.26	13.08±0.33	11.73±0.21
PCV (%)	44.34±0.69	31.18±0.23	32.13±0.19
ESR (mm/hr)	15.±0.70	11.6±0.54	8.8±0.37
MCV (fl)	63.60±0.95	85.69±0.31	87.21±0.23
MCH (pg)	20.18±0.72	33.44±0.23	34.04±0.19
MCHC (g/dl)	31.73±0.82	35.76±0.35	36.19±0.20

DISCUSSION

The hematological profile of individuals exposed to smokeless tobacco products, specifically snuff and gutkha, reveals significant deviations from the control group, indicating systemic alterations likely induced by the toxic constituents of these products.

A notable reduction in **Total Erythrocyte Count (TEC)** was observed in both the snuff (5.41 ± 0.044 million/mm³) and gutkha (5.84 ± 0.112 million/mm³) groups compared to the control (6.33 ± 0.331 million/mm³), suggesting a suppression of erythropoiesis or increased red blood cell destruction. Parallel to this, **Hemoglobin (Hb)** levels showed a significant decline, with the gutkha group (11.73 ± 0.21 g/dl) exhibiting a more profound reduction than the snuff group (13.08 ± 0.33 g/dl), relative to controls (15.89 ± 0.26 g/dl). These changes, coupled with the decreased **Packed Cell Volume (PCV)** values (gutkha: 32.13 ± 0.19%; snuff: 31.18 ± 0.23%) compared to controls (44.34 ± 0.69%), are indicative of **anemia**, potentially hypoproliferative in nature.

Total Leukocyte Count (TLC) was markedly elevated in the tobacco-consuming groups, with the gutkha group (10.5 ± 0.25 ×10⁹/L) showing the highest increase, followed by the snuff group (9.0 ± 0.21 ×10⁹/L), as compared to controls (7.2 ± 0.28 ×10⁹/L). This leukocytosis may reflect an ongoing systemic inflammatory response or immune modulation due to chronic exposure to tobacco toxins.

Interestingly, **Erythrocyte Sedimentation Rate (ESR)** demonstrated a declining trend in the experimental groups (gutkha: 8.8 ± 0.37 mm/hr; snuff: 11.6 ± 0.54 mm/hr) relative to controls (15.0 ± 0.70 mm/hr), possibly indicating altered plasma protein composition or reduced inflammatory protein production, which may have diagnostic relevance in chronic tobacco users.

The **Red Cell Indices**—namely, **Mean Corpuscular Volume (MCV)**, **Mean Corpuscular Hemoglobin (MCH)**, and **Mean Corpuscular Hemoglobin Concentration (MCHC)**—were significantly elevated in both snuff and gutkha groups. MCV increased from 63.60 ± 0.95 fL in controls

to 85.69 ± 0.31 fL and 87.21 ± 0.23 fL in the snuff and gutkha groups, respectively. Similarly, MCH rose from 20.18 ± 0.72 pg (control) to 33.44 ± 0.23 pg (snuff) and 34.04 ± 0.19 pg (gutkha), and MCHC increased from 31.73 ± 0.82 g/dl to 35.76 ± 0.35 g/dl (snuff) and 36.19 ± 0.20 g/dl (gutkha). These findings are suggestive of macrocytic anemia with hyperchromic characteristics, possibly due to disrupted erythropoiesis and defective hemoglobin synthesis caused by the cytotoxic effects of nicotine and other alkaloids present in smokeless tobacco.

Overall, the data demonstrate that chronic exposure to snuff and gutkha leads to significant hematological alterations, including anemia, leukocytosis, and modified erythrocyte indices, with gutkha exerting a more pronounced effect. These alterations underscore the systemic toxicity of smokeless tobacco products and warrant further investigation into their pathophysiological impact.

