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Original Research

Effects of *Myrtus communis* leaves decoction on biochemical and hematological disorders induced by cypermethrin chronic toxicity in rats

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ABSTRACT

Background: Uncontrolled and excessive uses of insecticides, in agriculture, will expose the human and animal health to a high risk of chemical toxicity. **Objective:** This study aimed to assess *Myrtus communis* (MC) effects against the toxicity induced by the Cypermethrin (CYP)in Wistar rat. **Methods:** The experimental period was 30 days, carried out on 50 rats, divided into five groups; Group I (controls), Group II orally administered with 20 mg/kg of CYP (< 1/10 LD50)dissolved in corn oil (CO), a Group III orally administered with CYP and treated with 1 mL of MC leaves decoction (50 g/L), a Group IV receiving 1 mL MC, and a Group V received 1 mL CO. **Results:** A decrease in mean body weight was observed in Group II (178 g)compared to Group III (190.66 g). Biochemical parameters were insignificant. Mean blood glucose and urea levels were, respectively, 0.94 ± 0.03 and 0.65 ± 0.06 g/L (Group II)and 0.72 ± 0.06 and 0.68 ± 0.05 g/L (Group III). Furthermore, liver transaminase activities as GPT were 93 ± 38.7 (Group II)and 36.6 ± 8.0 IU/L (Group III)but glutamic oxaloacetic transaminase and alkaline phosphatase were, respectively, 188.3 ± 55.1 and 73.3 ± 47.7 (II)and 210.3 ± 33.8 and 207 ± 5.1 IU/L (III). The hematological parameters (blood cells and Hb)were, respectively, 6.16 ± 0.26 $\times 10^5$/mm^3 and 13.52 ± 2.9 g/dL(II)and 7.37 ± 0.41$\times 10^5$/mm^3 and 14.14 ± 0.87 g/dL (III). **Conclusion:** The medicinal plant MC showed limited and partial beneficial effects against CYP negative effects in the animal model.

KEY WORDS: Agriculture, cypermethrin, insecticide, myrtus communis, toxicity

INTRODUCTION

World Health Organization has banned the use of pesticides such as neonicotinoides and glyphosate, classified as possibly carcinogenic [1]. These chemicals, used in agriculture, can cause long-term leukemia, neurological, and immunological disorders [2]. Farmers use pesticides without being provided with protective equipment making them more vulnerable to develop respiratory problems and skin diseases, and in another hand, they use the pesticides in anarchic, abusive, excessive, and uncontrolled way. Exposure to pesticides is a potential threat to public health due to the presence of chemical residues in vegetables, fruits, and poultry meat [1]. In Algeria cypermethrin (CYP), pesticide belonging to the perythroid family is extensively used in agriculture to check and neutralize pests of fruits and vegetables, particularly in tomato crops. CYP is moderately toxic chemical through a dermal exposure or ingestion [3]. Half-life of CYP is <100 days, in water and soil, whereas it is <16 days opposite the sunlight [4]. In mammals, CYP accumulates in adipose tissue, skin, liver, kidneys, ovaries, lungs, blood, and heart [2]. Studies suggested that CYP induces serum biological disorders altering significantly the blood glucose, urea, and creatinine levels [5]. Others studies revealed that animals, exposed orally to 19 mg of CYP per day for 2 months, recorded a decrease in testicular and epididymal sperm counts, fertility and reduction in blood follicle-stimulating hormone, luteinizing hormone, and testosterone concentrations [6]. CYP, highly hydrophobic, interacts with the phospholipid cell membrane. Metabolism of CYP is catalyzed, in the liver, by cytochrome P450 that is associated with the oxidative stress process [7]. Oxidative stress induces directly lipid peroxidation generating free radicals that damage proteins and DNA causing apoptosis [8]. It was

