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Protective Effect of Thyme Extract on Albino Rats Exposed to Paraquat

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ABSTRACT

*Paraquat (PQ), as a frequently used compound in many applications while the herbal providing is vigorously used in the curing of broad spectrum diseases such as liver disease, the influence of thyme extract or *Thymus vulgaris* L. (*T.vulgaris*) and their constituents was previously reported. The purpose of this study is to explore the influence of *T.vulgaris* extract toward PQ induced toxicity in male albino rats. The current study was conducted on thirty two male albino rats, they were randomly separated upon four equal groups, all groups were fed standard diet and tap water ad libitum as following; the first group was considered as control. The second group was treated with PQ (0.3ml/rats) orally by needle gavage, the third group was administered with PQ (0.3ml/rats), and 200ml/kg body weight (BW) of *T.vulgaris* extract orally by needle gavage, the fourth group was administered with 200ml/kg BW of *T.vulgaris* extract orally by needle gavage. The treatment duration was continued for eight executive days. Paraquat administration showed significant decrease in BW, food intake, liver, kidney weight also PQ increased malondialdehyde (MDA), low density lipoprotein (LDL), uric acid (UA) and alkaline phosphatase (ALP)*

significantly. The current results indicate that, the herbicide PQ showed adverse effects through initiation of lipid peroxidation, while T.vulgaris extract produced a significant recovery in ameliorating some aspects of PQ toxicity.

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1. INTRODUCTION

Paraquat (1,1'-Dimethyl-4,4'-bipyridinium dichloride) can be defined as a quaternary ammonium herbicide which cause its toxicity via ingestion, skin and respiratory route [1]. The systemic effects of it are attributed to promote oxidative stress through reactive oxygen species promoting in a redox cycling manner via microsomal nicotinamide adenine dinucleotide phosphate oxidase (NADPH-cytochrome P-450 reductase) [2]. Previous attempts demonstrated high mortality varying from 35-50%. Especially through the respiratory tract and multi organs failure [3]. Paraquat toxicity could result as a outcome of either by free radical impact or NADPH diminishing fr cellular [4]. Based on the previous study, *T. vulgaris* contains polyphenol, flavonoids, tannin, saponins and triterpenes. Furthermore favonoids are including luteolin, naringenin, apigenin, eriodictyol, cirsilineol, cirsimaritin, salvigenin, thymoine, thymusine. While, triterpenes are including oleanolic and ursolic acids as well [5].

2. LITERATURE REVIEW or RELATED WORK or INTRODUCTION

Recent studies suggested that, ROS elevation induce non-selective oxidation of biomolecules included lipids, proteins promoting cell degeneration as well [6]. Previous attempt has demonstrated that, PQ-exposed animals also promote its toxicity mechanism through mitochondrial, microsomal oxidation and reduction systems [7]. Previous attempts have demonstrated that, it also provokes many influences on amphibian's growth and development via orally drinking, and eventually it accumulates throughout body organs, particularly in lungs larger than it in plasma compartments due to their higher distribution volume. Therefore, in case of severe poisoning, death could occur due to respiratory failure especially, edema and fibrosis [8]. On the other hand, various medicinal plants are conducted around the globe as an alternative to the pharmacological products. Thyme (*T. vulgaris* L) in the mint family (*Lamiaceae*). Historically, it offered various uses in folk medicine for curing many diseases including bronchopulmonary and gastroenteric disorders [9]. Numerous attempts have suggested that, it provokes fungus expelling from digestive system included stomach and intestine route, and it able to enhance appetite due to its thymol component against bacteria and parasites. Different studies have demonstrated pharmacological ability of *T. vulgaris* containing biological components [10]. Therefore, the present study aimed to estimate *T. vulgaris* extract impacts on some physiological parameters in rats treated with PQ induced toxicity.

3. METHODS AND MATERIALS

Preparation of laboratory animals

Male albino rats *Rattus norvegicus* [11] were obtained and housed in Animal-house, Department of Biology, Faculty of Science/ Soran University, Iraq. Male albino rat's body weight about 150-250 grams. They acclimatized in controlled environment at stable temperature (22 ± 2 °C) on a lightening period (12 hours light and 12 hours darkness), Rats were kept at free access to normal pelleted food and drinking water. Cages were cleaned every three days through the experiment period.

