

The Effect of “Hookah Water” on Haematological Parameters in Common Toad

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Abstract

This study aimed to investigate the effect of Hookah Water (HW) on haematological parameters of Common Toad. Sixteen adult individuals of both sexes of common Toad were randomly assigned to four groups (n=4). The control group was exposed to room temperature and the experimental groups were exposed to HW injected intraperitoneally at the rate of 4cc/100gms of the body weight. Animals were sacrificed after 1,3,7 and 15 days following the treatment. For control series, the test animals were treated with water used by the HW smoker and were sacrificed after the same intervals as in treated series. After specific duration of exposure toads were anaesthetized with ether, the blood was collected by puncturing the heart. Immediately the smear was prepared and was dried in air for staining. The results showed that RBC count were significantly higher in HW injecting rats than the control group ($P < 0.001$). We found that WBC counts insignificantly increased ($P < 0.39$) but platelets count insignificantly decreased ($P < 0.13$) in HW injected toads compared with control group. The findings may help to raise awareness of tobacco smokers about the potential toxicities of Hookah Water; likewise, the results can be used by physicians and public health officials in tobacco prevention programs.

Keywords: Haematological Parameters, Health, Smoke, Hookah Water, Genotoxic, Cytotoxic.

Introduction

Tobacco is a commercial product obtained from dried and processed leaves of *Nicotiana tabacum* plant that is widely cultivated and grown in many countries around the world. According to the data of World Health organization (WHO), there are about 2.4 billion people worldwide that have consumed tobacco in the forms of smoking, chewing and snuffing. WHO also estimates that tobacco-related deaths will increase gradually and expected to one billion deaths during the 21st century.

Hookah Water (HW) is a classical device used for tobacco smoking attached with water bowl. The HW usage has a history about 400 years with the different names like as a shisha, narghile, hookah chillum and arghile. HW is often linked with social activity where two or more people may share the same pipe. In some cultures, children may smoke with their parents. It is estimated that approximately 100 million people use HW or tobacco smoke throughout the world. Due to the lack of awareness, there is a viewpoint in different cultures that HW is less dangerous than cigarette, so its prevalence is increasing, particularly among adolescent and young adults. This increasing trend can be attributed to the popular beliefs that the smoke is “filtered” by the water where harmful effect is believed to be reduced by the so-called “filtering” process. However the research findings highlight that HW smoking carries similar or higher risks than other forms of tobacco exposure. The research studies also indicate that HW has led to increase the risk of infectious diseases, cardiovascular disease, pulmonary illness, cancers and low foetal birth weight in pregnant women. The hematologic index alterations are used as physiological markers of organ and tissue damage. Therefore, the various pharmacological actions of nicotine and other materials led to change the status of hematologic and haemostatic parameters.

Objective of the Study

Several mutagenic agents are used for micro-nucleus test. Tobacco is one of the most dreadful mutagenic chemical. It contains many

mutagenic and carcinogenic chemicals. The most important chemical families of carcinogens are polycyclic aromatic hydrocarbons, aromatic amines and nitroso compounds. Some of these chemicals, in particular, aromatic amine and nitrosamines are potent carcinogens in animal system where they induce tumours in several organs. In India large number of people has the habit of smoking direct and indirect. Since long, pipe smoking or Hookah smoking has been the practice of Rajas, Maharajas and also village folk. Pipe (Hookah) smoking facilitates filtration of smoke in water. It is a common experience that HW quickly turns to brown colour and highly toxic in nature. In this way this water absorbs many harmful ingredients of smoke especially nicotine. The assessment of the genotoxicity of this HW would highlight the beneficial effects of pipe smoking over direct smoking. Considering above the present work "The Effect of 'Hookah Water' on Haematological Parameters in common toad has been undertaken.

