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Acute and sub-chronic toxicity of condensate produced from olive mill wastewater using solar energy in mice

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Abstract—Olive mill wastewater (OMW) is one of the environmental challenges associated with the olive oil industry. This study was carried out to investigate the potential acute and sub-chronic toxicity of oral treatment of OMW condensate in mice. Different doses (250, 500, 1000, 2000, 4000, 8000 mg/kg) were applied once to investigate acute toxicity. Sub-chronic toxicity was investigated using thirty mice; two groups with (500, 4000 mg/kg/body weight) doses along with one control group. Acute toxicity study results showed that the LD_{50} was greater than the highest tested dose with no signs of systemic toxicity, mortality, or behavioral changes. In addition, the sub-chronic investigation did not show significant changes in behavior, body weight, and vital organs weight/body weight ratio along with no observed differences in the studied hematological parameters. Condensate dose of 500 mg/kg did not show significant differences in the levels of blood urea nitrogen (BUN), alanine aminotransferase and aspartate aminotransferase (AST). However, the AST serum level was significantly decreased and the serum level of BUN was increased at the dose of 4000 mg/kg. Results suggest that single and repeated oral doses of olive condensate administered orally are safe in mice.

Keywords — Acute toxicity, Sub-chronic toxicity, Olive mill wastewater, Olive condensate, Wastewater management.

I. INTRODUCTION

The global consumption of olive oil has increased dramatically due to its nutritional value and its health benefits [1]. This trend led to an increase in the cultivation of olive trees to cover the increased global demand. Globally, more than eleven million hectares of agricultural lands, in about forty-seven countries, are planted with olive trees. The world's majority of olive trees (98%) are cultivated and harvested in the Mediterranean region, thus the olive oil extraction industry is growing continuously in almost all Mediterranean countries [2].

Olive oil extraction is associated with various environmental impacts including high levels of water and energy consumption, discharging of olive mill wastewater (OMW) and pomace (solid by-product) in addition to other environmental emissions such as noise and air pollution [2]. One of the challenges of the olive oil industry is the discharge of large volumes of the olive mill wastewater OMW [3]. The composition and organic load of the OMW are governed by the vegetation water of the olives, efficiency of the OMW in separating the oil from the OMW and storage time of the olives before processing. The organic load in the OMW contains phenols, polyphenols, chlorophyll, humic acids, polyalcohol, sugar and much more known and unknown compounds of

soluble, colloidal and suspended forms [4]. In general, OMW is acidic (pH 4.6-5.3) with high organic load (50 - >150 g/l) and intensive odor. OMW acidity is related to their continents of phenolic compounds and humic acids, while the phytotoxicity is related to the high concentration of phenols [5] and dissolved solids in the OMW (Condensate). The condensate is free of suspended solids, transparent, colorless and more acidic than OMW, it contains high concentrations of dissolved volatile organic compounds [6-8].

Different Strategies were proposed for safe disposal of olive condensate and realated pollutants [7, 9], however, biological effects of condensate on laboratory animals have not been investigated adequately.

Olive trees cultivation and olive oil production are one of the most important economic and agricultural activities in Jordan. Moreover, olive oil is considered as an important part of Jordanian culture being used in folk medicine recipes and as a main component of traditional Jordanian dishes. There are about 17 million olive trees in Jordan and more than 95% of the Jordanian olive production is used for the production of olive oil. Recently, more than 110 three-phase olive mills were reported in Jordan with more than140 production lines [3]. The yearly discharge of OMW was reported to be more than 400 000 m^3 via lagooning and the trend is increasing in Jordan [3]. Lagooning, anaerobic digestion, co-composting with pomace, irrigation, bentonite treatment and other methods were used for OMW treatment/disposal, but without finding the ultimate solution that is technically acceptable, economically feasible and meets all environmental demands.

OMW was recently separated into condensate and biofuel using solar still units [3]. The biofuel was evaluated [3], while the condensate still needs further studies. It is important to investigate the toxicity of olive condensate in laboratory animals. Hence this study aimed to evaluate the potential acute and sub-chronic toxicity of the olive condensate in laboratory mice.

II. MATERIAL AND METHODS

A. Condensate preparation and determination of acute toxicity (LD50)

OMW condensate separation procedure using the solar distillation technique was previously reported earlier in detail [3, 6]. All animals' procedures were ethically approved by the Yarmouk University Institutional Animal Care and Use Committee, following the guideline of the National Institutes of Health guide for the care and use of laboratory animals. Experiments were carried out on 4 to 7 weeks old females' albino Balb/c mice (n=60; weighing 24-33 g) supplied by Yarmouk University animal house facility. Mice were maintained in a controlled atmosphere of 12 hrs light-dark cycle, 25 ± 2C° room temperature with free access to food and water supply. To determine the LD_{50} of the condensate, mice (n=10 each group) were orally treated with different condensate doses (250, 500, 1000, 2000, 4000, 8000 mg/kg)[10]. The mortality rate and sign of toxicity were observed regularly for the first 24 hrs, and daily for two weeks. Toxicity was evaluated using an early proposed scale of Hodge and Sterner [11].