Preparation of standard rodent's diet

Standard diet constituents are determined in accordance of Pico Lab. Rodent diet (which assisted with expert in Erbil poultry project and Erbil animal diet factory) which involved 9.98% of wheat, 3.84% of soya, 65.25% of oil sun flower, 22.455% of lime stone, 95.1% of salt, 23.4% of methionine, 36.6% of lysine, 9.3% of choline chloride, 8.7% of vit CX lay, 96.3% of dicalcium phosphate, 12% of AZ /1200 ,and 7.5%.of trace elements

Preparation of paraquat dose

Paraquat herbicide was purchased from the local agricultural markets, 0.3ml/rats was determined and injected orally by gavage daily for eight executive days.

Preparation of *Thymus vulgaris* extract dose

The *T. vulgaris* was obtained from the kherezok countryside, Mergasor district, Iraq in summer 2019, the plant was washed, dried, crashed and dissolved into two solvents (70% ethanol and 30% water) then the solvents was evaporated by rotary evaporator vacuum system until the crude obtained (Butters & Whitehouse, 2003). The *T. vulgaris* extract dose was prepared according to the previous studies and the moderate dose (200 mg/kg BW) was selected against the PQ toxicity orally by needle gavage daily for eight executive days.

Phytochemical screening of *Thymus vulgaris* extract

Thyme extract was analyzed with Gas Chromatography-Mass Spectrometry (GC/MS) in a Research Center, Soran University, Soran, Iraq in order to estimate the phytochemical component of *T. vulgaris* hydro-ethanolic extract.

Experimental design

Group I (control), Rats were fed standard diet, and tap drinking water *ad libitum*.

Group II (PQ group), Rats were fed standard diet with tap drinking water *ad libitum* and PQ (0.3ml/rat) daily via needle gavage orally.

Group III, Rats were fed standard diet with tap drinking water *ad libitum* and PQ (0.3ml/rat) and *T. vulgaris* extract 0.3ml/ kg BW daily via needle gavage orally.

Group IV, Rats were fed standard diet with tap drinking water *ad libitum* and 0.3ml/kg BW of *T. vulgaris* extract daily via needle gavage orally.

Blood sampling and absolute organ weight

At the last day of the experiment and 24 hours of rats fasting, rats were anesthetized via injection of ketamine hydrochloride 0.7 ml and xylazine 0.3 ml. Blood was collected via cardiac puncture then it chilled into tubes after that, they were centrifuged with 3000 revolution per minute about 15 minutes, for measuring serological parameters. Then the liver , right kidney and spleen are weighted (gm) with electronic balance.

Measurement of malondialdehyde

Serum MDA (nmol/L) level was measured depending on the previous method, the 150 μ L of serum followed adding 1ml of trichloroacetic acid (17.5%) and 1ml of thiobarbituric acid (0.66%), mixed quite with vortex, and it incubated via boiled water till a quarter hour, then after it left to be cooled. Eventually 1 ml of trichloroacetic acid (70%) had added, the mixture was left to stand at room temperature till further 20 minutes, and then it centrifuged at 2000 rpm for 15 minutes, the supernatants were used for spectrophotometric scanning at 532nm according to equation (No1) [12].

Measurement of serum lipids parameters

Serum lipids included total cholesterol, TG, HDL and LDL were measured (mg/dL), serum total cholesterol was determined with according to the laboratory kit obtained from Centronic GmbH (Germany).

Measurement of liver function test and kidney function parameters

Serum parameters included ALT and ALP (UI/L) of liver and CR and UA of kidney (mg/dL) were measured with automatic instrument.

Data analysis

Data are analyzed with statistical package for the social sciences (version 16.0) using one-way ANOVA and Duncan test as a post hoc. The level of significant is fixed ($p < 0.05$), and data are showed as Means \pm standard errors. The similar superscript letters indicate no significant, while the different superscript letters indicate significance ($* = p < 0.05$).

4. RESULTS

Phytochemical properties of *Thymus vulgaris* extract

The results obtained by GC/MS analysis of the essential biological components it characterized by its richness and its variety (table 2).

Effect of paraquat and *Thymus vulgaris* extract on body weight

The impact of PQ and *T. vulgaris* on BW parameter are presented in table 1. Animals were treated with PQ produced significant level ($p < 0.05$) in declining BW against normal group. On the other hand, the administration of PQ and *T. vulgaris* elevated BW significantly ($p < 0.05$) as compared with PQ group. Meanwhile, the non-significant changes were observed in rats treated with PQ + *T. vulgaris* and *T. vulgaris* from control group.