Review of Literature

The micronucleus was recognized in the end of the 19th century when Howell and Jolly found small inclusions in the blood taken from cats and rats. The small inclusions, called Howell-Jolly body, are also observed in the erythrocytes of peripheral blood from severe anaemia patients. These are the first description of the micronucleus itself. In 1959, Evans et al. reported that gamma-rays induced micronuclei in root tips of kidney beans, and tried to evaluate the chromosomal aberration quantitatively. This was the first report to evaluate chromosomal aberration by the frequency of cells harbouring micronucleus among normal cells and they estimated that about 60 % of the chromosomal fragments contributed to micronucleus formation. In 1970, Boller and Schmid developed a test method to evaluate the frequency of micronucleated erythrocytes among normal erythrocytes, which lack their own nuclei during haematopoiesis, using bone marrow and peripheral blood cells of Chinese Hamster treated with a strong alkylating agent, trenimon. In the paper, they named this method as "Mikrokern-Test (micronucleus test)". Up to the mid 1970's Schmid and Heddle's group built up the basics of the micronucleus test. In 1976, Countryman and Heddle reported a method using human cultured lymphocytes. Modifications have been introduced by Fenech and Moley using cytocharasin B and now the method is widely used for human monitoring. Inorganic biochemistry, biological inorganic chemistry or bioinorganic chemistry is a multidisciplinary scientific field which combines biological inorganic chemistry, biochemistry, biology, medicinal chemistry, microbiology and other fields (Latsis et al., 2018). Now a days, the research on the design and development of new metallodrugs [Small Bioactive Molecules (SBAMs) or Conjugates of Metals with Drugs (CoMeDs)] against numerous cancer types, includes their biological activity towards cancerous and non cancerous models (Banti et al., 2016; Sainis et al., 2016; Banti et al., 2018; Chrysouli et al., 2018a and 2018b; Latsis et al., 2018; Milionis et al., 2018; Stathopoulou et al., 2018; Banti et al., 2019; Polychronis et al., 2019).

Especially, the development of new SBAMs or CoMeDs imposes the need to assess their potential *in vitro* and *in vivo* toxicity against experimental models in order to check their potential risks (Banti et al., 2016; Sainis et al., 2016; Banti et al., 2018; Chrysouli et al., 2018a and 2018b; Latsis et al., 2018; Milionis et al., 2018; Stathopoulou et al., 2018; Banti et al., 2019; Polychronis et al., 2019). Therefore, the micronucleus assay has been developed in monitoring genetic damage in normal human cells as it is a sensitive tool for toxicity screening and it is capable in reducing the use of animals in toxicological testing (Sahu et al., 2014). The presence of micronucleus (MN) is a biomarker of mutagenic, genotoxic, or teratogenic agent influence (Torres-Bugarin et al., 2014). The *in vitro* genotoxicity is calculated by checking the micronucleus frequency, upon treatment of normal cells by an agent at the concentration of its IC₅₀ value (Chrysouli et al., 2018a and 2018b; Latsis et al., 2018; Milionis et al., 2018; Sainis et al., 2016; Stathopoulou et al., 2018).

Concepts & Hypothesis

Micronucleus is known as the Jolly bodies. The first serious attempt to use micronuclei as a monitor of cytogenetic damage reported by Evans et al. (1959). They reported that mutagens create acentric fragments during mitosis. These fragments are frequently excluded from the daughter nuclei and appear in the following interphase as MN. Beginning in 1970, Schmid and co-workers studied to determine which parameters might serve as the most useful indicators of cytogenetic damage in bone marrow *in vivo* (Schmid and Staiger, 1969; Boller and Schmid 1970. Matter and Schmid 1971, Von Ledebur and Schmid 1973, Matter et al., 1973, Heddle 1973). This work led to the conclusion that the incidence of MN – PCE was a useful index of *in vivo* bone marrow cytogenetic damage (Von Ledebur and Schmid 1973). The bone marrow cells of mutagen treated animal show micronucleus after completion of the last mitosis when on expulsion of the main nucleus from the erythroblasts, the MN if present would disappear as a minute chromatin structure in the free cytoplasm which was formed generally from anaphase laggards, asymmetrical exchange etc (Schmid, 1976). The MN could be detected for long time until it was made to disappear by the cytoplasmic enzymes, nucleases and proteases.