Nicotine and Nitrosamine are most commonly used component of smokeless tobacco in snuff and gutkha respectively. The evaluation of haematological parameters plays a vital role in assessing the toxicity of any foreign compounds in the body's system. The toxic effect of a compound is usually seen as an increase or decrease in the blood values indicates excess or suppression of blood cells production respectively or an imbalance between blood cells production and destruction. Consumption of smokeless tobacco products can cause minimal physiological effects, but it is irritating to respiratory tract and stressful during induction period. After consumption and it is rapidly transfer to blood and animal shows signs of distress, discomfort and respiratory depression. The various chemical agents when consumpted can interact with specific tissue in the body and cause alteration in haematological parameters viz. total erythrocyte count (TEC), total leucocyte count (TLC), heamoglobin concentration, paced cell volume (PCV), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

CONCLUSION

In conclusion, the present data indicated that consumption of tobacco snuff at sub-lethal doses in albino rats decreased TEC, HC PCV, and ESR and increased TLC, MCV, MCV and MCHC. Gutkha doses in comparison to snuff treated albino rats decreased HC, ESR and increased TEC, TLC, PCV, MCV, MCH, MCHC. These changes may put the body at risk of adverse health conditions such as inflammatory conditions, bleeding tendency, infections and poor blood flow.

REFERENCES

Dorathy CO, Euphoria CA, Sabrina ME & Patience EO (2022). Outcome of red and white cell indices of smokeless tobacco (snuff) consumers in Nigeria. *Journal of Medical Sciences*, **22**, 196–202.

Dacie SJV & Lewis SM (1975). *Practical Hematology*. London: E1BS.

Gilman SL & Xun Z (2004). *A Global History of Smoking*. The University of Chicago Press, Chicago, IL, USA.

Kathuria NS, Agarwal G, Gupta S & Patel NP (2022). A pilot study on the effects of chewing tobacco on hematological parameters. *Journal of Cardiovascular Disease Research*, **13**(7), 975–976.

Kumar D, Jaiprakash, Dubey P & Sharma M (2024). Impact of smokeless tobacco on red cell indices in southern Rajasthan population. *International Journal of Academic Medicine and Pharmacy*, **6**(2), 921–925.

Mesembe O, Bisong S, Ekong M & Ekeoma A (2008). Neurobehavioural activity in albino Wistar rats in the open field maze following long term tobacco diet ingestion. *International Journal of Neurology*, **10**(2), 345–363.

Nath Barbhuiya S, Barhoi D, Giri A & Giri S (2020). Arsenic and smokeless tobacco exposure induces DNA damage and oxidative stress in reproductive organs of female Swiss albino rats. *Journal of Environmental Science and Health Part C: Toxicology and Carcinogenesis*, **38**(4), 384–408.

Richter P, Hodge K, Stanfill S, Zhang L & Watson C (2008). Surveillance of moist snuff: total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines. *Nicotine and Tobacco Research*, **10**, 1645–1652.

Singhavi HR, Singh A & Chaturvedi P (2021). Smokeless tobacco and its effects. *Indian Journal of Medical and Paediatric Oncology*, **12**(2), 122–128.

Shukla AK, Khaitan T, Gupta P & Naik S (2019). Smokeless tobacco and its adverse effects on hematological parameters: a cross-sectional study. *Advances in Preventive Medicine*, **10**, 1155.
Ukoha U, Uchechukwu D & Stephen M (2012). The effect of sub-lethal doses of smokeless tobacco (snuff) on certain haematological and haemostatic parameters in Wistar rats. *Journal of Experimental and Integrative Medicine*, **2**(3), 225–230.
Vidhuwala E, Pisinger C, Basumalik B & Prabhakar DS (2016). The ban on smokeless tobacco products is systematically violated in Chennai, India. *Indian Journal of Cancer*, **53**(2), 225–230.
Wintrobe MM, Boggs GR, Bithell TC, Foerster J, Athens JW & Lukens JN (1981). *Clinical Hematology* (8th edn). Lea and Febiger, Philadelphia, 1882 pp.