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Acute and Repeated-Doses (28 Days) Toxicity of Thymol Formulation in Male Albino Rats

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Abstract: The objective of this study was to evaluate the acute and repeated-doses toxicity (28 days) of thymol oil-water emulsion formulation (O/W) via oral route in male albino rats. For the acute toxicity study, thymol formulation was administrated to rats by oral route at doses ranging from 500 to 4000 mg/kgbw and general behavior, adverse effects and mortality were recorded for up to 14 days post treatment. The results revealed that, the median lethal dose (LD_{50}) of thymol formulation was found to be 2462.23 mg/kgbw. In the repeated-doses toxicity study, thymol formulation was administrated to rats at doses of 15.39, 30.78 and 61.55 mg/kgbw (which represent of 1/160, 1/80, and 1/40 of LD_{50}) for 28 days. Our results demonstrated that all treatments did not induce any statistically significant changes in body weight gain or organs weight compared with control group. Also, no significant alteration was observed in haematological and biochemical parameters in treatment groups throughout the experimental period. However, the histopathological analyses, revealed normal architecture of kidney and liver tissues of treatment groups. Therefore, the no-observed adverse effect level (NOAEL) for repeated-doses administration of thymol formulation was considered to be 61.55 mg/kg body weight.

Key words: Thymol, formulation, acute oral toxicity, repeated dose toxicity, rats.

INTRODUCTION

The dramatic increase in the human population over the past 50 years has put great stress on increasing crop yield. Therefore, in recent years, scientists have focused on the increase of food production needed for the fast expansion of world population. Unfortunately, substantial yield losses occur due to insects and plant diseases caused by fungi, bacteria and viruses. Fungi furthermore have unfavorable effects on quality, safety and preservation of food. (Fletcher *et al*., 2006).

Synthetic fungicides are known to be highly effective to control the plant diseases in various vegetables and fruits. The widespread use of pesticides in the croplands, urban environment, and water bodies to get rid of noxious pests has resulted in an increased risk of pesticide resistance and enhanced pest resurgence. Also, toxicological implications to human health and increased environmental pollution, increased cost, handling hazards have to be considered. Efforts are thus being made world wide to replace these synthetic chemical fungicides with other alternatives of natural origin such as Essential oils. (Karmen *et al*., 2003 and Salima *et al*, 2009).

Essential oils (EOs) are commonly used as natural preservatives and fragrances in cosmetic products. More recently, thanks mainly to their antimicrobial properties, new applications as food preservatives. (Burt, 2004), growth promoters in livestock. (Calsamiglia *et al*., 2007), natural pesticides in organic agriculture. (El-Shafei *et al*., 2010) and insecticides. (Phillips *et al*., 2010) are emerging. EOs typically are volatile and they rapidly evaporate from surfaces. It is thus desirable to formulate them, in its effective concentration, in a way that allows minimizing the evaporation and protecting the oil, from high temperature, oxidation and UV light, at the same time. Besides, such formulations should allow for a selective release and for the increase of the shelf life of the oil. For this purpose, oil-in- water (O/W) emulsions are one of the preferred formulations of EOs. (Rodríguez-Rojo *et al*., 2012).

In recent years a great concern on environmental protection has evoked on the use of O/W emulsion as carriers in pharmaceutical, cosmetics, biotechnological and food industries. (Trindade *et al.*, 200 8). For agrochemicals, O/W emulsions using plant-based materials have numerous advantages: (1) water-dilutable emulsion which can replace volatile organic compounds (VOCs) is cost economical, commercially viable and environmentally acceptable; (2) vegetable oils are green-friendly alternative solvents to replace toxic and non-renewable chemicals such as xylene and petroleum oil adjuvants in the conventional pesticides; (3) plant-based biodegradable surfactants can be added to stabilize emulsions and control its particle size to optimum value which are crucial for pesticide penetration. Water is essentially used to hydrate hydrophilic pesticide, prevents crystal formation and drying for increasing the pesticide uptake (Hess and Foy, 2000).

