

ORIGINAL ARTICLE

Water Pipe A Safe Alternative To Cigarette? A Comparative Study on Hematological and Biochemical Parameters in Mice Exposed to Cigarette and Water Pipe Smoke

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ABSTRACT

Objective: To compare the toxic effects of cigarette and water pipe/shisha smoking on hematological parameters such as hemoglobin (Hb) and Total Leukocyte Count (TLC) and also on serum Triglycerides levels (TAG) in BALB/c mice.

Study Design: Experimental randomized controlled study.

Place and Duration of Study: The study was carried out at Islamic International Medical College, department of Anatomy in collaboration with National Institute of Health. Duration of the study was six months, from October 1st 2013 till March 30th 2014.

Materials and Methods: Forty adult male mice of strain BALB/c were obtained from National Institute of Health and were randomly divided into three groups. They were kept in whole body smoke exposure chamber. Control group C was exposed to fresh air. Cigarette smoke group CS and Shisha/water pipe smoke group SS were exposed to cigarette and water pipe smoke respectively in separate chambers for 8 weeks. After 8 weeks, blood samples were collected by cardiac puncture in separately labeled test tubes. Blood was analyzed for hematological parameters including Hemoglobin and Total Leukocyte count and also Triglycerides and results were compared among three experimental groups. Data was analyzed using SPSS version 20.0. Mean \pm S.D was used for quantitative hematological and biochemical parameters. A p-value of ≤ 0.05 was considered as statistically significant.

Results: Average hemoglobin was 11.95 ± 0.792 g/dl in control group C, 13.80 ± 1.095 g/dl in group CS and 13.66 ± 1.349 g/dl in group SS. Control group had significantly lower average hemoglobin as compared to group CS ($p < 0.05$) and group SS ($p < 0.05$). Average serum Triglyceride level was 102.50 ± 31.785 mg/dl in control group, 127.80 ± 13.487 mg/dl in group CS and 147.00 ± 18.326 mg/dl in group SS. All the groups were significantly different from each other ($p < 0.05$). Median Total Leukocyte count was $5900/\mu\text{l}$ (IQR: 5150-6875 μl) in control group, $5200/\mu\text{l}$ (IQR: 4200 – 7200 μl) in group CS and $9900/\mu\text{l}$ (5300 – 13300 μl) in group SS. Difference among three groups was significant ($p < 0.001$).

Conclusion: Water pipe smoking is more toxic than cigarette smoking in terms of its adverse effects on Hemoglobin, Total Leukocyte Count and Serum Triglycerides levels.

Key Words: Cigarette Smoking, Hemoglobin, Total Leukocyte Count, Triglycerides, Water Pipe Smoking.

Introduction

Researches show that life span of a tobacco smoker, on an average, is reduced by approximately ten years as compared to people who do not smoke.¹ There are

multiple ways of tobacco smoking with cultural differences around the globe. Common ways of smoking include cigar, cigarettes, hookah, water pipe, vaporizers and kretek.¹ Tobacco, smoked by any means, contains more than 4000 compounds and free radicals among which, nicotine and carbon monoxide are responsible for most of the deleterious effects associated with long term smoking. In addition to many known cardiovascular, respiratory and neurological disorders, these free radicals are believed to cause derangements in hematological parameters like hemoglobin, white cells count and triglycerides levels.²

Carboxy hemoglobin is formed when carbon

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monoxide binds with hemoglobin resulting in left sided shift of the oxygen-hemoglobin dissociation curve. This results in reduced ability of the hemoglobin to carry and deliver oxygen to the tissue leading to compensatory high levels of hemoglobin in smokers.^{2,3}

Total leucocyte cell count is a useful, relatively inexpensive biomarker used to assess endothelial damage and is also an important predictor of atherosclerosis and cardiovascular disease. Nicotine in tobacco smoke induces an inflammatory response in the tracheobronchial tree along with an increase in the inflammatory markers in the blood. Smokers with high TLC count might be at greater risk for developing atherosclerosis and cardiovascular diseases as compared to non-smokers.³