Research Design: (Materials and Methods)

In the present study Hookah Water was collected from the village people of Midnapore district in West Bengal. At the time of collection the smokers were asked about its preparation, no of smoking per day and the amount of water they used for smoking. The smoking water age was determined on the basis of their declaration. In this study different days old Hookah water was collected and stored in labelled vials. This was used as the test chemical. Common toad, *Bufo melanostictus* of both sexes were collected from the adjacent areas of Bankura serve as the test animal for the present study. The toads were kept in the rearing chamber and fed with small earthworm. Each toad was injected with test chemical at the rate of 4cc/100gms of body wt. Animals were sacrificed

after 1, 3, 7 and 15 days. Normal water used by the Hookah smokers was treated to the animals for control series. After specific duration the animals were sacrificed and immediately blood was collected by puncturing the heart. The smear was drawn and dried in air. The tissues are fixed with methanol and stained with Giemsa solution for 1½ hrs and washed with distilled water. Slides were dried and stored in a dust free slide box. 4000 cells (1000cells/ slides) were examined at random for the occurrence of micronucleus. Other nuclear and cellular anomalies were also recorded. Anomalies found were drawn with the help of camera Lucida and some were also photographed.

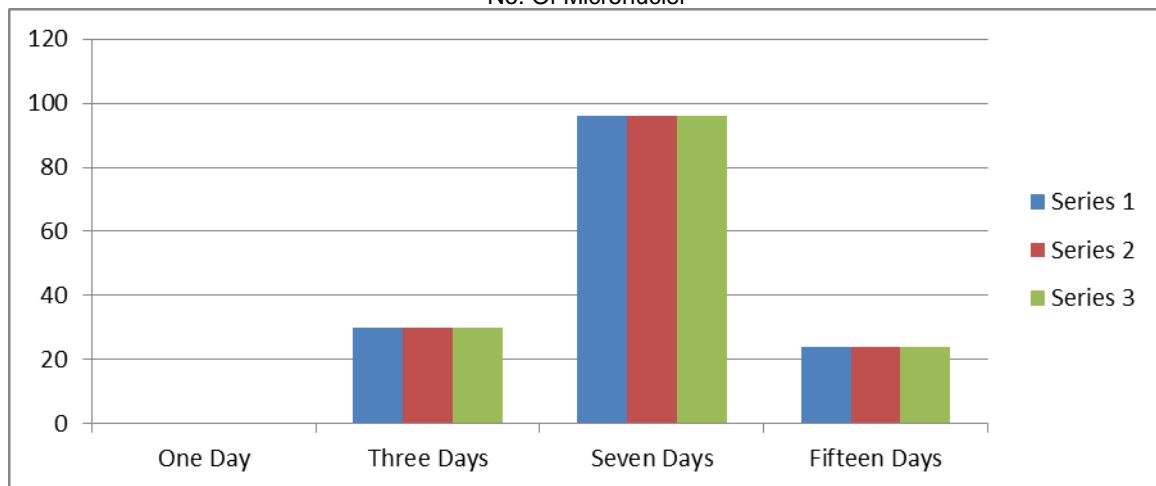
Findings

The results from the present investigation indicate that the aqueous absorbent of tobacco smoke could induce MN of erythrocytes of blood of toad. The induction of MN by any agent is indicative for its genotoxic, cytotoxic or aneugenic properties. Micronucleus may be formed due to chromosomal fragments or due to the elimination of a whole

