REPORT

Significance of herbal medicine in removing excessive iron content in human

Wan Mohd Azhar bin Wan Ibrahim[1*], Eraj Humayun Mirza[2] and Syed Fahad Akbar Ali[3]
[1-2]Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, Kuala Lumpur, Malaysia
[3]Sir Syed University of Engineering & Technology, Karachi, Pakistan

Abstract: Heavy metals in cigarette tobacco such as iron may cause a serious damage on human health. Surveys showed that the accumulation of certain toxic heavy metals like cadmium, mercury, iron is very often due to the effect of smoking. This work involved 15 volunteers in two randomly divided groups having the habit of cigarette smoking over 15 cigarettes / day. Concentration level of iron in blood and urine before and after treatment using the herbal medicine, widely used in Europe, is analyzed. Determination of Iron concentration in blood and urine was calculated by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) according to the procedure DIN EN ISO 11885 ("E22" from April 1998). The analysis shows that the concentration of iron in blood and urine samples in both groups increased in some volunteers instead of decrease. The independent T-test shows that the mean of iron concentration in the group A and group B had no significant difference ($p>0.05$). The results suggested that the herbal medicine under test does not have significant influence on reduction of iron concentration levels.

Keywords: Iron concentration, cigarette smoking, herbal medicine, heavy metals, toxicology.

INTRODUCTION

According to World Health Organization (WHO), half of the people who smoke tobacco are killed by it. WHO also express its concerns of more than 8 million deaths per annum from smoking tobacco by 2030, if urgent action is not taken to prevent it (WHO, 2012). Smoking tobacco increases the risk for various acute and chronic diseases (Weinberg, 2009). In the smoke of cigarette, there are various metals present, like Cadmium, Zinc, Copper, Iron, and Magnesium (Unkiewicz-Winiarczyk *et al*., 2009). Heavy metals from the tobacco in cigarette may cause serious and irrevocable damage on human health (Mussalo-Rauhamaa *et al*., 1986). It was also found that certain toxic elements, like cadmium and iron in blood of smokers were much higher when compared to those who do not smoke. Hence monitoring of heavy metals in tobacco is essential for good health (Willers *et al*., 1992). Iron plays a pivotal role physiologically in humans but its excess can be of great danger. Excessive storage of iron in the body may lead to generation of free radicals that propagate to cause damage to wide range of cellular structure; this may lead ultimately to cancer and various heart related disorders. Therefore it is of sheer importance to have optimal concentration of iron in the body for stable health (Tuomainen *et al*., 1998). Studies reveal that one pack of cigarette per day leads to 1μg of iron intake per day (Weinberg, 2009). It was also revealed that alveolar macrophages of smokers had 6 times more iron than nonsmokers (Mateos *et al*., 1998). In a more recent study cause of unexplained fetal and infant death was evaluated from the effect of maternal cigarette smoke. This study showed that maternal cigarette smoking habit may cause unexplained fetal and infant death due to free iron deposition in the brain. This may also result due to nicotine absorption (Lavezzi *et al*., 2011). Recent studies also showed that cigarette smoke encouraged the release of iron and it may ultimately modify iron metabolism in the lungs of chronic smokers, this leads to increase in total oxidative burden on the lungs of the smokers (Kim *et al*., 2012). Research has also shown that smoking cigarette causes impaired hearing, lung cancer, oral cancer, hepatic cancer, osteoporosis and other vascular diseases (Weinberg, 2009). Now the question is how iron affects the health of the living being. In a study to explore the role of iron overload in non-alcoholic subjects, rats were examined for serum cholesterol, triglyceride, transaminase, hepatic homogenate iron content and liver tissue disease changes in the characteristics of science. The results showed that higher iron concentration plays an important role in the process of non-alcoholic steatohepatitis development (David A, 2005). In another review in March 2012, the increase in iron content was considered to be the mediator of oxidative stress in Alzheimer disease (Castellani *et al*., 2012). How can level of increased iron be reduced or controlled is another issue. There are various medicines available in the market for this purpose and are more commonly known as chelators. The chelants work to remove the toxic agents by forming soluble, complex molecules with certain metal ions, resulting in inactivating the ions, hence they cannot interact normally with other elements or more precisely

