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Toxicity of biomass pollutants in Wistar rats

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Abstract

Biomass pollutants are an important source of environmental pollution. The use of traditional fireplaces leads to high concentrations of pollutants in the smoke from wood fires.

The objective of this study was to evaluate the acute and subchronic toxicity of biomass pollutant in Wistar rats. The different gas concentrations were measured before and after acute and subchronic inhalation exposure in rats. Motor activity, behavorial change and weight development of the animals were evaluated.

The results showed a decrease of pollutant after inhalation, a disturbance of motor activity of animals and toxic symptoms are recorded.

Smoke from biomass released during fish smoking activity is the cause of the appearance of several diseases predisposing to cardiorespiratory diseases.

Keywords: Behavorial Change, Biomass, Exposure, Toxicity, Rat

Introduction

Through his activities, man emits numerous pollutants into the air which have significant health effects in terms of cardio-respiratory mortality and morbidity [1]. In underdeveloped countries where electricity and gas are scarce and expensive, many families, both rural and urban, use wood (biomass) as their main source of energy for cooking. More than half of the world's population uses the combustion of biomass (wood, charcoal, straw, animal dung, crop residues) as the main source of energy for heating and cooking [2-3]. These fuels and cooking methods produce high levels of air pollution in houses, involving a multitude of pollutants harmful to health, including fine particles that penetrate deep into the lungs. In poorly ventilated houses, the content of fine particles in household smoke can reach a concentration 100 times higher than acceptable levels [2]. Wood is a renewable source of energy that limits greenhouse gas emissions. Wood energy has long been regarded as the sole source of heat for heating and cooking human food. The first reason is economic [1]. Women who smoke fish in urban and rural Benin use smoke from biomass. These smoking operations are carried out in thick layers of smoke, exposing the subjects to respiratory, ocular and even cardiovascular problems [4].

In fact, in the latest estimates, published in March 2014, the World Health Organization (WHO) indicated that more than 7 million premature deaths in 2012 could be attributed to exposure to air pollution, of which about 2.6 million could be attributed to outdoor pollution. That is one in eight deaths worldwide. The vast majority of deaths due to air pollution (51%) were mainly related to cardiovascular and respiratory diseases [5]. Several other studies have demonstrated the effect of biomass pollution on respiratory health. Exposure to smoke from biomass is responsible for respiratory aggravations such as rhinitis, chronic bronchitis, obstructive ventilation disorders and asthma symptoms [4, 6].

This work aims to determine the chemical composition of biomass pollutants and to identify toxic symptoms in Wistar rats.

Materials and methods

Type and scope of the study: This is a longitudinal study of an experimental type. It aims to evaluate some symptoms related to the inhalation of smoke from biomass combustion in rats.

Wistar, following acute and subchronic exposure. It is carried out in Research Unit in Effort Physiology (URPEF) from February to May 2020.

Protocol

Gas detection and variation: The animals are divided into a control batch and a batch exposed to the fumes from biomass combustion under actual fish smoking conditions. Exposures are carried out for 10 minutes. The animals are placed in a closed cylindrical jar made of transparent glass for inhalation of the smoke from biomass. The closed cylindrical jar is connected to the fireplace used for the fish smoking activity by a pipe.

The concentration of gases inside cylindrical jar is taken before and after exposure of rats using a SENKO Portable Multi Gas Detector and Analyser. The gas analyser indicates on the screen the concentration of 4 gases (oxygen O_2, carbon monoxide CO, hydrogen sulphide H_2S, and methane CH_4) simultaneously.

Animal material

Sixty Wistar rats (60) aged 10-week-old male and female nulliparous Wistar strain with an average weight of 185.55 ± 6 and divided into 06 batches of 10 rats were used. They were supplied by the Research Unit in Effort Physiology of the National Institute of Youth, Physical Education and Sport (INJEPS). The animals were raised in rooms with a stable temperature (24 ± 2°C). In these rooms, the photoperiod was 12 hours and the hygrometry 50%. The animals had ad libitum access to water and food (pellets, 15% protein, 5.3% fat). They were kept in wire mesh cages of 50 × 30 × 20 cm3 with drinking troughs.

Toxicity assessment

Behavorial change during acute toxicity: Acute toxicity test was conducted according to OCDE Test Guideline 433, No. 39 [7].