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One of the most promising essential oils is thymol (2-isopropyl-5-methylphenol) is a compound classified among the group of monoterpenes; however, thymol is an active ingredient in pesticide products registered for uses an animal repellent. Also, it is widely use as antibacterial. (Cosentino *et al*., 1999 and Venturini *et al*., 2002), antioxidant. (Aeschbach *et al*., 1994), antifeedant. (Gonzalez-Coloma *et al*., 2002), insecticidal activity. (Mansour *et al*., 2000 and Hummelbrunner *et al*., 2001) and widely used as a general antiseptic in the medical practice, agricultural cosmetics and food industry. (Manou *et al*., 1998)**.** Thus, the present study was undertaken to investigate the acute and sub-acute toxicity of thymol formulation in male albino rats. An additional aim was to identify non-observed adverse-effect level (NOAELs) of thymol formulation toxicity.

MATERIALS AND METHODS

2.1.Materials:

Thymol was purchased from Loba Chemie. PVT. LDT. India. Diethyl ether was obtained from Fisher Scientific Company. Fair lawn, New Jersey, USA. Potassium ethylene diaminetetraacetic acid (K-EDTA) was purchased from Sigma-Aldrich Chemie GmbH Steinheim, Germany. Tween 40 and Tween 60 emulsifiers were purchased from Sigma-Aldrich Chemie GmbH, Riedstr, Steinheim, Germany and water used in all preparations obtained from Water distillatory LABCONCO water PROTM PS LABCONCO Cooperation, KANSAS City, Missouri 64132.

2.2. Preparation of Thymol Formulation (10 % EW):

The oil-in-water emulsion (EW) was prepared by a two-step procedure, progressively adding oil phase in water phase under stirring. The oil phase was prepared with amount of active ingredient of thymol, and a mixture of two nonionic emulsifiers and antifoam agent, while the water phase was prepared with water and antifreeze agent. The oil phase was added in the water phase under high shear mixing. For homogenization high shear mixer (X120, speed of 20,000 rpm/min, duration for 2 minutes) was used.

2.3. Animals:

Male Wistar albino rats (Rattus. norvegicus vara albinus), weighing (150-170 g), provided by the Department of Mammalian and Aquatic Toxicology at Central Agricultural Pesticides Laboratory, (CAPL), Dokki, Giza, Egypt. The animals were housed in plastic cages and were kept five per cage and maintained in standard environmental conditions: controlled temperature 25± 2°C relative humidity 60±5%, photoperiod 12:12 h dark/light cycle and fed with standard pellet diet and water ad libtum. The animals were acclimatizing to the laboratory conditions for two weeks before commencement of experiment. All animals received human care and our study complies with the instructions guidelines. The local committee approved the design of the experiments and the protocol confirms to the guidelines of the National Institute of Health "Guide for the Care and Use of Laboratory Animals" (NIH).

2.4. Acute Toxicity Study:

An acute oral toxicity study was performed according to guidelines of Organization for Economic Co-Operation and Development (OECD) OECD Guideline 401(1987). Healthy male albino rats, fasted overnight, but with access to water ad libtum. They were randomly divided into five groups (n=5). The first group (control group) received 0.9 % NaCl of 1.00 ml/kg body weight. Groups 2-5 were orally treated with thymol formulation with doses of 500, 1000, 2000 and 4000 mg/kg body weight, respectively.

The animals were observed individually for acute toxicity signs and behavioral changes 1h- after treatment, and at least once daily for 14 days. The median lethal dose (LD_{50}) was calculated according to the method described by Weil (1952).

2.5. Repeated-Doses Toxicity:

The repeated-doses toxicity (28 day) study was conducted in accordance with protocol of OECD Guideline 407 (OECD, 1995), based on the acute toxicity results. Animals were randomly divided into four groups, each had animals. The rats of group I was administrated normal saline (0.9 % NaCl) of 1.00 ml/kg body weight via stainless steel ball-Tipped gavage needle and served as a control group, where Groups II- IV administrated thymol formulation at the doses of 15.39, 30.78 and 61.55 mg/kgbw (i.e.1/160, 1/80 and 1/40 of the LD_{50}, respectively). Weekly, the body weight of rats in all experimental groups was recorded. Animals were observed for signs of toxicity during the treatment period.