chromosome. It is known that time factor is critical for the induction of any mutagenic event in the biological system. For such events especially in vivo condition the physiological factors like absorption, elimination and retention of the chemical are largely responsible. The chemical induced higher frequency of micronucleus over the control in treated series. Micronucleus size was small or dot shaped, sometimes it was larger. It is shown that the nucleus was divided into two halves (Fig; 8 & 9) or sometimes into three halves (Fig; 3, 7&10). The middle was larger than the others. In a number cells protruding cytoplasmic materials are observed (Fig; 4 &5). In some cells erythrocytes were observed with pointed cytoplasmic protrusions assuming the shape of balloon (Fig; 5). Cytoplasmic bridge also formed in Fig; 4. Quantitatively hookah water generates 30, 96, 24 number of MN after 3, 7 and 15 days exposure respectively (Table: 1). Cellular and nuclear anomalies are 42, 65,129, 127 after 1, 3, 7 and 15 days exposure respectively (Table: 2)

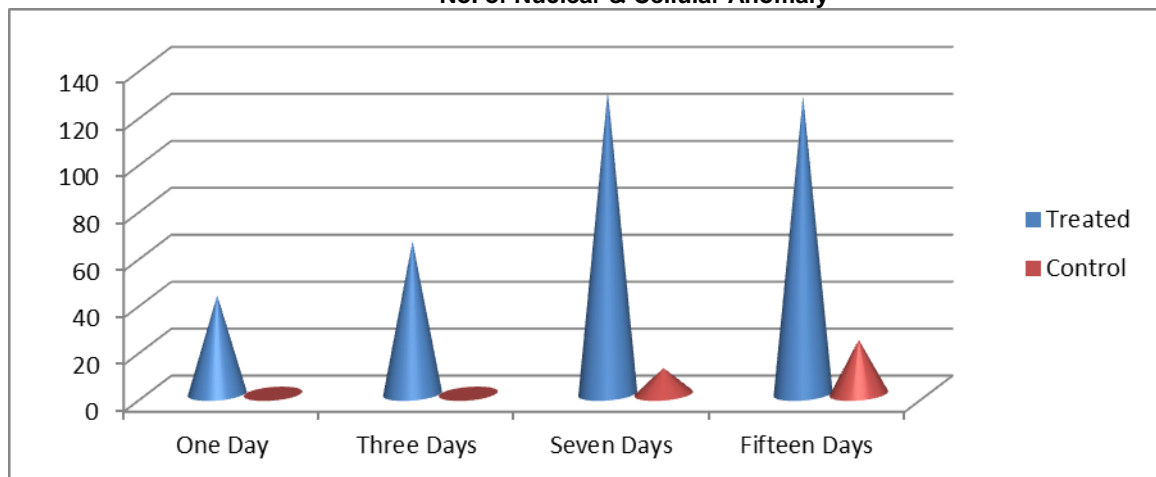
Frequency of Micronuclei

(Table-1)
No. Of Micronuclei

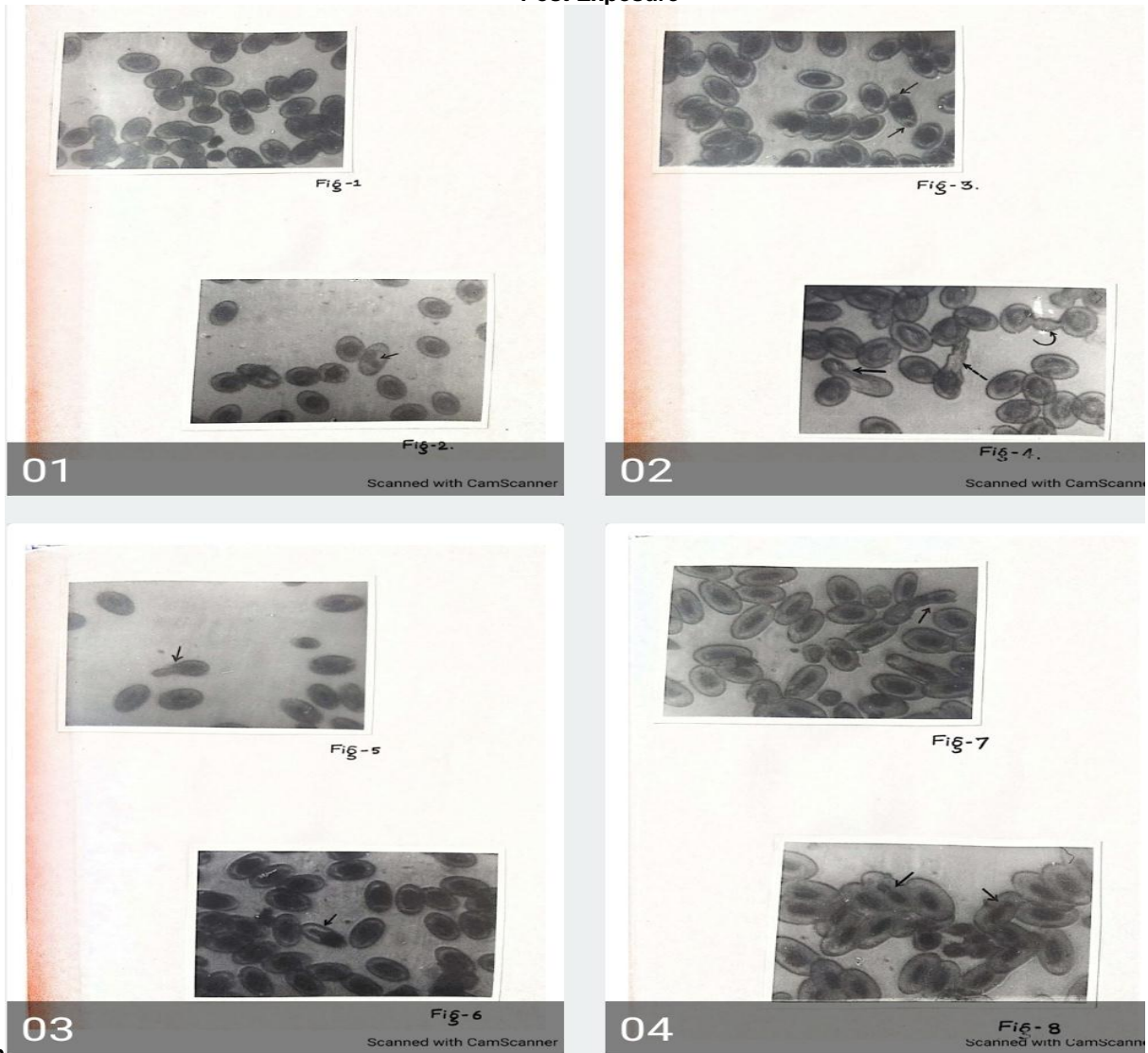


Post Exposure Time
Frequency of Cellular and Nuclear Anomaly
(Table-2)