**Corresponding author:* e-mail: wmohdazhar@yahoo.com

they no more remain a threat for the human being. In 1991 U.S. FDA approved the use of chelants. This is therapy is now called as chelation therapy. In a study, increased iron uptake was related to toxicity and to control this was considered necessary. The most often role of iron is the toxicity linked with catalytic decomposition of hydrogen peroxide leading to the formation of reactive oxygen species that cause damage to biomolecules, this include lipids, proteins and DNA. The iron specific chelators bind with nitrogen, oxygen or sulphur donor atoms resulting in blocking the ability of iron to catalyze the formation of free radicals (Jomova and Valko, 2011). The aim of this research is to study the efficacy of herbal medicine that is widely available in the European market. The producers of this medicine claim that it contains a concentrated plant extract which contains polyphenols and it targets the gastrointestinal tract. Ultimately the herbal medicine helps in release of heavy metals through urine which were taken orally or by cigarette smoke inhalation.

METHODOLOGY

Experimental Setup

This study was performed on 15 volunteers who smoked more than 15 cigarettes per day. Initially, 20 volunteers were recruited, among which 15 agreed to participate actively in herbal medicine study. Every treatment or trial have some kind of risks associated, but in case of this herbal medicine, there is no damaging response till now, it is extensively presented in European market as chelation therapy. Volunteers were told that no adverse risk was associated with the use of this herbal medicine. Orange juice is considered to have iron content reduction effect but it is immensely slow (Franke *et al*., 2006). In this study the orange juice is used only to reduce the bitterness of this herbal medicine.15 volunteers were divided in 2 groups (A & B) by using scientific tables of Geigy 1959 with arbitrary numbers. Blood and urine samples of all the 15 volunteers were taken prior to treatment. Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) was used to measure the iron content in the collected samples before treatment.

In the first stage of the study, Group A comprising 7 volunteers were asked to use 50 ml of orange juice. On the other hand Group B comprising of 8 volunteers were asked to use 100 ml of herbal medicine mixed with orange juice in 1:1 ratio, twice a day. The treatments were followed for 4 weeks. After this treatment, immediately a second sample of blood and urine were obtained and iron concentration was determined with ICP -AES technique.

In the second stage of the study, both the groups were asked to interchange the treatment and continue for 4 weeks. At the end of this phase, urine and blood samples were collected from all 15 volunteers. Using ICP-AES iron concentration was determined another time.

Experimental implementation for iron determination

Concentration of iron was plotted against absorbance, using iron standard solution of 1g/Liter having with an iron content of 20 mg /Liter and further six standards with the concentration of 0.01 mg/Liter, 0.250 mg/Liter, 2.0 mg /Liter, 5.0 mg/Liter, 10.0 mg /Liter and 20.0 mg /Liter were prepared.

Iron content was determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) according to Ibrahim *et al*. (Ibrahim WMA, 2012). Blood sample was prepared by taking 10 ml of lyophilized blood into 25 ml flask. 0.4 ml of 30% Hydrogen Peroxide (H_2O_2) and 7 ml of 65% Nitric Acid (HNO_3) were then added. The sample was transferred into a vacuumed steel chamber at 140^oC and kept for 3 hours for decomposition. Iron content was determined by transferring the mixture in to the ICP -AES. Urine sample was prepared by taking 20 ml of urine into 25 ml flask and adding 0.5 ml of 65% Nitric Acid (HNO_3). The solution was then transferred into a small polystyrene beaker which was kept on the heating plate for 2 hours. The sample volume was adjusted by adding 20 ml of deionized water. The iron content was determined using ICP -AES technique.