Effect of paraquat and *Thymus vulgaris* extract on some organs weight

The PQ and *T. vulgaris* influences on organs weight liver kidney weight are presented in table 3. Animals were treated with PQ produced non-significant decline included liver, kidney and spleen absolute weight from normal rats. While rats administered with PQ and *T. vulgaris* showed non-significant elevation include liver and kidney weight, with non-significant decrease in spleen weight from PQ group. Furthermore, the supplementation of rats with *T. vulgaris* produced non-significant changes in all mentioned organs weight as compared versus control group.

Effect of paraquat and *Thymus vulgaris* extract on food intake

The PQ and *T. vulgaris* impact on food intake are presented in table 4. The administered group with PQ produced declined food intake ($p < 0.05$) from control group. In contrast, rats administered with PQ and *T. vulgaris*, increased significant ($p < 0.05$) food intake from PQ group. Meanwhile, no change was observed in food intake of rats administered with *T. vulgaris* from control group.

Effect of paraquat and *Thymus vulgaris* extract on malondialdehyde level and lipid profile

The impact of PQ and *T. vulgaris* extract on lipid profile and serum MDA level are presented in table 5. In the PQ group, the level of MDA was elevated significantly ($p < 0.05$) from control rats, while the MDA level of the PQ- *T. vulgaris* group showed a decrease significantly ($p < 0.05$) from rats administered with PQ. On the other hand, animals were supplemented with *T. vulgaris* produced non-significant change of MDA from control group. While, serum lipids parameters (LDL level) showed significant increase ($p < 0.05$) of PQ rats from control. At the same time the significant increase ($p < 0.05$) changes were occurred in LDL of rats treated with PQ and *T. vulgaris* extract from second group. In the thyme group the significant ($p < 0.05$) changes also was occurred with LDL which confirmed the action of *T. vulgaris* extract.

Effect of paraquat and *Thymus vulgaris* extract on renal tests

The impact of PQ and *T. vulgaris* extract on renal test are shown in table 6. The administration of rats with PQ elevated significantly ($p < 0.05$) of UA level, and nonsignificant increase in creatinine parameter from control group, while both parameters were decreased non-significantly, in PQ with *T. vulgaris* group from PQ group. Furthermore *T. vulgaris* administration alone produced significant ($p < 0.05$) declination in both parameters from control rats.

Effect of paraquat and *Thymus vulgaris* extract on liver function tests

The impact of PQ and *T. vulgaris* extract on liver function test are shown in table 7. Animals were administered by PQ produced significant elevation change ($p < 0.05$) included AST and ALP level from control group, besides that ALT level was increased non-significantly against control group. In addition, rats were administered by PQ- *T. vulgaris* together produced decreased both of AST and ALP level significantly ($p < 0.05$) from PQ group. While, ALT was declined non-significantly from PQ group. Furthermore, the administration of rats with *T. vulgaris* extract declined AST and ALP significantly ($p < 0.05$) from control rats, but it increased ALT level significantly ($p < 0.05$).

5. DISCUSSION

The decreasing of BW, (table 2) under PQ impact was in accordance with the attempt of [13] who proposed that, the single dose of LD₅₀ caused loss in rats BW sharply. In addition, this loss appeared as an indicator from a declined food intake. On the other hand, *T. vulgaris* extract ameliorated the BW loss in rats were treated with both PQ and thyme extract and this action of thyme is confirmed by [14] who proposed that, albino rats were received *T. vulgaris* extract, 400 mg/kg lead to gain weight loss against another toxic substance. On the other hand, BW increase in group II may be attributed to increase of food intake in the same rats (table 4). The significant decrease was observed in the liver and spleen weight (table 3) of rats treated with PQ and the present data are confirmed with the attempt of [15, 16], who demonstrated that female Wistar rats were injected daily of PQ included 10, 15 and 25 mg/ kg BW, via gavage about 28 days caused declined organ weight. *T. vulgaris* extract positively recovered organ weight loss in both liver and kidney while in spleen it remained as non-significant change. Previous studies have suggested that, orally gavage treatment of *T. vulgaris* ethanolic extract in sub-acute toxicity attempt raised mice liver weight [17]. It has been reported that, rats treated with *T. vulgaris* offered less protective effect on the internal organ against another toxic substance [18]. Rats treated with PQ showed significant decrease in their food intake our result is supported with previous finding that demonstrated that rats administered with diet contained 20% PQ showed significant decrease in the food intake [19], another study reported that, PQ administration generate oxidative damage to such organs involved lungs, liver, kidneys and heart as well [20, 21]. In contrast, rats were fed with diet combined with thyme extract caused to increasing of food intake, Accordingly, Hassan and Awad, [22] found that *T. vulgaris* diet of broilers at 5 g/kg BW improved ood intake. Furthermore, we attributed to contain many bioactive substances besides their essential oil to enhance food intake and hormones amelioration such as thyroid gland. In the present result, the significant decrease of serum MDA (table 5) with PQ is agreed with the finding of Melchiorri et al, 1996 who reported that both strains (Sprague-Dawley and Wistar) rats were treated with PQ at dose 50 mg/kg BW/ rat [23]. In contrast, the significant decrease of elevated MDA level with thyme is supported with the attempt who investigated that, quail treatment with diet contained thyme extract showed significant decrease in MDA level, also it has been reported that male albino rats administered with thyme extract against paracetamole produced protective role as antioxidant [10]. On the other hand, the lipid peroxidation was confirmed by the non-significant increase in cholesterol level and TG, it has been demonstrated that rabbit treated with graded administration doses (3 and 6 mg/kg) of PQ daily for a period (14 days) caused