It was carried out on male and female nulliparous Wistar rats, divided into 04 group of 10 rats per sex (02 control group and 02 exposed group). Each animal inhaled smoke from the biomass in a single exposure for 10 minutes. They were then observed regularly for the first four (04) hours after exposure and every hour for 24 hours and then every six (06) hours for 14 days. Control group are not exposed. Behavorial change [8-9] have been noted. They such as noze mobility,

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sensitivity to noise and pinching, feeding, breathing, irritation, possible mortality and the appearance of excrement [10]. Changes in body mass was also recorded.

Behavorial change during Subchronic toxicity

The study was carried out in accordance with Guideline 413 of the revised OCDE standard [11]. The rats were divided into 02 group of 10 rats per sex each (10 males and 10 females). Each animal was exposed daily for 90 days to smoke from the biomass. Each exposure lasted 10 minutes. The rats' behavorial changes were observed daily and their weight was measured weekly [11].

Motor activity

The determination of motor activity is carried out according to the method of Martin et al [12] with slight modifications. Thirty minutes (30 minutes) after smoke inhalation, each animal is placed in a rectangular cage, 60 cm long and 40 cm wide, the floor of which is divided into 24 squares. Motor activity is assessed by the number of squares each animal passes through in 10 minutes. All the rats have undergone this test.

Statistical analysis

The statistical analyses of the results were carried out using Graph Pad prism Version 8.0.2 software. Comparisons of mean values were performed using parametric tests, including paired t-series tests after performing the Anova test on repeated measurements. The significance level is set at $p < 0.05$.

Results

Gas detection and variation: The different values of pollutants concentrations found in the smoke before and after inhalation are recorded in Table 1. After exposure, there is a significant decrease in the pollutants for carbon monoxide, oxygen, methane, and hydrogen sulphide, respectively (p = 0.0134; 0.0139; 0.0047 and 0.0002) compared to the starting quantities.

Table 1: Gas detection and variation

Gaz	Before exposition	After exposition	P value
Carbon mooxide (C0)	170,13 ± 46,46	106,75± 30,15	*P = 0, 0134**
Oxygene (O2)	20,04 ± 0,40	19,24 ± 0,75	*P = 0 ,0139**
Methane (CH4)	3,5 ± 2,45	0,75 ± 1,39	*P = 0,0047***
Hydrogen sulphide (H2S)	5 ± 1,39	2,37 ± 1,17	*P = 0,0002****

*p< 0,05; **p<0,01; ***p<0,001

Behavorial change during

The motor activity of rats after exposure reveals signs of shortness of breath in these animals. In the immediate aftermath of smoke exposure, rats are almost motionless, sometimes giving impression of death. For the first four hours after inhalation, animals are very sluggish and do not regain normal mobility for several hours.

Behavorial change such as noze irritation after exposure, lack of appetite, reduced mobility during the first weeks (Tables II and III). Even though, there is an absence of mortality, diarrhea and hair loss in animals.

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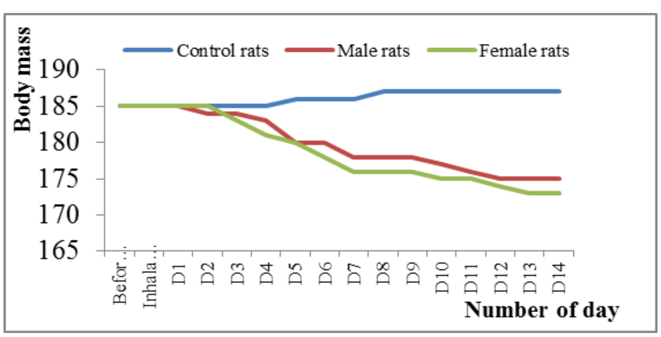
Table 2: Behavorial change during acute toxicityLegend: (+): presence; (-): absence, inh = inhalation

Events	Before Inh	Days after inhalation 30min	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appetitet	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Mobility	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Hair loss	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Irritation	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: Behavorial change during subchronic toxicity

Events	Before in h	30min	D1	D2	D3	D4	D5	D6	D7	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
Appetitet	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mobility	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hair loss	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Irritation	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

