

ORIGINAL RESEARCH

**THE EFFECT OF SMOKING MODELS ON THE SERUM LIVER ENZYMES**Suha Khaiery Ababneh<sup>1\*</sup>, Ziad Shraideh<sup>1</sup>, Darwish H. Badran<sup>2</sup><sup>1</sup>Department of Biological Sciences, Faculty of Science, The University of Jordan, Amman, Jordan<sup>2</sup>Department of Anatomy and Histology, Faculty of Medicine, The University of Jordan, Amman, Jordan

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**ABSTRACT:**

**Introduction:** Smoking has been associated with adverse health effects including cardiovascular system and other body organs.

**Study objectives:** The main objective of the present study is to evaluate the effects of smoking models on the level of liver enzymes.

**Methods:** This experimental study was carried out on 30 male albino rats. Male albino rats (*Rattusratas*) weighted 50-180 g (6-8 weeks old) were used. Rats were randomly assigned to one of 3 groups (n= 10 per group), group 1 was negative control exposed only to fresh air, groups 2 exposed to the most commonly used cigarette brands in the Jordanian market (red LM cigarettes) as 1 cigarette/rat/day for 30 consecutive days. Rats in groups 3 were exposed to flavored water-pipe coming from the complete burning of 20 g from one of moassal for a period of 30 days one session a day for whole body. A digital smoking machine was used. Liver enzymes, LDH, AST, and ALT were evaluated in study groups. Data obtained were analyzed using SPSS version 20.

**Results:** Liver enzymes were significantly increased as a result of exposure to both smoking models: cigarette smoking and water-pipe smoking. It was interestingly found that smoking cessation restore liver enzymes close to control group.

**Conclusion:** Smoking models can induce liver injury as reflected by increased levels of liver enzymes and this injury is reversible if smoking is quitted.

**KEYWORDS:** cigarette smoking, water-pipe smoking, liver enzymes, ALT, AST, LDH

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## INTRODUCTION

Tobacco smoking is a global epidemic phenomenon in developing countries, and considered as the earliest example of noninfectious disease that causes preventable deaths in the world. It has been estimated that tobacco smoking is responsible for killing more than six million people in 2010<sup>1</sup>, while it is expected that by 2030 the death exceeds million per year in developing countries<sup>2</sup>.

There are different methods to smoke tobacco including cigarettes, cigars, chew, pipes or water-pipe. Water-pipe smoking and cigarette smoking are currently considered a fashionable way of tobacco leaves consumption, especially among young and middle aged males and females<sup>3</sup>.

Primarily, there are two types of narghile tobacco mixtures: the flavored one could be either moassel (also known as tobamel) or jurak, and the unflavored type called tumbak (or ajamy). The tobacco used in water-pipe system (WPS) typically weighs 10 to 20 gms. "Muessel" or "maasel" (literally, "honeyed") contains 30% tobacco and 70% honey or molasses (treacle). "Tumbak" or "ajami" is a pure, dark paste of tobacco. "Jurak," mainly of Indian origin, is an intermediate form that often contains fruits or oils but that may also be unflavored. "Muessel" is usually flavored with apple, mango, banana, strawberry, orange, grape, mint, cappuccino, or other additives. It is generally sold in cardboard boxes or plastic jars decorated with fruit or alcohol are often added to the tobacco<sup>4</sup>.

It has been estimated that approximately 1300 million people are engaged in various types of smoking internationally<sup>5</sup>. Furthermore, smoking is considered the second leading cause of death and the fourth major risk factor for disease worldwide<sup>6</sup>.

Studies showed that smoking is behind the death of five million adults annually, besides the expectations that about 650 million current smokers are likely to die due to their addiction to smoking<sup>7</sup>.

Epidemiological data revealed variations in youth smoking prevalence according to regions of the world and this is thought to be due to existing difficulties in defining age of youth, smoker and an insufficient reliable data from developing countries<sup>8</sup>.

At the international level, it has been estimated that about one hundred million fatalities were recorded as a result of tobacco smoke last century (Yauk, et al<sup>9</sup>). It has been estimated that tobacco contains more than 400 chemical compounds of which many compounds have toxic and tumorigenic nature<sup>10</sup>. It is believed that nicotine, the chemical agent of cigarette smoking, is beyond the behavior of addiction, and with other chemicals participate to smoking related diseases<sup>11</sup>.