decreased in TG level [22]. Along with the TG elevation, the LDL increased was also agreed with the previous study who demonstrated that, rats treated by 10 mg/kg BW, before decapitation produced a significant LDL elevation, HDL could speed up cholesterol diminishing in peripheral tissue back to liver in order catabolism excretion process. Besides that, raised HDL may collaborate with LDL in smooth muscle cells arterial receptors therefore, it partially inhibits its uptake, and the lipids in HDL are preferentially oxidized before those in LDL as well. The significant difference in serum LDL between the PQ with thyme and PQ alone groups. However, as it mentioned previously, the present results can be expressed by the same significant along with MDA elevation. In the present result the PQ administration induce nonsignificant elevation of creatinine significant increase in uric acid are supported by the attempt of [24] who demonstrated that, the administration of rats by PQ (10 mg/kg BW) led to decrease kidney function parameters. Creatinine is considered as more specific than the others in renal function tests. Therefore, the damage in kidney is considered the only significant indication to the level creatinine may it attributed to toxic effect resulted from vasoconstriction and renal injury respectively [25]. On the other hand, the kidney parameters are unchanged sharply after thyme and PQ administration, which is in accordance with results of [26]. The significant increase in ALP level with PQ administration from our result is in accordance with finding of [27] who reported that, the intraperitoneally administration of PQ sub-lethal dose (1.5 mg/kg/BW) caused impaired in liver enzymes. Previous attempts proposed that, cellular molecules oxidative damage under PQ exposure produces both of functional and biochemical changes along with liver damage [28] with liver enzymes elevation [29]. Furthermore, it has been demonstrated that PQ administration provoke the same observation and produced the liver enzyme activity due to liver toxicity [30]. In addition, *T. vulgaris* extract and PQ intake administration against PQ action showed the reversion of serum liver enzymes, and it confirmed by the study of [30] who suggested that, *T. vulgaris* extract (200 mg/kg BW) orally by gavage for 21 days treatment promote slight decrease in the liver enzymes. Previous study indicated that thyme administration reduced MDA for their appropriate antioxidant content [31]. It has been reported that, rats treated with thymol (one of the most phytochemical substance of thyme in table 1) for 15 days offered strong ameliorative impact against oxidative stress induced rats in hepatic tissues [32].

5.1. Tables

Table 1: Phytochemical properties of *Thymus vulgaris* extract

Components	Molecular weight
p-Cymene	134.11
o-Cymene	134.11
.gamma.-Terpinene	136.125
Thymoquinone	164.084
Phenol, 2-methyl-5-(1-methylethyl)-	150.104
Thymol	150.104
Phenol, 2-methyl-5-(1-methylethyl)-	150.104

Table 1: Effect of paraquat and *Thymus vulgaris* extract on rats body weight

Parameters	Before treatment (gm)	After 8 executive days * (gm)
Groups		
Control	183.861 ± 7.2257 ^a	201.864 ± 8.081 ^a
PQ	180.292 ± 8.340 ^a	187.145 ± 7.781 ^b
PQ + <i>T. vulgaris</i>	185.003 ± 6.813 ^a	207.146 ± 6.060 ^a
<i>T. vulgaris</i>	182.864 ± 6.441 ^a	209.293 ± 6.824 ^a