Similarly, a proatherogenic lipid profile is also related to an increased incidence of coronary artery disease in smokers. Studies have shown high levels of triglycerides in smokers posing a greater risk of atherosclerotic changes in cardiovascular system.⁴ Nicotine stimulates adrenal-sympathetic system which releases catecholamine. This surge in catecholamines induces lipolysis, thus increasing levels of serum free fatty acid, promoting cholesterol synthesis, and secretion in the liver.⁵

Recently, water pipe smoking has regained popularity especially in young population. When compared to a cigarette, water pipe tobacco smoking is associated with almost four-fold more carboxy hemoglobin, and more than 50-fold inhaled volume of smoke. Thus, water pipe smoking is associated with more risk of dependence, disease, and death.⁶

It is a common misconception that water pipe smoke is comparatively less harmful than cigarette smoke because the smoke first passes through water before being inhaled through the mouth piece. Thus, it filters the smoke from tar, nicotine and other carcinogenic substances. On the contrary, the water only cools down the smoke³ and this leads to deeper inhalation by the smoker. This causes deep penetration of smoke into the lungs leading to adverse health effects.⁷ In fact, water pipe smoking has been suspected to be a risk factor for a number of diseases such as lung and esophageal cancer, cardiovascular disease and adverse pregnancy outcomes.⁸ Mainstream hookah smoke contains several toxicants including tar, nicotine, carbon

monoxide, ammonia, polycyclic aromatic hydrocarbons (PAHs), aldehydes, phenolic compounds and heavy metals, to name a few.⁹ The difference in heating process and charcoal combustion might lead to more toxicants in water pipe smoke. A complete session of water pipe smoking usually lasts 60 minutes, with smokers taking up to two hundred puffs. On the other hand, cigarette smoking takes about 7 minutes with 8-12 puffs/cigarette. There is thus possibility of inhaling higher levels of toxicants in water pipe smoking than cigarette smoking.¹⁰

This study was thus done with an objective to compare the toxic effects of cigarette and shisha/water pipe smoking on hematological parameters such as hemoglobin (Hb) and Total Leukocyte count and on biochemical parameter Serum Triglycerides levels (TAG) in BALB/c mice.

Materials and Methods

This randomized controlled trial was conducted at Islamic International Medical College, Rawalpindi, in the department of Anatomy in collaboration with National Institute of Health Islamabad.^{10,11} The Institutional Review Committee of Riphah International University approved the study design and duration of the study was six months, from October 1st 2013 till March 30th 2014. Sample size consisted of forty male BALB/c mice, weighing 35-45g which were obtained from animal house of NIH, Islamabad and were kept in separate cages. Mice with any pathology and less than 3 months old, weighing less than 35g or more than 45g were not included. Female mice were not used because of risk of pregnancy. All mice were kept in a room at 24°C with 12 hours light and dark cycle and were given pelleted diet. They were acclimatized for one week before smoke exposure. They were randomly divided into 3 groups; Control Group C n =10, Shisha Smoke (SS) Group n =15 and Cigarette Smoke (CS) Group n =15. Simple randomization was done using random allocation cards through computer-generated random numbers.

For water pipe smoke commercial *Ma'assel* was used that contains 30% tobacco and 70% honey or molasses.¹¹ Nonfiltered cigarettes of a local brand with known percentage of nicotine content were used for cigarette smoke. Locally made plastic chamber were used for whole body inhalational

exposure.¹² These chambers were designed according to the WHO specifications.^{13,14}

Approximately 10g of water pipe flavor was placed in the plate on the top of water pipe apparatus and covered with aluminum foil. Hot coal biscuit was placed over it and smoke was introduced as puffs into the chamber using a manual vacuum pump.