No. of Nuclear & Cellular Anomaly

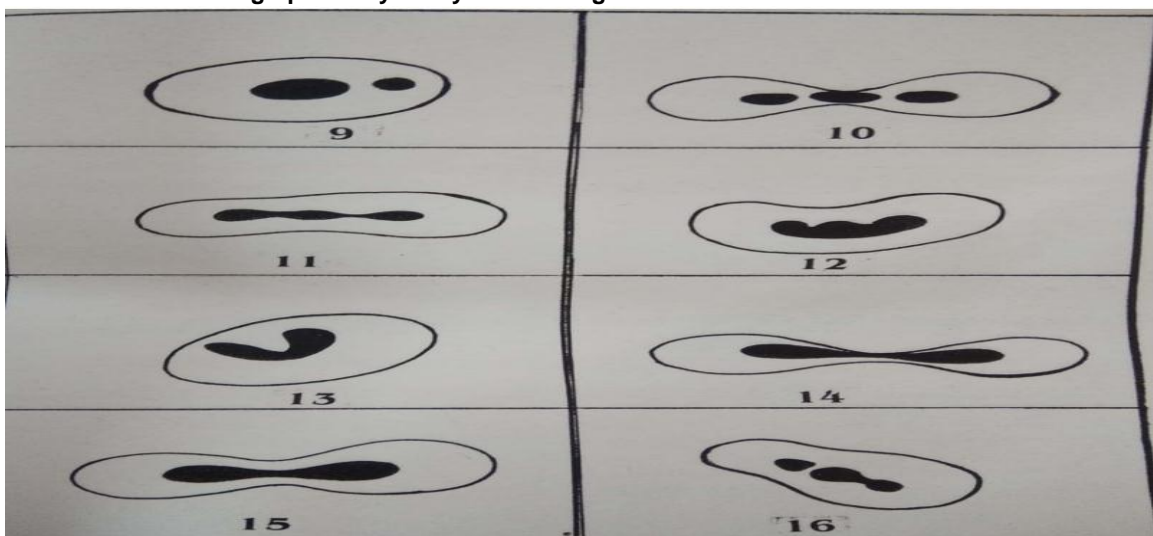


Post Exposure



Tim

Photomicrograph of Erythrocytes Showing Some Nuclear and Cellular Anomalies



Camera Lucida Diagrams Showing Some Nuclear and Chromosomal Anomalies

Conclusion

Since cigarette smoking causes several health problems in people, the findings clearly show that HW smoking has severe adverse effects on hematological parameters among common Toad. Results indicated that HW smoking led to increasing WBC count compared to control group. Kurtoglu reported that the mean leukocyte counts were significantly greater in smokers compared to nonsmokers. Friedman observed that WBC counts in smokers were about 20-25% higher than nonsmokers and also they increased with intensity of smoking. Watanabe found that not only WBC counts but also tumour necrosis factor (TNF) system activities increased in current smokers than nonsmokers. Light smoking associated with WBC counts, while heavy smoking related to TNF system activities. A group of researchers suggested that the increased leukocyte count might be due to nicotine-induced release of catecholamines, resulting in an increase in blood lymphocyte counts. In addition, the irritant effect of cigarette smoke on respiratory tree with resultant inflammation might be a contributory factor for higher WBC count. Also, it has been suggested that inflammatory stimulation of the bronchial tract induces an increase in inflammatory markers in the blood circulation. Another study showed that nicotine increased soluble Kit ligand, consistent with stem cell activation, followed by increase WBC count. The high WBC count may be a marker of smoking-induced tissue damage, and can be a risk factor for development of cardiovascular diseases through multiple pathologic mechanisms such as mediate inflammation, plug the microvasculature, induce hypercoagulability and promote infarct expansion.

The results indicated that RBC count values were significantly high in HW smoking. A normal carbon monoxide (CO) level in the blood stream is less than 8 PPM. A person that smokes one or two pack of cigarettes per day raises a blood CO level to 20 PPM. When a smoker stops smoking, the CO level in their blood stream typically returns to normal level within a few days. Hakim et al. compared the acute effects of a single 30-min session of HW smoking on concentration of carboxyhemoglobin (COHb) before and after smoking. The survey showed that COHb concentration significantly increased after HW smoking ($1.47\% \pm 0.57\%$ to $9.47\% \pm 5.52\%$). COHb is the molecule formed from the combination of CO and hemoglobin. The affinity of Co is 200-fold more than O₂ to Hb. Thus, CO displaces oxygen from Hb in RBC to produce COHb, which reduces the transfer of oxygen to tissues. When body tissues do not receive a continuous and adequate supply of oxygen; they starve and begin to suffocate, malfunction, and finally die. Oxygen affects RBC membranes because they have more polyunsaturated fatty acids than other body tissues. Cigarette smoke increased 2, 2'-azobis-(2-amidinopropane) dihydrochloride-induced erythrocyte haemolysis by 281.7%. Nicotine leads to 13.8% increase in erythrocytes membrane peroxidation at the highest concentration and its

effects are dose-dependent. The combination of CO in tobacco with effects of nicotine disrupts oxygen delivery to tissue and stimulates the bone marrow to produce more RBCs and thereby increase HCT and Hb. Stopping smoking should cause a slow return to pre-smoking levels. In people who smoked WP secondary polycythemia caused by CO poisoning and all blood parameters returned to normal ranges within six weeks after stopping smoking.