On the last day of experimentation (Day 29), all surviving animals were fasted overnight, and were anesthized with diethyl ether for blood collection from the retro-orbital sinus vein. (Stone, 1954) using capillary tubes in two clean eppendorf tubes. The one set of clean eppendorf tubes, containing the potassium ethylene diaminetetraacetic acid (K-EDTA) for haematological analysis. The other set of dry nonhepernized clean eppendorf tubes, were kept under refrigeration (4°C) allowed to clot, and serum was separated by centrifugation

at 3000 rpm for 15 minutes. [B y using Centrifuge MSE, Super-Minor, England]. The serum samples were kept at -40°C for biochemical analysis.

2.6. Organ Weights:

After collecting blood samples, rats were euthanized by diethyl ether and selected vital organs such as brain, heart, lungs, liver, spleen, kidneys, testes and epididymis were quickly removed and weighed individually. The organ to body weight ratio was calculated, the organ weights were expressed in absolute and relative terms (g and g/100 g of bodyweight, respectively).

2.7. Hematology and Blood Biochemistry:

Haematology was assayed with whole blood for determination of Haemoglobin concentration (HGb) by the cyanomethoglobin method. (Dacie and Lewis, 1991), and the total erythrocyte (RBCs) and packed cell volume (PCV) by using micro-haematocrit technique were determined by Schalm, 1993. Wintrobe erythrocyte indices, i.e., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated (Schalm, 1993). Furthermore the total leukocyte count (WBCs) and platelets counts were determined. (Schalm, 1993). The differential leukocyte count was performed with an optical microscopy after staining. (Hayhoe, 1964) and, in each case, 100 cells were counted. For biochemical analysis, blood urea nitrogen (BUN) and creatinine levels were measured according to the methods of Patton and Crouch, (1977) and Cook, (1975), respectively. Also, the aspartate and alanine aminotransferases (AST and ALT)) activities were determined by using the method of Reitman and Frankel, (1957).The gamma- glutamyl transpeptidase (GGT) and alkaline phosphates activities were determined by using the method of Szasz and Persijng, (1974) and of Hausdman et al., (1967), respectively. The total Protein profile, viz., serum total protein (TP) and albumin (Ab) concentrations were determined by using the methods of Henry, (1974) and Young, (1975), respectively. The globulin (G) concentration was determined by subtract the albumin from serum total protein and albumin/globulin (A/G) ratio was calculated mathematically according to Coles, (1986). The total and conjugated bilirubin levels were determined according to the method of Jendrassik and Grof, (1938) and un-conjugated bilirubin calculated by subtracting conjugated bilirubin from total bilirubin. The optical density at the appropriate wavelength was measured using a spectrophotometer (ThermospectronicHeλlosα, Helios Alpha, England).

2.8. Histopathological Examination of the Liver and Kidney:

Small Portions of liver and kidney were fixed in 10 % neutral -buffered formalin, dehydrated in ethanol, cleaned in xylene and embedded in paraffin wax. Thin sections (4µm) were cut and stained with Hematoxyl and Eosin (H&E) for histopathological observation under light microscopy. Banchroft *et al*. (1996).

2.9 Statistical Analysis:

Results were expressed as Mean ± Standard Deviation (S.D.). Multi-group comparisons of the means were carried out by compared one-way analysis of variance (ANOVA) test. The means were compared by least significant difference (LSD). Statistical analyses were done by using the (SAS software, 1998).

3. Results:

3.1. Acute Toxicity Study:

Results indicated that the median lethal dose (LD_{50}) of thymol formulation (10% EW) after a single dose by oral route in male albino rats was found to be 2462.23 mg/kg body weight. (Table1). In addition; daily cage side observation, did not demonstrate any change in the skin, eyes, respiratory systems and behavioral patterns throughout the experimental period (14 days). Also, there was no significant change in the body weights of treated rats with thymol formulation when compared with control group, during the experimental period (data not shown).

Table 1: Acute oral toxicity of thymol formulation for male albino rats.

Dose level (mg/kg body weight)	D/T	Mortality (%)
(Control)	0/5	0.0
500	0/5	0.0
1000	0/5	0.0
2000	1/5	2.0
4000	5/5	100

D/T: dead/treated rats

3.2. Repeated-Doses Toxicity Study:

3.2.1. Body and Organ Weights Change:

There were no toxicologically or statistically significant changes in body weight gain in any treatment group (Table 2). Also, this trend was observed in the absolute and relative weights of the selected organs, such as, brain, heart, lungs, liver, spleen, kidneys, testes and epididymis in thymol-treated rats during the experimental period. (Table 3)

Table 2: Effect of thymol formulation (10 % EW) treatments for 28 days on the mean of body weights of male albino rats.