Twenty commercial non-filtered cigarettes were ignited and placed in a plastic stand and placed in the chamber of CS group. The smoke-exposure group CS was exposed to nicotine concentrations equivalent to Shisha/water pipe smoke group. The flavor used in Shisha/water pipe contains approximately 2.5 mg of nicotine.¹⁵ The nicotine content in side stream smoke of one cigarette is 0.12 mg. So, nicotine content in 20 cigarettes is 2.4 mg.¹⁶

The mice in experimental groups were exposed to smoke until all water pipe flavor and cigarettes were consumed, which took approximately two hours. Mice were exposed to water pipe and cigarette smoke in morning and evening for two months. All mice were sacrificed at the end of 2nd Month. They were anaesthetized 24 hours after the last dose of smoke by placing them in a closed glass container with cotton balls soaked in chloroform, until they lose consciousness.

Blood samples were collected by cardiac puncture in separately labeled test tubes. The blood for hemoglobin and Total Leucocyte counts was collected in test tubes with anticoagulants while the blood for triglycerides was collected in plain test tubes and serum was extracted after centrifugation. The values were obtained by using commercially available kits. (Hemoglobin meter hb-101 certeza Germany for Hb and Coulter® DxH 800 analyzer for TLC).

Statistical analysis was done using SPSS version 20.0. Mean \pm S.D was used for quantitative biochemical parameters. The quantitative variables Hb, TLC count and TAG were checked for normal distribution by applying Kolmogorov-Smirnov test that showed that Hb and TAG were normally distributed variables. One Way Analysis of Variance (ANOVA) was used to compare the mean differences of normally distributed data of Hb and TAG levels among three groups. Post-hoc tukey test was applied to compare which group mean differs individually from other two groups. A p-value of ≤ 0.05 was considered as

statistically significant.

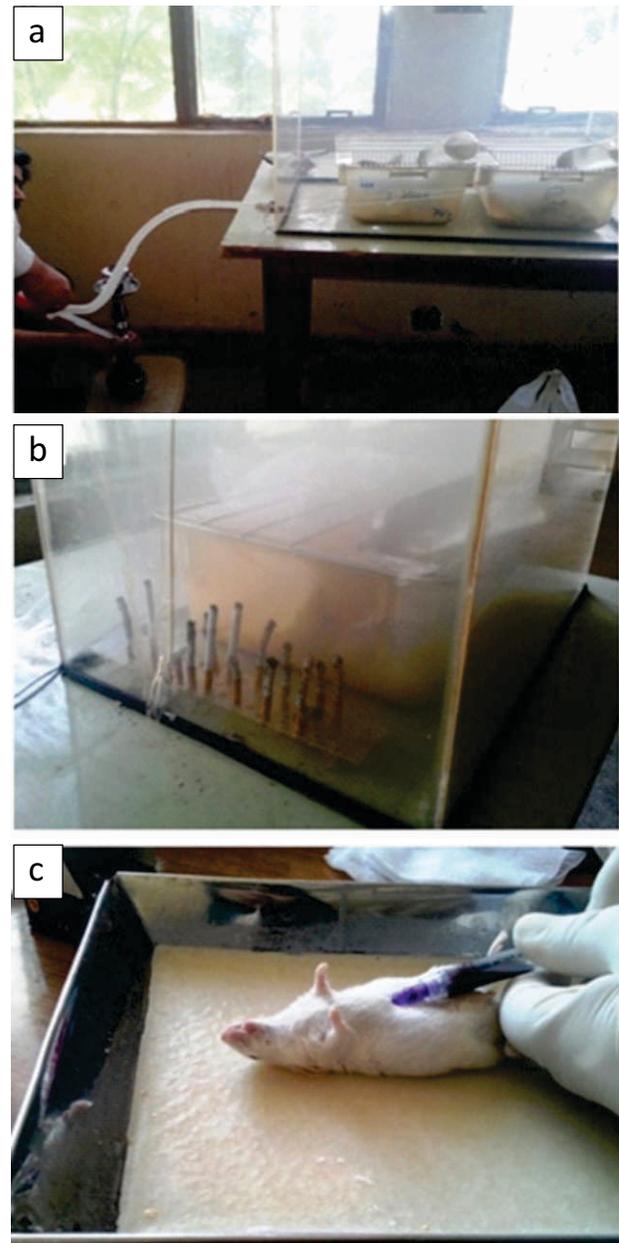


Fig 1 (a) Photograph showing generation of water pipe smoke with manual vacuum pump^{11,12}, (b) whole body cigarette smoke exposure chamber showing burnt cigarettes and (c) blood collection through cardiac puncture.