Nicotine is metabolized in the liver; it alters the metabolism of oxidants/ antioxidants and stimulates the production of free radicals and ROS, which affects antioxidants defense system and generate oxidative stress<sup>12</sup>.

### Study objectives

The main objective of the present study is to evaluate the effects of smoking models on the level of liver enzymes.

## METHODOLOGY

### Experimental Design

This experimental study was carried out on 30 male albino rats (females were Excluded to avoid the possible effect of hormonal changes). Male albino rats (*Rattusratas*) weighted 50-180 g (6-8 weeks old) were obtained from animal house at the Jordan University of Science and Technology and maintained under optimal conditions of diet and temperature .

Rats were randomly assigned to one of 3 groups (n= 10 per group), group 1 was negative control exposed only to fresh air, groups 2 exposed to the most commonly used cigarette brands in the Jordanian market (red LM cigarettes) as 1 cigarette/rat/day for 30 consecutive days. Rats in groups 3 were exposed to flavored water-pipe coming from the complete burning of 20 g from one of moassel for a period of 30 days one session a day for whole body.

A further period of one month non-exposure (cessation) to smoking was done as a recovery stage from the effects of cigarette and water-pipe smoking. Following each period, histological, immunohistochemistry and biochemical studies were performed.

### The Digital smoking machine

A digital smoking apparatus was designed that have a special smoking topography, suitable for the exposure of rats to water-pipe /cigarette smoke<sup>13</sup>. The smoking machine is composed of the following components as illustrated in (Figure 1).

Inhalation chamber made of Plexiglas (8 mm thick) with the dimensions 30 cm length × 22.5 cm width × 10.5 cm height that can host five rats rats weighting 100-150 gm. Time cotroller. Valve allowing fresh air to pass inside the inhalation chamber, vacuum pump, 30% and 50% alcohol traps connected in series by rubber and glass connectors.

### The smoking regimen:

Each cycle of smoking run lasted for 90 seconds and consisted of the three followig steps:

- a. Continous withdrawing of water-pipe /cigarette smoke for 30 seconds .

b. Washing out of the smoke for 30 seconds, with fresh air.

c. Finally, rats were allowed to breath normal fresh air for 30 seconds.



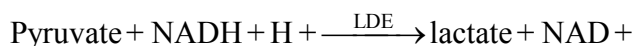
**Figure 1:** Five rats placed in the digital smoking machine and exposed to the smoke of 5 cigarettes

### Biochemical investigations

The activity of selected antioxidant enzymes of trachea, heart, lung and blood will be tested. Using a 5cc syringe, blood was collected by heart puncture using plain tubes. Blood was left to clot at room temperature for thirty minutes, and then centrifuged at 3000 rpm for 10 minutes. After that, the supernatant (serum) was stored at -20°C until used for subsequent measurement of liver enzymes tests and another biochemical tests.

The Following biochemical parameters were examined.

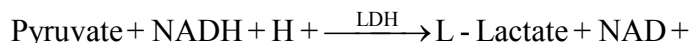
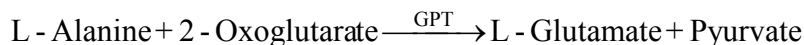
**Lactate Dehydrogenase (LDH):** It is an oxidoreductase stable enzyme, used to evaluate the presence of damage and toxicity of tissues and cells. Lactate dehydrogenase catalyzes the reduction of pyruvate by NADH, to form lactate and NAD<sup>+</sup>. The catalytic concentration is determined from the rate of decrease of NADH measured spectrophotometrically<sup>15</sup>.



### Alanine Aminotranferase (ALT)

It catalyzes chemical reactions by transfer the amino group from alanine to 2-oxoglutarate forming pyruvate

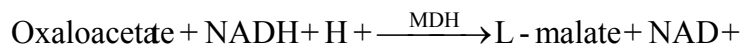
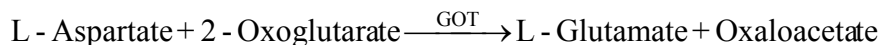
and glutamate. The catalytic concentration is determined from the rate of decrease of NADH measured by a spectrophotometer at 340nm, by means of the LDH- coupled reaction<sup>14</sup>.



### Aspartate Aminotransferase (AST)

Aspartate aminotransferase catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate. The principle is

determined from the rate of decrease of NADH measured by a spectrophotometer at 340nm, by means of the malate dehydrogenase (MDH)-coupled reaction<sup>15</sup>.