B. Sub-chronic toxicity study

Thirty mice were randomly divided into 3 groups: two groups that were treated orally with two different condensate doses (500, 4000 mg/kg body weight) for 30 days along with a control group (10 animals) treated with distilled water [12]. During the experiment, animals were observed for mortality and any changes in general behavioral patterns and physical appearance. By the end of the treatment period, all mice were killed and blood samples were collected for further hematological and biochemical parameters analysis. Then, vital organs (heart, lung, liver, and kidney) were removed and weighed.

C. Hematological and biochemical analysis

Different hematological parameters were investigated. Those include white blood cells (WBC), red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), which were determined using automated hematology analyzer. Serum samples were separated by centrifugation at 3000 rpm for 15 minutes and the obtained serum was stored at -80 ºC for further analysis. Serum was analyzed for blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using an automated biochemistry analyzer.

D. Statistical analysis

Results were expressed as the mean ± Standard Error of mean (SEM). Results were analyzed by one-way analysis of variance (ANOVA) using SPSS software (version 22.0) [13]. The *P*-value was considered to be significant when it is value < 0.05.

III. RESULTS

A. Acute toxicity studies

The LD_{50} was greater than the highest tested dose (8000 mg/kg). Also, animals did not show any signs of systemic toxicity (such as irritability, ataxia, sedation, seizures, and diarrhea), mortality, changes in body weight or behavioral changes during the next 14-days after the exposure time. This indicates that acute exposure to the condensates can be considered non-toxic and safe.

B. Sub-chronic toxicity studies.

Sub-chronic oral administration of condensate at different doses (500, 4000 mg/kg) of body weight for 30 days did not produce apparent variation in common behavior of the mice, significant changes in feed and water intake compared to the control group and all animals were survived throughout the study time.

C. Body weight and organ weight/body weight ratio

The change in mean body weight and vital organs (heart, lung, liver, spleen, and kidney) weight/body weight ratio at the end of the period of treatment in both control and condensate treated groups are shown in Table 1. Results showed no significant difference ($p > 0.05$) in the body weight between condensate treated and control animals. Similarly, there were

no significant changes ($p > 0.05$) in the organs weight/body weight ratios in the condensate treated groups compared to the control group ($p > 0.05$; Table 1).

16]. In the present study, sub-chronic oral administration of two different doses of the condensate showed no harmful impact on the relative vital organs weight and on the total body weight,

Table 1. Effect of condensate on vital organs/body weight ratio.

		Organ weight/body weight ratio				
	Body Weight (g)	**Heart**	**Lung**	**Liver**	**Kidney**	**Spleen**
Control	26.2 ± 0.58	0.117 ± 0.01	0.218 ± 0.01	1.136 ± 0.05	0.262 ± 0.01	0.152 ± 0.01
Condensate (500mg/kg)	27.5 ± 0.80	0.127 ± 0.01	0.258 ± 0.01	1.184 ± 0.07	0.267 ± 0.01	0.156 ± 0.01
Condensate (4000mg/kg)	27.1 ± 0.53	0.127 ± 0.01	0.213±0.01	1.194 ± 0.07	0.251± 0.01	0.133 ± 0.01

D. Hematological and biochemical analysis

The effect of sub-chronic administration of condensate on selected hematological indices is shown in Table 2. The results showed that there was no significant difference ($p > 0.05$) in all parameters measured between the condensate treated group and control group. In addition, 500 mg/kg condensate dose did not significantly affect the serum AST, ALT and BUN levels. However, 4000 mg/kg dose significantly ($p < 0.05$) decrease the AST serum level and increase BUN level ($p < 0.05$; Table 2).

Table 2. Effect of different doses of condensate on different hematological parameters, BUN levels and serum AST and ALT enzymes in mice.

Parameter	**Control**	**Condensate (500mg/kg)**	**Condensate (4000mg/kg)**
WBC (×10^3/µl)	10.65 ±1.39	11.3 ± 2.24	8.075 ± 1.05
RBC(×10^6/µl)	9.57 ± 0.272	11.2 ± 0.333	9.72 ± 0.56
HB (g/dl)	13.5 ± 0.191	15.866 ± 0.332	13.875 ± 0.925
HCT (%)	40.3 ± 0.497	50.133 ± 1.275	42.75 ± 2.604
MCV (fl)	42.175 ± 1.23	44.433 ± 0.635	44.075 ± 1.844
MCH (pg)	14.125 ± 0.45	14.066 ± 0.152	14.275 ± 0.417
MCHC (g/dl)	33.475 ± 0.11	31.666 ± 0.629	32.45 ± 0.861
AST(U/L)	70.8 ± 9. 7	62.6 ± 5.2	53.4 ± 4.6*
ALT(U/L)	47.6 ± 7.3	54.3 ± 4.6	44 ± 6.9
BUN(g/dL)	31.3 ± 6.0	34 ± 2.3	53.6 ± 7.6*

* $P < 0.05$ in comparison to the control group.