The results from the present investigation indicate that the aqueous absorbent of tobacco smoke "Hooka water" could induce MN of erythrocytes of blood of toad. The indication of MN by any agent is indicative of its genotoxic, cytotoxic or aneugenic properties. Micronuclei may be formed due to chromosomal fragments or due to the elimination of a whole chromosome. It is known that time factor is critical for the induction of any mutagenic event in the biological system. For such events especially in- vivo condition the physiological factors like absorption, elimination and retention of the chemical are largely responsible.

Genotoxic effects of some types of tobacco product have been studied earlier. Dash and Das (1992) studied the genotoxicity of "gudakhu" in mice in vivo and recorded significant increase of chromosome aberration, MN and sister chromatid exchanges. Earlier Stich and Anders (1989) reported higher incidence of MN in exfoliated cells of buccal mucosa of 7 habitual users of "gudakhu". Again Stich and his co-workers (1982), Brunnemann et al (1987), Stich and Anders (1989) recorded a higher incidence of MN in buccal mucosa cells of habitual users of several tobacco preparation like "khaini", "snuffs", "nass", "betel quid" with tobacco. Similarly a higher incidence of sister chromatid exchange have been noted in peripheral lymphocytes of persons chewing tobacco (Ghosh and Ghosh 1984). So the "Hooka" water containing nicotine and other ingredients of tobacco preparation proves to be genotoxic in the tested system.

In sum, RBC, Hb, HCT and WBC counts are higher in HW smokers, and these biomarkers might be associated with a greater risk for developing different diseases. Reduction in smoking improves some of these biomarkers. Additional research is necessary to determine which biomarkers are more sensitive to measure improved health and to what extent smoking needs to be reduced to obtain health benefits. We hope that these findings will be used by physicians and public health officials to inform HW tobacco smokers of the risk of tobacco-induced nicotine addiction and cardiovascular disease.

Suggestion:

Cigarette smoking causes several health problems in people, the findings clearly show that Hookah water smoking has severe adverse effects on haematological parameters among common Toad. Results indicated that HW smoking led to increasing WBC count compared to control group. Kurtoglu reported that the mean leukocyte counts were significantly greater in smokers compared to nonsmokers. Friedman observed that WBC counts in

smokers were about 20-25% higher than non-smokers and also they increased with intensity of smoking. It is found that not only WBC counts but also tumour necrosis factor (TNF) system activities increased in current smokers than non-smokers. Light smoking associated with WBC counts, while heavy smoking related to TNF system activities. A group of researchers suggested that the increased leukocyte count might be due to nicotine-induced release of catecholamines, resulting in an increase in blood lymphocyte counts. In addition, the irritant effect of cigarette smoke on respiratory tree with resultant inflammation might be a contributory factor for higher WBC count. Also, it has been suggested that inflammatory stimulation of the bronchial tract induces an increase in inflammatory markers in the blood circulation. The high WBC count may be a marker of smoking-induced tissue damage, and can be a risk factor for development of cardiovascular diseases through multiple pathologic mechanisms such as mediate inflammation, plug the microvasculature, induce hypercoagulability and promote infarct expansion. Additional research is necessary to determine which biomarkers are more sensitive to measure improved health and to what extent smoking needs to be reduced to obtain health benefits. We hope that these findings will be used by physicians and public health officials to inform HW tobacco smokers of the risk of tobacco-induced nicotine addiction and cardiovascular disease.

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