Results

The average hemoglobin was 11.95 ± 0.792 g/dl in control group C, 13.80 ± 1.095 g/dl in group CS and 13.66 ± 1.349 g/dl in group SS. ANOVA test showed a significant p- value and post hoc test was then applied to compare all the three groups individually with each other. The CS and SS groups were

significantly ($p < 0.05$) different from control group C. Control group had significantly lower average hemoglobin as compared to group CS ($p < 0.05$) and group SS ($p < 0.05$). While the difference in hemoglobin between experimental group CS and experimental group SS was not significantly ($p > 0.05$) different. Group CS although had higher average hemoglobin level than group SS but this difference was insignificant statistically (Table-I).

The average serum triglyceride level was 102.50 ± 31.785 mg/dl in control group, 127.80 ± 13.487 mg/dl in group CS and 147.00 ± 18.326 mg/dl in group SS. All the groups were significantly different from each other ($p < 0.05$). Control group had significantly lower average serum triglyceride level as compared to group CS ($p < 0.05$) and group SS ($p < 0.05$) while the group SS had significantly ($p < 0.05$) higher level of TG as compare to CS group (Table-I).

The Total Leukocyte count (TLC) was normal in the control group C (normal range: $5500-11,000/\mu\text{l}$). The data of TLC count did not follow the normal distribution based upon Kolmogorov Smirnov test of normality so Interquartile range (IQR) was calculated for this data. Median TLC count was $5900/\mu\text{l}$ (IQR: $5150-6875/\mu\text{l}$) in control group, $5200/\mu\text{l}$ (IQR: $4200 - 7200/\mu\text{l}$) in group CS and $9900/\mu\text{l}$ ($5300 - 13300/\mu\text{l}$) in group SS. Difference among three groups was significant ($p < 0.001$) (Table-II).

Table I: Post Hoc Comparison of Hemoglobin (G/Dl) and Serum Triglycerides (Mg/Dl) Between the Groups.

Groups	Hemoglobin(g/dl)			Serum Triglycerides(mg/dl)		
	Mean	SD	p-Value	Mean	SD	p-Value
Group C vs. Group CS	11.95	.792	0.001*	102.50	31.785	0.015*
	13.80	1.095		127.80	13.487	
Group C vs. Group SS	11.95	.792	0.002*	102.50	31.785	0.000*
	13.66	1.349		147.00	18.326	
Group CS vs. Group SS	13.80	1.095	0.939 ‡	127.80	13.487	0.044*
	13.66	1.349		147.00	18.326	

* Difference is Significant at 5% level of confidence

‡ Difference is not Significant at 5% level of confidence

Table II: Comparison of White Cell Count between the Group CS and SS

Groups	Total Leukocyte Count/ μl Median (IQR)
Group C (n = 10)	5900 (5150 – 6875)
Group CS (n = 15)	5200 (4200 – 7200)
Group SS (n = 15)	9900 (5300 – 13300)
p-value	0.031*

* P-value by Kruskal-Wallis test is Significant at 5%

Confidence level

Discussion

The tobacco smoke is known to cause many biochemical changes in the body including alterations in hemoglobin, white cell counts and triglycerides levels. Free radicals and peroxides generated during smoking are reportedly linked with physiological changes in the body such as increased synthesis of prostaglandins and thromboxane and advanced pathological changes like atherosclerosis, inflammation and metastatic changes. Effects of smoking on antioxidant status and hematological parameters have been extensively studied but results on comparative effects of cigarette and water pipe smoking are inconsistent. In this study, hematological parameters were used for comparative analysis among study groups.

The hemoglobin levels showed significant differences between control and experimental groups. While it was normal in control group C, the value was higher in both experimental groups SS and CS. Similar results have been shown by Asif³, Shah BK¹⁶, and Kumar¹⁷ in their studies done on effects of cigarette smoke on hemoglobin levels. According to Rabbe¹⁸ normal value of hemoglobin in male mice is 13.3-16.1g/dl.