Table 2: Effect of paraquat and *Thymus vulgaris* extract on some organs weight

Parameters	Liver (gm)	Kidney (gm)	Spleen (gm)
Groups			
Control	5.266 ± 0.349 ^a	0.666 ± 0.061 ^a	0.916 ± 0.054 ^a
Paraquat	4.480 ± 0.399 ^b	0.500 ± 0.054 ^b	1.040 ± 0.191 ^a
Paraquat + Thyme	5.495 ± 0.742 ^a	0.657 ± 0.020 ^a	0.771 ± 0.042 ^a
Thyme	5.885 ± 0.288 ^a	0.775 ± 0.242 ^a	0.850 ± 0.290 ^a

Table 3: Effect of paraquat and *Thymus vulgaris* extract on food intake

Groups	Parameters	Food intake * (gm)8day
Control		100.571 ± 1.461 ^a
PQ		60.000 ± 1.812 ^b
PQ + <i>T. vulgaris</i>		90.000 ± 1.712 ^a
<i>T. vulgaris</i>		110.000 ± 1.212 ^a

Table 4: Effect of paraquat and *Thymus vulgaris* extract on lipid profile and malondialdehyde level

Parameters	MDA* (nmol/L)	Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL* (mg/dL)
Groups					
Control	0.4521 ± 0.1224 ^a	27.8333 ± 2.93731 ^a	24.8333± 2.40023 ^a	18.3333± 2.65414 ^a	26.6667± 0.88192 ^a
PQ	1.6850 ± 0.0804 ^c	29.4000± 2.24944 ^a	35.4000± 4.30813 ^{ab}	21.8000± 4.44297 ^a	34.6000± 0.81240 ^b
PQ + <i>T. vulgaris</i>	1.0542 ± 0.0218 ^b	28.0000 3.27327 ^a	30.0000± 2.57275 ^{ab}	18.0000± 2.36039 ^a	30.5714± 1.13089 ^c
<i>T. vulgaris</i>	0.5437 ± 0.0711 ^a	32.7500 2.49583 ^a	26.5000± 0.28868 ^b	28.7500± 6.65050 ^a	23.0000± 0.91287 ^d

Table 5: Effect of paraquat and *Thymus vulgaris* extract on kidney function tests

Parameters	Creatinine * (mg/dL)	Uric acid * (mg/dL)
Groups		
Control	0.1500± 0.023 ^a	0.516± 0.016 ^a
PQ	0.160± 0.040 ^a	0.960 ± 0.263 ^b
PQ + <i>T. vulgaris</i>	0.162± 0.045 ^a	0.600 ± 0.403 ^{ab}
<i>T. vulgaris</i>	0.140± 0.110 ^b	0.410 ± 0.018 ^a

Table 6: Effect of paraquat and *Thymus vulgaris* extract on liver function test

Parameters	AST * (U/L)	ALT * (U/L)	ALP * (U/L)
Groups			
Control	63.833 ± 1.922 ^a	16.500± 1.47761 ^a	97.000± 1.31656 ^a
PQ	68.400 ± 2.1587 ^a	23.800± 1.15758 ^a	121.400± 1.36382 ^c
PQ + <i>T. vulgaris</i>	52.714 ± 4.02796 ^b	20.714± 3.04501 ^a	112.572± 1.08797 ^b
<i>T. vulgaris</i>	52.750 ± 0.62915 ^b	32.250± 2.80995 ^b	119.000± 0.40825 ^c

5.2. Equations

$$MDA(nmol/L) = \frac{\text{absorbance at } 532 \text{ nm}}{L \times E_0} \times D \times 10^6 \quad (1)$$

MDA: Malondialdehyde

L : light path (1 cm)

E₀ : Extinction coefficient 1.56×10⁵ M⁻¹.Cm⁻¹

D : Dilution factor = 1 ml Vol. Used in ref./0.15= 6.7

6. CONCLUSION

The present result concluded that, rats with PQ administration induce loss of body weight and consumed diet, and also liver weight. Besides that, TG parameter was elevated along with many serological toxicity (liver and kidney function tests). Furthermore, the oral administration of *T. vulgaris* hydro-ethanolic extract offered many physiological benefits included antioxidant potential and lipid peroxidation declination. The present study indicated that, *T. vulgaris* improved preventive action against unwanted free radicals generated from PQ exposure in mammals.

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