established a positive relationship between the use of medicinal plants and the reduction of toxic effects of environmental contaminants such as pesticides. *Myrtus communis* (MC)is an aromatic and medicinal plant, belonging to the Myrtaceae family. It is cultivated and grown in Mediterranean areas. It has been used in traditional medicine, food and spice applications. MC, also called in Arabic *Reyhan*, is widely used by the Algerian population in culinary preparations. MC leaves and fruits are used as antiseptic, antibacterial, antihyperglycemic, and anti-inflammatory agents [9]. In Algeria, the MC leaf decoction is recommended as an antihypertensive remedy [10]. Studies suggested antioxidant activities of different extracts of MC. It was revealed that bioactive compounds, extracted from MC, induce beneficial effects for the treatment of diseases including polyphenols, flavonoids, and tannins isolated from this plant [11]. Therefore, this present study was designed and performed to investigate the preventive effects of MC on biochemical alterations and oxidative stress induced by chronic exposure of CYP in rats.

MATERIALS AND METHODS

Chemicals

CYP (97% purity) [Cyano-(3-phenoxyphenyl)methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate was purchased from TASMID industry, Tunisia.

Plant Material

The MC was harvested at the flowering stage in February 2017 from Tlemcen region (Western Algeria; latitude 34°52'41"N; longitude 1°18'53"W, altitude 811 m)[Figure 1] [12]. MC leaves were isolated manually from the aerial parts in plant biology laboratory, for teaching, to obtain a weight of 300 g. The plant was identified by Professor Pedro Sanchez Gomez (Botanical Laboratory, University of Murcia, Spain). The authentication document, issued by the University of Murcia, was deposited in the Research Laboratory of Water Resources and Environment, Biology Department, Faculty of Sciences, Dr Tahar-Moulay University of Saida, Algeria.

MC Leaves Decoction

To prepare an aqueous decoction, 50 g of powder fresh leaves of MC have been used in a flask containing 1000 ml of distilled water. Decoction has been maintained under continuous reflux for 2 h at 80°C [13]. Decoction has been filtered through a funnel containing cotton wool and then centrifuged at 2500 rpm for 5 min.

Experimental Design

Fifty healthy male albinos rats, 4 months old and weighting 120-200 g, were used. They were kept under standard environmental conditions at 25°C with 12:12 h light-dark cycle