### Statistical analysis

The Statistical Software Package for the Social Sciences (SPSS version 20) was used for data management and analyses. All data in the physiological part are presented as means  $\pm$  SEM. Student's t-test for independent samples was used to determine whether differences between the mean values of the responses of the control and the experimental tissues, were significant. The differences were considered significant if the probability was less than 0.05 ( $P < 0.05$ ).

### RESULTS AND DISCUSSION

#### The Effect of Chronic Exposure to Cigarette/Water-pipe Smoking and its Recovery on the Serum Liver Enzymes

As shown in tables (1-3), the statistical analysis of three serum liver enzymes activity (ALT, AST, and LDH level), following chronic exposure to water-pipe and cigarette smoking, and after cessation. (Data are expressed as Mean  $\pm$  SEM of 6 rats) are presented.

**Table 1: Effect of cigarette smoke on ALT level in albino rats**

Animal groups	After exposure U/L	P- values	After cessation U/L	P-values
Control (Fresh air)	51 $\pm$ 3.2	1.0	51 $\pm$ 3.2	1.0
Cigarette	71 $\pm$ 2.3	0.00*	33 $\pm$ 4.2	0.01*
Water-pipe	76 $\pm$ 1.8	0.00*	24 $\pm$ 2.2	0.00*

\* Mean significant  $P < 0.05$ .

**Table 2: Effect of cigarette smoke on AST level in albino rats**

Animal groups	After exposure U/L	P- values	After cessation U/L	P-values
Control (Fresh air)	222 $\pm$ 7.5	1.0	222 $\pm$ 7.5	1.0
Cigarette	267 $\pm$ 12.8	0.01*	267 $\pm$ 12.8	0.01*
Water-pipe	314 $\pm$ 9.3	0.00*	264 $\pm$ 17	0.05*

\* Mean significant  $P < 0.05$ .

**Table 3: Effect of cigarette smoke on LDH level in albino rats**

Animal groups	After exposure U/L	P- values	After cessation U/L	P-values
Control (Fresh air)	186 $\pm$ 1.2	1.0	186 $\pm$ 1.2	1.0
Cigarette	502 $\pm$ 11.5	0.00*	395 $\pm$ 30	0.00*
Water-pipe	498 $\pm$ 17.4	0.00*	477 $\pm$ 13	0.00*

\* Mean significant  $P < 0.05$ .

### The Effects of Cigarette Smoking on Liver Enzymes

The levels of ALT were significantly elevated from  $51 \pm 3.2$  U/l in control group to  $71 \pm 2.3$  U/l in cigarette smoking group ( $p=0.000$ ). Following smoking cessation, the level of ALT significantly lowered to  $33 \pm 4.2$  U/l ( $p=0.001$ ). These results indicated the adverse effects of cigarette smoking on liver and are consistent with previous studies in which the level of ALT increased significantly because of cigarette smoking<sup>16</sup>.

Cigarette smoking significantly elevated the level of AST compared with control group ( $p=0.01$ ). After smoking cessation, the level of AST was significantly lowered. These findings showed that cigarette smoking induces adverse effects on livers cells reported by other studies<sup>16</sup>.

### The Effects of Water-pipe Smoking on Liver Enzymes

Water-pipe smoking significantly elevated the level of ALT ( $p=0.000$ ). The level of ALT was insignificantly increased with cessation water-pipe smoking ( $p=0.13$ ). Its continuous treatment after water-pipe cessation significantly lowered the level

of ALT (0.006). Water-pipe smoking increased significantly the level of AST ( $p=0.000$ ). Quitting water-pipe smoking significantly lowered the level of AST ( $p<0.05$ ). These findings suggested that water-pipe smoking induces oxidative damage in the liver<sup>17</sup>. Several studies have reported the pathologic effects of smoking in general on liver enzymes<sup>15</sup>. The findings of the present study help in setting up the impacts of smoking models on liver and other study parameters.

Cigarette smoking and water-pipe smoking significantly increased the level of HDL ( $p=0.000$ ). Cessation of cigarette and water-pipe smoking significantly reduced the level of LDH ( $p=0.000$ ).

**CONCLUSION:** The results of the present study demonstrated that smoking can induce liver injury through creating alterations in liver enzymes. Smoking cessation can reverse liver injury and restores liver enzymes to normal-levels.

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CONFLICT OF INTEREST REPORTED: NIL;

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