RESULTS

Table 1 shows the results of independence T-test for blood samples. The result for the first stage was the comparison between the iron concentration from the first and second blood samples collection. The means and standard deviation of iron concentration before treatments in group A and B were 2.37(0.22) and 2.34(0.25) respectively. After 4 weeks of treatments, the means and standard deviation of iron concentration were 2.35(0.12) and 2.31(0.11) respectively. Independence T-test between group A and B before the treatment started gave $p > 0.05$. The same results were revealed for the test after 4 weeks of treatment. The results for the comparison before and after treatments for group A also gave $p>0.05$, similar results were obtained for group B.

The result for the second stage was the comparison between the iron concentration from the second and third sample. The means and standard deviation of iron concentration of the second blood samples collection are mentioned earlier. After 4 weeks of treatments, the means and standard deviation of iron concentration in blood was 2.40(0.09) for group A and 2.34(0.10) for group B. Independence T-test between group A and B gave $p> 0.05$.Similarly for the comparison between iron concentration from second and third blood samples collection for group A gave p 0.05. The t-test also showed the same result for the group B. Table 2 shows the results for the urine samples. The first, second and third measurements of iron concentration in urine samples for group A, revealed the mean value and the standard deviation of

Table 1: Result for blood samples

Test Comparison	Group A	Group B	Level of Significance Equality of Means
	Mean (Std Dev)mg/L	Mean (Std Dev)mg/L	
First Sample Measurements Second Sample Measurements	2.37 (0.22) 2.35 (0.12)	2.34 (0.25) 2.31 (0.11)	P > 0.05
Second Sample Measurements Third Sample Measurements	2.35 (0.12) 2.40 (0.09)	2.31 (0.11) 2.34 (0.10)	P > 0.05

Table 2: Result for urine samples

Test Comparison	Group A	Group B	Level of Significance Equality of Means
	Mean (Std Dev)mg/L	Mean (Std Dev)mg/L	
First Sample Measurements Second Sample Measurements	0.057 (0.015) 0.076 (0.008)	0.071(0.035) 0.069 (0.033)	P > 0.05
Second Sample Measurements Third Sample Measurements	0.076 (0.008) 0.13 (0.074)	0.069 (0.033) 0.11 (0.033)	P > 0.05

0.057(0.015), 0.076(0.008) and 0.13 (0.074) respectively. Similarly the means and standard deviation of iron concentration for group B were 0.071(0.035), 0.069 (0.033) and 0.11(0.033). The t-test also showed similar results as for the blood samples. Statistical analysis were performed with 95% confidence level with the IBM's software SPSS. The level of significance by equality of means was found and the results revealed that the herbal medicine showed no significant effect in lowering the heavy metals from the blood or through the urine.

DISCUSSION

This study was based on the efficacy of herbal medicine that claims to remove heavy metals from the human body. All the volunteers were confirmed cigarette smokers who smoked more than 15 cigarettes a day. The efficacy of herbal medicine was found lacking in removing the heavy metal, in our case iron content from the blood stream or through the urine. Form table I and II, no significant deviation was found in the iron level where samples were treated in very similar way specified in case of other heavy metals by using Inductively Coupled Plasma-Atomic Emission Spectroscopy to analyze the influence of this herbal medicine on iron concentration in blood and urine samples. From the statistical analysis it was clear that herbal medicine cannot efficiently lower the heavy metal concentration from the subject's blood. This greatly arise a concern on the efficacy of other herbal medicines available in the market. This observation could be a step towards eliminating the use of this herbal medicine as an effective medication for removing the heavy metals from body. Also this study can form a base for further research on other brands who claim to produce the same results.

CONCLUSION

The aim of this study was that to see the effect of herbal medicine as a reducing agent of increased concentrations of the heavy metal such as iron in blood and urine. The study was performed among 15 volunteers with the habit of cigarettes smoking. The study was continued until 8 weeks. The analysis showed that this herbal medicine had no significant effect on removal of heavy metal from the body.

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