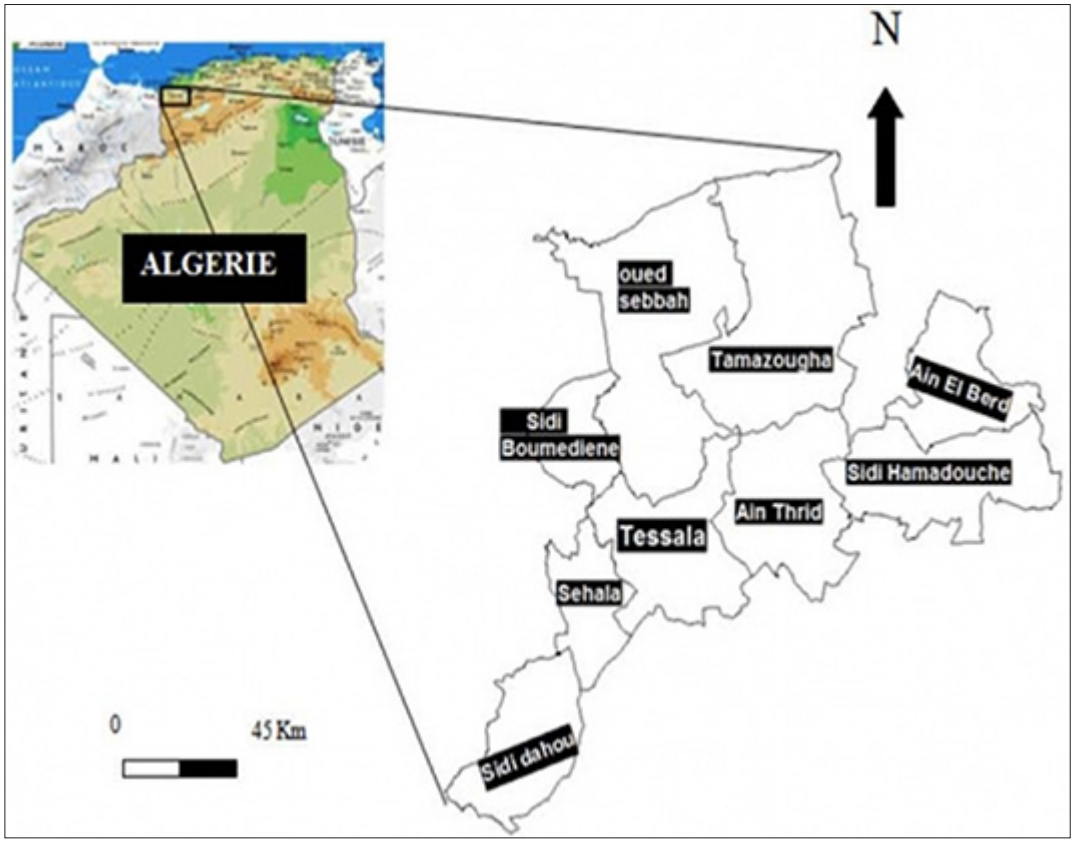

Figure 1: Study area of *Myrtus communis* crop in Tlemcen region, located in Western Algeria [12]

in ventilated plastic cages. The rats were fed with standard feed livestock and water *ad libitum*. Animals were divided into five groups (ten rats per group)as follow [Figure 2].

Group I: As normal controls received a tap water and standard diet,

Group II: As experimental controls were orally administered CYP (20 mg/kg)daily for 30 days, CYP was dissolved in corn oil (CO),

Group III: Rats were orally administered CYP and 2 h later treated with 1 mL MC leaves decoction (50 g/L),

Group IV: Rats were orally administered with MC leaves decoction in the same conditions,

Group V: Orally received 1 mL of CO in the same conditions.

The selected daily dose of CYP was <1/10th of reported oral LD50 [14]. Every 5 days, body weight gain (g)was measured and blood samples were obtained by an ocular puncture to collect between 0.5 and 3 ml of blood in EDTA tubes. They should be used in the biochemical and hematological analysis. The experimental protocol was approved by the Animal Ethics Committee of the UFPE (Process No. 012974)in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [15].

Biological Analysis

The serum levels of fasting blood glucose were measured by biochemical analyzer using commercial kits (VIDAS, Biomerieux, and France). Blood samples were centrifuged at 2500 rpm for 10 to 15 min and the sera isolated were used for estimation of the blood liver enzymatic activities such as serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP)and gamma-glutamyl transférase (gamma-GT), and other markers such as creatinine and urea. Another part of the blood was used to establish the blood count formula showing the number of all blood cells (red, white cells, and hemoglobin)using controller Coulter STKS®.

Statistical Analysis

Data are expressed as mean ± SEM (standard error of mean), with a value of $P < 0.05$ considered statistically significant. Statistical evaluation was performed by one-way analysis of variance (ANOVA)followed by the Tukey's test for multiple comparisons. All statistical analyses were conducted with the statistical software Sigmaplot (Version 11.0).

RESULTS

The effects of CYP chronic toxicity and the treatment of the animals with MC leaves decoction on the body weight gain, and hepatic and pulmonary tissues weights are shown in Table 1. This study revealed that CYP at the dose 20 mg/kg, in Groups II and III, slightly and non-significantly ($P < 0.05$)decreased the body weight of animals whereas in Group IV it was recorded that animals preserved their weights. Regarding the hepatic and pulmonary tissue, Group II showed non-significantly ($P < 0.05$) increase in tissue weights. Group III, administered with the MC leaves decoction (50 g/L), maintained stable hepatic and pulmonary tissue weights around the values of 37 and 26 g, respectively.

The effects of CYP, as a pesticide, and the treatment of the animals with MC leaves decoction on the blood glucose (or glycaemia), urea and creatinine (as blood kidney markers)are shown in Table 2. Groups III and IV, treated with the medicinal plant MC, showed a hypoglycemic effect (0.72 ± 0.06 and 0.75 ± 0.04 g/L, respectively)in contrast to Group II which presented moderately high glycaemia (0.94 ± 0.03 g/L)due to the chronic toxicity of the CYP. Neither the CYP toxicity nor the MC prevention had a significant impact on the urea metabolite, whereas creatinine level was highly significantly elevated in Group III (20.8 ± 13.4 mg/L)probably due to the reaction and the synergistic or complementary effects of CYP and MC.