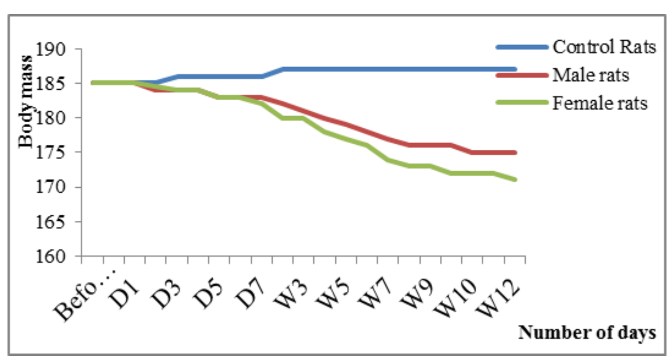
Legend: (+): presence; (-): absence, D: day; S: week, inh = inhalation



D: day *p<0,01; ***p<0,0001

Figure 1: Evolution of body mass after acute inhalation

Figure 1 shows the changes in body mass of exposed and control rats before and after inhalation. Rats exposed to biomass smoke show a decrease in body mass. This decrease is significant in female rats (p = 0.000034).



D: day; S: week

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Figure 2: Changes in rat body mass after subchronic exposure

The body mass of animals exposed to biomass smoke showed a significant decrease according to sex (p males=0.0030; p females=0.0016).

Discussion

Analysis of the smoke from the combustion process inhaled by the rats revealed four different gases: methane (CH_4), carbon monoxide (CO), oxygen (O_2) and hydrogen sulphide (H_2S). These different gases come from the incomplete combustion of biomass used by the women during this activity. The conditions of fish smoking in this study are similar to the real conditions of the activity. In order to avoid the calcination of fish, water is often used to lower intensity of fire, which generates a thick smoke involving incomplete combustion of biomass. This explains high carbon monoxide (CO) content. These results corroborated those obtained by several authors.

In his work, Raphael [13] explains that any situation leading to incomplete combustion, due to a lack of oxygen, of a substance containing carbon causes the production of CO. According to the World Health Organisation (WHO), this is one of the most widespread air pollutants. It is the result of natural phenomena and the incomplete combustion of carbonaceous organic compounds attributable to human activities. The work carried out by Mathis [14] and Rouvière [15] confirms the presence of the various gases obtained. Indeed, for these authors, smoke emitted by coal or wood-fired cookers and fireplaces contain fine particles with a diameter of order of a micron, non-volatile compounds such as tars and volatile compounds such as: nitrogen oxides (NOX), carbon monoxide (CO), volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs), some of which are carcinogenic [14]. Heating and cooking are sources of emissions of pollutants including CO, PAHs and CO [15].

The significant decrease in gases obtained after exposure confirms the inhalation of these gases by the rats which led to the clinical manifestations revealed by the behavorial change during toxicity. However, for some authors [16], the first signs of exposure to biomass fumes are headache, nausea, vomiting, confusion, disorientation, visual disturbances and polypnoea. This explains the torpor observed in these animals after exposure. This assertion is supported by a study [17] which has explained that the immediate effects of inhalant intoxication are similar to the first classical stages of anaesthesia. Initially, the user feels stimulated, disinhibited and prone to impulsive behaviour. Analysis of the results shows that the exposed rats have from the very first hours an inappetence, a redness of the mucous membrane, a reduction in motor activity and a significant reduction in body mass. Several studies have shown the correlation between exposure to pollutants and health effects. In a Japanese study [18], the authors demonstrated that gases from biomass smoke combustion are caused respiratory diseases, such as asthma and respiratory infections. It is also justified that the extent of exposure depends on the type of fuel, the ventilation often absent in primitive dwellings without chimneys and the duration of exposure.

The observed inappetence and significant decrease in body mass are also signs of an infection of digestive tract. In French, the authors [13] demonstrated that weight loss is the result of organic disease and respiratory failure is also a cause of weight loss. Thirty to 70% of patients with severe chronic obstructive pulmonary disease will lose weight significantly.

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However, several other studies [4, 19, 20] have demonstrated the relationship between exposure to biomass smoke and development of chronic obstructive pulmonary disease. Ahounou et al [4] and Kwas et al [19] have shown that women engaged in fish smoking suffer from obstructive ventilatory disorders. The weight loss observed in rats is believed to be the cause of respiratory diseases and other lung infections. Indeed, under the action of heat induced by wood smoke, particularly magnesium and calcium from the blood, come out of the body through perspiration (sweating). The skin becomes considerably dehydrated and weakened, leading to severe dehydration and drying of the skin [21].

Conclusion

Biomass smoke contains various gases and exposure to this smoke causes toxic manifestations, reduced motor skills and body mass, which are likely causes of the development of cardio-respiratory diseases.

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