Mean Body weight (g)						
Dose level mg/kg bw	Initial body weight	Day 7	Day 14	Day 21	Day 28	Body weight gain (g)
Control	184.5 ± 5.10	199.5 ±6.9	213.0 ± 8.96	223.8 ± 9.03	238.5 ± 11.50	54.0 ± 10.92
15.39	184.0 ± 8.18	195.0 ± 6.08	214.0 ± 8.92	231.0 ± 14.24	251.0 ± 17.03	67.0 ± 11.46
30.78	187.0 ± 6.69	205.0 ± 7.04	226.0 ± 11.35	235.0 ± 10.25	257.0 ± 16.36	70.0 ± 12.04
61.55	179.0 ± 5.44	190.0 ± 6.08	205.0 ± 12.48	212.0 ± 12.48	236.0 ± 7.38	57.0 ± 4.46
123.11	183.0 ± 2.72	195.0 ± 7.87	214.0 ± 4.17	224.0 ± 6.48	239.4 ± 6.24	56.4 ± 6.08
246.23	181.0 ± 4.17	194.0 ± 6.48	210.0 ± 6.08	220.0 ± 6.08	232.0 ± 7.55	51.0 ± 8.92

All Values represent the mean ± S.D.
a weight gain represent the body weight difference between 28 days and the first dosed day.
No Statistical differences between control and treated groups.

Table 3: Effect of thymol formulation (10 % EW) treatments for 28 day on the absolute and relative organ weights of male albino rats.

Thymol formulation (mg/kgbw)				
Organs	Control	15.39	30.78	61.55
Absolute organs weight (g)				
Brain	4.68 ± 0 .11	4.57± 0.22	4.48 ± 0.22	4.79 ± 0.18
Heart	3.14 ± 0.11	3.21 ± 0.06	3.12 ± 0.11	3.16 ± 0.06
Lungs	4.11 ± 0.13	4.16 ± 0.09	4.19 ± 0.11	4.18 ± 0.13
Liver	9.63 ± 0.49	9.73 ± 0.44	10.06 ± 0.64	10.01 ± 0.18
Spleen	3.17 ± 0.18	3.16 ± 0.09	3.12 ± 0.09	3.31 ± 0.06
Kidneys	4.33 ± 0.15	4.46 ± 0.22	4.48 ± 0.24	4.45 ± 0.09
Testes	5.94±0.17	5.79±0.11	5.71±0.44	6.01±0.17
Epididymis	2.33±0.22	2.19±0.15	2.25±0.02	2.34±0.09
Relative organ weights to final body weight (g/100 g)				
Brain	0.673 ± 0.02	0.639 ± 0.06	0.647 ± 0.02	0.683 ± 0.02
Heart	0.304 ± 0.02	0.316 ± 0.15	0.302 ± 0.02	0.308 ± 0.01
Lungs	0.521 ± 0.02	0.525 ± 0.02	0.541 ± 0.02	0.558 ± 0.02
Liver	2.840 ± 0.29	2.870 ± 0.29	3.075 ± 0.42	3.110 ± 0.04
Spleen	0.320 ± 0.02	0.304 ± 0.02	0.303 ± 0.02	0.337 ± 0.01
Kidneys	0.576 ± 0.22	0.612 ± 0.06	0.621 ± 0.06	0.607 ± 0.02
Testes	1.11± 0.06	1.06±0.11	1.01±0.15	1.18±0.43
Epididymis	0.17± 0.02	0.15±0.02	0.16±0.01	0.17±0.02

All Values represent the mean ± S.D.
Values are not significantly differ at $p < 0.05$ (one way ANOVA).

3.2.2. Haematological and Biochemical Measurements:

The results of haematological measurements in treated rats with thymol formulation are shown in (Table 4). There was no significant alteration in all haematological parameters in rats following treatment with differentselected doses of thymol formulation when compared with control group. However, the biochemical measurements of rats post-treatment with thymol formulation and control group are presented in (Table 5). No statistically significant differences were detected in any of the biochemical parameters in thymol-treated rats in compared with control group.

Table 4: Effect of thymol formulation (10 % EW) treatments for 28 days on haematological Parameters of male albino rats.