The CYP and MC effects on liver enzymatic activities in animals are shown in Table 3. CYP induced an increased blood GOT and GTP levels in Group II (188.3 ± 55.1 and 93 ± 38.7 IU/L, respectively), but it was without effect on ALP and γ-GT in the same group. However, what remains unexplained is the elevation of GOT and ALP markers in Group III treated with MC (210.3 ± 33.8 and 207 ± 5.1 IU/L, respectively)compared to controls (179.6 ± 2.0 and 194 ± 1.7 IU/L)and Group II (exposed to CYP). Moreover, the treatment of animals with MC alone (Group IV)had the expected effect by decreasing blood GOT,

Table 1: Effects of cypermethrin and MC on body weight gain, hepatic and pulmonary tissue weight in the rat

Treatment	Hepatic tissue	Pulmonary tissue	Body
	Weight (g)	Weight (g)	Weight gain (g)
Group I (controls)	31.2±0.16^a	27.1±0.01^a	7.5±2.9^a
Group II (CYP)	42±0.16^a	29.1±0.01^a	5.4±1.0^a
Group III (CYP+MC)	37.1±0.08^a	26.5±0.04^a	3.3±1.0^a
Group IV (MC)	35.3±0.14^a	26.2±0.01^a	5±1.5^a
Group V (CO)	32.5±0.02^a	26.1±0.03^a	4.3±0.4^a

Each value in the table is represented as mean±SEM. Mean sharing the same letter are not significantly different at $P<0.05$ probability level in each column. MC: *Myrtus communis*, CO: Corn oil, CYP: Cypermethrin

Table 2: Effects of cypermethrin and MC on blood glucose, creatinine, and urea in rat

Treatment	Blood glucose (g/L)	Urea (g/L)	Creatinine (mg/L)
Group I	0.95±0.01^a	0.64±0.02^a	7.76±0.2^a
Group II	0.94±0.03^a	0.65±0.06^a	8.1±0.2^a
Group III	0.72±0.06^b	0.68±0.05^a	20.8±13.4^b
Group IV	0.75±0.04^b	0.47±0.13^a	6.5±1.3^a
Group V	0.82±0.04^a	0.61±0.01^a	7.9±0.17^a

Each value in the table is represented as mean±SEM. Means not sharing the same letter are significantly different at $P<0.05$ probability level in each column. MC: *Myrtus communis*