Carboxyhemoglobin, formed after binding of carbon monoxide with oxygen, is an inactive compound that shifts the dissociation curve of hemoglobin to the left side. This reduces the ability of hemoglobin to deliver oxygen to the tissue. Tissue hypoxia leads to an increased secretion of erythropoietin causing enhanced erythropoiesis. Smokers, thus, maintain a higher hemoglobin level than non-smokers^{2,3} to compensate the tissue hypoxia.

The difference between the values of group CS and SS was highly significant. A single water pipe session

delivers higher levels of CO than a single cigarette⁷, which explains the higher levels of hemoglobin in group SS as compared to group CS in this research. In a comparative study by Eissenberg¹⁹, the first five minutes of water pipe smoking produced more than four times more carboxyhemoglobin as compared to an entire cigarette.

The normal range of TLC count in mice is 5000-12,000/ μ l.²⁰ TLC count was increased in smoke exposure groups as compared to the control. An increase in TLC count in smokers has been reported previously by Kume^{16, 21}, Shenwai,²² Asif³ and Malenica.²³ All of them have observed an increased total leukocyte count in smokers as compared to nonsmokers. According to Shenwai,²² toxicants in smoke cause marked release of inflammatory cytokines from the epithelial cells which influence the growth, differentiation and activation of leucocytes. Inflammation also cause endothelium damage and increased aggregation of leukocytes that further predispose to microcirculatory occlusion and vascular damage. This can be the underlying mechanism of leukocytosis in smokers. Nicotine also causes a surge in circulating catecholamine which can increase the total white cell count.²²

The difference between group CS and SS was statistically significant. This could be possible due to large volume of smoke per puff in Shisha/water pipe smoking as compared to cigarettes. According to Chaouachi¹⁵, cigarette smokers on an average take 7-10 puffs, inhaling a total of 550 ml of smoke. In contrast, complete water pipe session usually encompasses 60 minutes with around 150 puffs. The smoker inhales 500 ml of smoke in each puff.^{11,12} Thus one complete session of water pipe smoking produces 50,000ml of smoke¹⁵ causing much higher degree of systemic inflammation and leucocytosis.

The comparative analysis of serum triglycerides (TAG) also showed differences among study groups. The normal range of serum triglyceride in mice is 102-188mg/dl.²⁴ The control group C showed a normal average level of 126mg/dl while group CS and SS showed an increase in triglyceride levels. This has been reported earlier in cigarette smokers by Yan-Ling²⁵, Rashidi²⁶, Alharbi²⁷ and Lietz.⁴ Shafique²⁸ observed a significantly increased levels of TAG in water pipe smokers. Sahnoun²⁹ in his comparative research on water pipe and cigarette observed an

elevated TAG levels with no significant difference between the two groups. This supports the finding in this research in which the difference between group CS and SS was statistically insignificant ($p=0.078$).

According to Rashidi²⁶, smokers are susceptible to metabolic disorders characterized by insulin resistance, which increases postprandial triglyceride and low density lipoprotein. Similar results are reported by Mc Govern³⁰ who observed that high doses of nicotine lead to resistance to insulin. This causes rapid surge in the levels of catecholamines. Smokers have low levels of adiponectin, an adipose-derived protein which modulates insulin sensitivity and has anti-inflammatory effects. The insulin resistance alters body composition leading to an increased fat in viscera.

Conclusion

Water pipe/Shisha smoke contains higher levels of chemical irritants that cause respiratory tract inflammation as well as derangements in many hematological and biochemical parameters that are indicators of several systemic disorders. The damage is more as compared to cigarette smoke given in same quantity and for same duration. Thus, water pipe smoking is not a safe alternative to cigarette smoking.

Limitation of the study Baseline values of hematological and biochemical parameters could not be recorded in experimental animals because of their small age and size. Thus, values were compared with those of control group.

Future recommendations Further experimental studies can be carried out on rabbits or even humans comparing effects of cigarette and water pipe smoke with baseline parameters.

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