IV. DISCUSSION

A global environmental concern in the olive mill industry is the disposal of large quantities of byproducts. OMW is a serious problem in public health with a negative impact on soil and water quality. OMW contains different compounds with biological activity [14]. Previous study provided interesting information on a novel technique that can be used to solve this problem in a safe way and low economic cost via OMW processing into condensate and biofuel using solar still units [6] . However, acute and sub-chronic toxicity of the condensate in laboratory animals were not assessed. The main aim of this study, therefore, was to investigate acute and sub-chorionic toxicity of olive condensate and the effect of condensate on hematological, biochemical and histological parameters and tissue structures in mice.

During the acute study, animals did not show any signs of acute systemic toxicity or mortality and the LD_{50} was greater than the highest tested dose (8000 mg/kg). The LD_{50} > 8,000 mg/kg classified the condensate as practically non-toxic according to the Hodge and Sterner scale [11]. Therefore, condensates can be considered non-toxic.

Body weight changes and vital organs weight are considered among important indices of pathological and physiological status in laboratory animals. Heart, liver, kidney, spleen and lungs are the vital organs that could be affected by different metabolic reactions caused by drugs and chemicals toxicity [15,

concluding that the condensate is non-toxic on these vital organs.

Liver is the main site of metabolism reactions and elimination of foreign compounds. Due to its unique metabolic functions and its relationship with the gastrointestinal tract, liver is an important target of drugs and xenobiotic (chemicals that are not normally produced or expected to be present in the body) detoxification processes [17]. Metabolic alteration of

chemical compounds into reactive intermediate species, such as free radicals, prompted liver injuries via the initiation of free radicals, leading to oxidative stress. The disturbing oxidative stress causes deregulation of cell signaling pathways, dysfunction of biomolecules, and could lead to cell death [17]. The tested blood levels of the aminotransferases ALT and AST are often used as marker enzymes to indicate liver damage [18-20]. Both ALT and AST catalyze the transfer of an amino group from an amino acid to α-ketoglutarate [20]. ALT localized in the liver mainly is released into the bloodstream as the result of liver injury, thus is a more specific marker of hepatocellular cell injury than AST. On the other hand, AST is widely distributed in other organs in addition to the liver including cardiac muscle, skeletal muscle, kidney and brain tissue and it is released into the serum when any one of these tissues is damaged [21]. Severe (> 20 times, 1000 U/L) or moderate (3-20 times) elevations of serum aminotransferases levels are observed most often in cases with diseases that affect hepatocytes such as hepatitis and toxin-induced liver necrosis [18, 19]. The results of the present study suggest that the condensate did not cause any hepatocellular injury as the values of serum ALT and AST enzymes were almost similar in all the groups and no significant difference was observed between the groups. This was supported by the absence of histopathological changes in the liver of treated mice (data not shown).

Kidneys play a major role in eliminating waste metabolites and xenobiotics from circulation. Since xenobiotic excretion occurs mainly in proximal tubules and during this process, the kidney may become susceptible to nephrotoxicity [22]. BUN is a nitrogen-containing compound produced by the liver as the end product of protein metabolism and the urea cycle. When renal clearance decreases in acute and chronic renal failure, serum urea is increased [23]. In the current study, BUN levels as an indicator of kidney function showed no significant differences between the experimental and control groups at a low dose (500mg/kg). However, 4000 mg/kg dose significantly increased BUN, which indicates that the sub-chronic administration of high condensate dose may impair renal function. However, the histological analysis confirmed that the condensate at both doses (500 and 4000mg/kg) has no negative effect on normal kidney histology (data not shown).

Hematological parameters, such as hematocrit, hemoglobin, and numbers of RBCs and WBCS, can be used as indicators of toxicity. RBCs are exposed to high xenobiotic concentration, and are limited in their ability to respond to injury since they are anucleate cells. Therefore, decreased RBC mass and RBC indices (MCV, MCH and MHCH) are a common finding in toxicity studies [24]. Decreased RBC production during a toxicity study is associated with changes in RBC indices, decreased reticulocytes, and decreased erythropoiesis. In the present study and after 30 days of treatment with the condensate there was no significant change in the hematological parameters between the control and treatment groups, excluding the occurrence of anemia.

V. CONCLUSIONS

The results of the present study suggest that single and repeated oral dosing with the condensate that separated from the OMW using the solar system is safe in mice. Renal impairment may result from the administration of a high condensate dose. Further studies are needed in order to determine the chronic toxicity effect of olive condensate. Higher doses should be also applied to assess the acute and sub chronic toxicity of the olive condensate. In addition, the genotoxicity effect of the of the olive oil condensate should be tested.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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