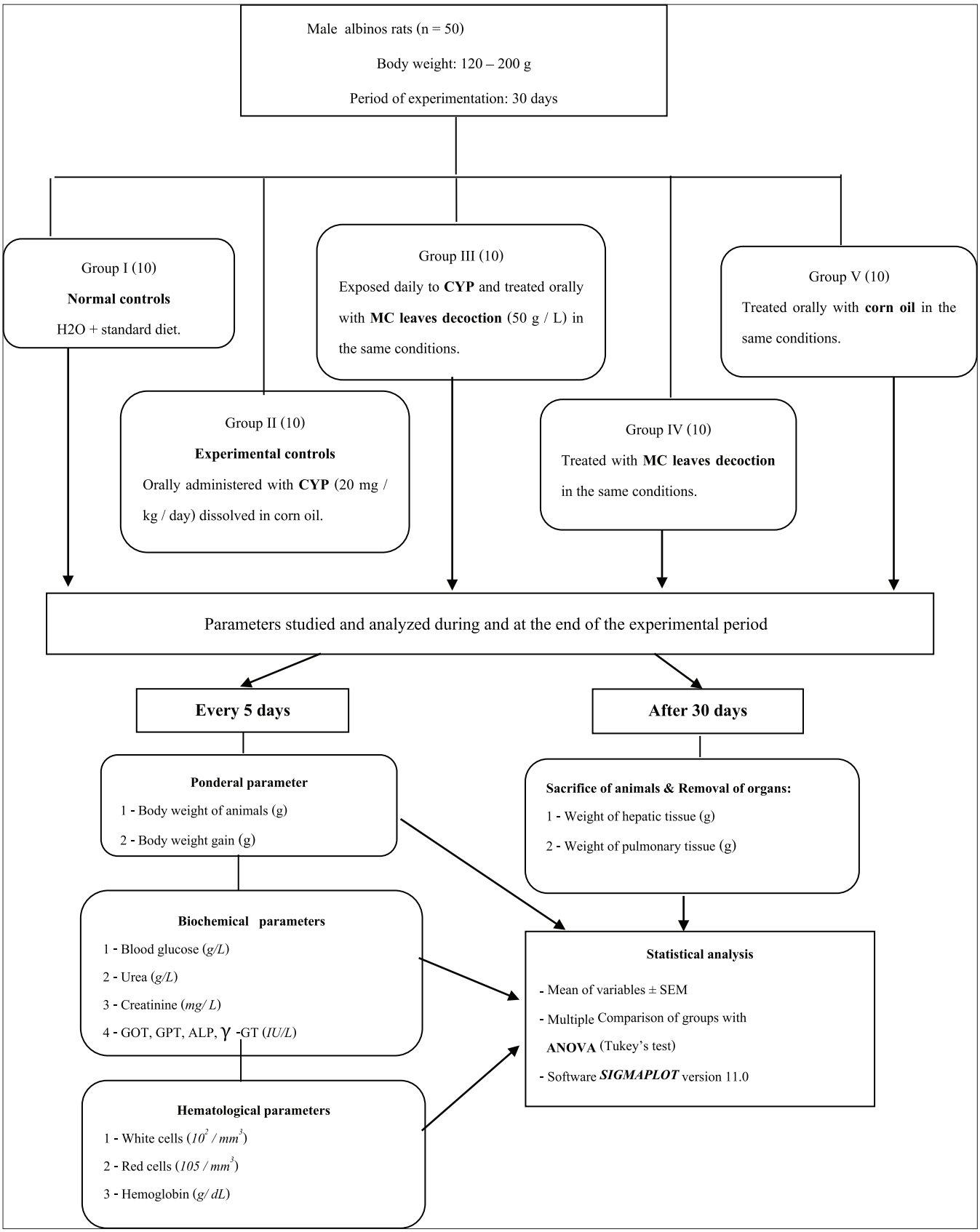

Figure 2: Experimental design of the study of *Myrtus communis* leaves decoction effects on biochemical and hematological disorders induced by cypermethrin chronic toxicity in rats

Table 3: Effects of cypermethrin and MC on blood liver enzymatic activities in rat

Treatment	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	γ-GT (IU/L)
Group I	179.6±2.0[a]	49.3±1.4[a]	194±1.7[a]	10.6±1.2[a]
Group II	188.3±55.1[a]	93±38.7[a]	73.3±47.7[a]	10.6±0.8[a]
Group III	210.3±33.8[a]	36.6±8.0[b]	207±5.1[b]	10±4.5[a]
Group IV	148.3±8.1[a]	33±6.6[b]	172±65.1[b]	5.66±0.8[b]
Group V	176±2.3[a]	47±2.5[a]	192±2.0[b]	11±0.5[a]

GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, ALP: Alkaline phosphatase, γ-GT: Gamma-glutamyl transferase. Each value in the table is represented as mean±SEM. Means not sharing the same letter are significantly different at $P<0.05$ probability level in each column. MC: *Myrtus communis*

Table 4: Effects of cypermethrin and MC on blood count formula (number of blood cells) in rat

Treatment	White cells (10^2/mm^3)	Red cells (10^5/mm^3)	Hemoglobin (g/dL)
Group I	6.77±0.0[a]	8.68±0.06[a]	16.79±0.15[a]
Group II	22.41±0.05[b]	6.16±0.26[b]	13.52±2.9[a]
Group III	35.44±0.8[b]	7.37±0.41[a]	14.14±0.87[a]
Group IV	7.4±0.21[a]	7.28±0.63[a]	13.71±1.17[a]
Group V	6.3±0.07[a]	8.5±0.3[a]	16.99±0.27[a]

Each value in the table is represented as mean±SEM. Means not sharing the same letter are significantly different at $P<0.05$ probability level in each column. MC: *Myrtus communis*

GPT, and γ-GT (148.3 ± 8.1, 33 ± 6.6, and 5.66 ± 0.8 IU/L, respectively)compared to Groups II and III.