Thymol formulation (mg/kg BW)				
Parameters	Control	15.39	30.78	61.55
RBCs (106/µl)	7.31 ± 0.11	7.35 ± 0.22	7.40 ± 0.08	7.22 ± 0.04
HGB (g/dl)	15.57 ± 0.44	15.39 ± 0.42	15.67 ± 0.31	15.59 ± 0.75
PCV (%)	43.20 ± 1.13	43.00 ± 2.72	43.2 ± 0.82	42.80 ± 2.85
MCV (fl)	60.70 ± 2.05	58.54 ± 3.96	58.35 ± 1.6	59.27 ± 4.14
MCH (pg)	21.87 ± 1.11	20.95 ± 1.02	21.16 ± 2.00	21.58 ± 0.95
MCHC (g/dl)	34.26 ± 2.45	35.89 ± 2.25	35.98 ± 1.85	36.52 ± 3.83
PLT(103/µl)	580.2 ± 27.20	549.4 ± 36.12	548.6±27.42	604 ±20.07
WBC(103/µl)	8.80 ± 0.22	8.76 ± 0.26	8.65 ± 0.26	8.57 ± 0.53
LYM (103/µL)	7.18 ± 0.35	7.13 ± 0.17	7.12 ± 0.93	7.06 ± 0.04
NEV (103/µL)	1.52 ± 0.24	1.29 ± 0.15	1.17 ± 0.51	1.38 ± 0.08

RBCs, erythrocyte count ; HGB, haemoglobin concentration; PCV; packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets count; WBC, total leukocyte count; LYM, lymphocytes and NEU, Neutrophils
All Values represent the mean ±S.D.
Values are not significantly differ at $p < 0.05$ (one way ANOVA).

Table 5: Effect of thymol formulation (10 % EW) treatments at selected doses for 28 days on the different biochemical Parameters of male albino rats.

	Thymol formulation (mg/kgBW)			
Parameters	Control	15.39	30.78	61.55
Glu (g)	106.7 ± 57	109.4 ± 4.17	105.9 ± 1.20	109.2 ±8.42
AST (U/L)	156.9 ± 3.56	156.4 ± 4.9	154.6 ± 5.39	165.8 ± 11.03
ALT (U/L)	156.9 ± 3.56	156.4 ± 4.9	154.6 ± 5.39	165.8 ± 11.03
γ-GT (U/L)	0.0	0.0	0.0	0.0
ALP (U/L)	222.1 ± 2.94	220.0 ±0.69	218.4 ±25.19	223.2 ± 5.5
TB (mg/dl)	0.194 ± 0.06	0.196 ±0.01	0.196 ± 0.01	0.204 ± 0.01
CB (mg/dl)	0.02± 0.0	0.02± 0.0	0.02± 0.0	0.02± 0.0
UB (mg/dl)	0.170 ± 0.006	0.176 ±0.01	0. 176 ± 0.01	0.184 ± 0.01
TP (g/dl)	6.61 ± 0.06	6.55 ± 0.06	6.68 ± 0.17	6.68 ± 0.37
Alb (g/dl)	3.45 ± 0.02	3.44 ± 0.13	3.48 ± 0.06	3.48 ± 0.24
Calculated A/G ratio	3.09 ± 0.02	3.1 ± 0.11	3.12 ± 0.11	3.20 ± 0.35
Creatinine (mg/dl)	0.8±0.06	0.74±0.04	0.74±0.04	0.72±0.04
BUN (mg/dl)	17.00±0.98	17.8±1.29	17.00±0.69	16.6±1.11

AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, glutamyl-transpeptidase; ALP, alkaline phosphates; TB, total bilirubin; CB, conjugated Bilirubin; UB, un-conjugated Bilirubin; TP, total protein; Alb, albumin; A/G , Albumin/Globulin ratio and BUN, blood urea nitrogen.

All Values represent the mean ±S.D.

Values are not significantly differ at $p < 0.05$ (one way ANOVA).

3.2.3. Histopathological Study:

None of the macroscopic observations were considered to be treatment related. No gross abnormalities were attributed to treatment with thymol formulation was observed. The histopathological section of liver and kidneys revealed normal architecture in comparison with the control group. (data not shown).