Effects of CYP and MC on the number of blood cells in rats are displayed in Table 4. Animals of the Group II, administered with CYP at the dose 20 mg/kg, showed a significant increase in white cells number (22.41 ± 0.05 × 10^2/mm^3)compared to other groups. The pesticide had no real and concrete effects on the other hematological parameters (red cells and hemoglobin). The treatment animals with CM (Groups III and IV)led to a weak immune response and consequently reduced the white cells number in Groups III and IV (35.44 ± 0.8 and 7.4 ± 0.21 × 10^2/mm^3).

DISCUSSION

A slight decrease in the weight of animals, in this study, could be explained by the toxic effect of the CYP on the intestinal tract leading to a low appetite and intestinal absorption of nutrients [16]. Our results are similar to those of Yousef *et al.*, in 2003 that showed a statistically significant reduction in weight of the rats and changes in weight of their different tissues [17].

A high blood liver markers, as GOT and GPT, were revealed in CYP-exposed animals. The CYP toxicity damaged the liver cells inducing tissue necrosis and metabolic dysfunction of this organ. These findings are consistent with the studies conducted by Grupta and bhaumik, in 1988, on the CYP toxicity in rats and rabbits [18].

CYP did not affect hepatic metabolites such as urea and creatinine or blood glucose. These results are inconsistent with those of studies [17]conducted by Yousef *et al.*, in 2003. In toxicological literature, oral exposure to a pesticide induces an increase in urea and creatinine. Protein catabolism and the conversion of ammonia to urea also contribute to an elevation of blood urea level [19].

The chronic toxicity of CYP leads to oxidative stress in the animal's organism. CYP damaged the phospholipid composition of the cell membrane and generated free radicals or reactive oxygen species (ROS). Glutathione (GSH)is an antioxidant system providing cellular defense against harmful free radical activity through the detoxification process of xenobiotics [20]. According to recent studies, CYP inhibits GSH activity and decreases its cell concentration [21].

MC, as a medicinal plant, is rich in antioxidant compounds such as polyphenols, flavonoids, terpenes, and tannins. In addition to their pharmacological, antibacterial and anti-tumor properties, they have already shown their preventive effects against stress oxidative [22,23]. In this study, MC leaves decoction showed its insignificant hypoglycemic effect and its antioxidant role against an increased urea and GPT levels. Hydroxyl radical, as a potent free radical, can induce lipid peroxidation and biological damage. Therefore, the scavenging of ROS by MC leaves decoction may provide prevention against free radicals [24]. The main antioxidant activity of MC is mainly due to its richness in phenolic compounds [25]. MC-induced, in animals previously exposed to CYP, high levels of creatinine, GOT, and ALP. These results remain incomprehensible. It is probably due to a synergistic reaction between CYP and MC or maybe the CYP abolished the MC antioxidant effect. The mechanism of molecular synergy remains to be elucidated. These results are encouraging and suggest an eventual medical application of aqueous extracts of MC, but further investigations are needed to isolate and purify of more active compounds in MC extract as well as clarification of their mode of action.

CONCLUSION

The results of this study showed that the use of the CYP (20 mg/kg) may induce non-significant disturbances in the biological profile whereas the treatment with MC leaves decoction (50 g/L)partially improved the condition of animals. The aerial plant parts of MC are rich in polyphenols, flavonoids, terpenes, alkaloids, tannins, and unsaturated fatty acids. These bioactive compounds are endowed with antioxidant power and pharmacological properties that will limit the harmful effects of the free radicals generated by the CYP toxicity. This medicinal plant, abundant in our country, can join other local aromatic plants and will 1 day mitigate the toxic effects of CYP an insecticide generally used to protect our tomato fields.

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