Discussion:

Toxicity tests are essentially performed on either mice or rats because of their availability, low cost and the wealth of toxicology data in the literature already available for these species (Rollo, 1977). Thymol toxicity data reported in available literature acute oral LD_{50} of thymol material (ai) was found to be 980 mg/kg for rats. (Sax, 1984). In our study, the LD_{50} o f thymol formulation was 2462.23 mg/kg body weight, indicating that the formulation is less toxic than the active ingredient. This may be related to the other substances present in the formulation. According to Loomis and Hayes, (1996) classification, chemical substance with LD_{50} within the range of 1000-5000mg/kgbw is considered low toxic.

Generally, the reductions in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances. (Raza *et al*., 2002; Teo *et al*., 2002 and Hilaly *et al*., 2004). Since, no significant changes were observed in the body weights and organs weights of rats in the treated groups as compared to the control group after 28-day of treatment, it suggested that at the sub-acute oral doses administered, thymol formulation has no deleterious effects on health status, growth or development of the animals.

The haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in human and animals (Mukinda and Syce, 2007). The analysis of blood parameters is relevant for risk evaluation, as any change in the haeamatological system have higher predictive value for human toxicity when data are translated from animal studies.(Olson *et al*.,2000).In this study, the data of the haematological parameters showed no significant differences between the control and the treated groups of animals, indicating that thymol formulation had no effects on the circulating blood cells or their production ALT and AST are important serum enzymes in the human liver and monitoring their concentrations usually help to detect chronic liver diseases. (Witthawaskul *et al*., 2003; Burger *et al*. 2005 and Wang *et al*., 2007). ALT is a cytoplasmic enzyme that is found at a very high concentration in the liver, and an increase in the level of this specific enzyme suggests hepatocellular damage. (Tennekoon *et al*., 1991). However, AST is an enzyme that is present in high quantity in the cytoplasm and mitochondria in different tissues, including the liver, heart, skeletal muscle, kidney and brain. (Evan, 2009). Moreover, bilirubin elevation is also confirmation to varying liver functions. A small elevation in serum bilirubin is an important indicator of liver damage in laboratory animals or could be a sign of biliary duct obstruction. (Rasekh *et al*., 2008). Furthermore, it has been reported that an increase in the level of serum proteins is indicative of tissue injury. (Solomon *et al*., 1993). Serum proteins like albumin can act as a criterion for assessing the synthetic capacity of the liver, since nearly all are synthesized in hepatocytes. A reduction in serum proteins therefore tends to reflect chronic damage in hepatocytes. (Rasekh *et al*., 2008). In the present study, there were no statistically significant differences in the usual markers of liver function between control and treated animals at any dose. Hence, thymol formulation does not cause hepatic toxicity. This was further confirmed by histopathological examination of the liver of treated and control rats. (data not shown).

Usually, creatinine is known as an effective indicator of renal function and a rise in creatinine level is a reliable indicator of a negative impact on kidney function or impaired glomerular filtration. (Lameri *et al*. 2005; Hassan *et al*., 2007; Rhiouani *et al*., 2008 and Evan 2009).

A blood urea nitrogen (BUN) test, measures the amount of nitrogen in the blood that comes from the waste product, urea and other nitrogenous wastes. Urea nitrogen undergoes renal tubular re-absorption by specific transporters. This tubular re-absorption limits the value of BUN as a marker for glomerular filtration. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, and shock and bleeding in the digestive tract. (Pagana, 2002). In the present study, the data of the kidney function showed no significant differences in the BUN and creatinine between the control and the treated groups indicating that thymol formulation does not cause nephrotoxicity. This was cofinrmed by histopathological examination of kidneys of treated and control rats, showing normal architecture (data not shown).

Conclusion:

The oral dose administration of thymol formulation at 61.55 mg/kg bw for 28 day did not induce any significant change in the body weight gain and organ weights as well as the haematological and biochemical measurements, in addition, to histopathological findings in the treatment groups of animals, it can be defined as the no-observed adverse effect level (NOAEL) for male albino rats in the current study. However, it should be emphasized that this NOAEL was derived from repeated-doses toxicity study only. For a more reliable safety evaluation performed on the basis of the acceptable daily intake concept, data on the chronic toxicity, reproductive toxicity, genotoxicity and carcinogenicity of thymol formulation would also be required.

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