

Comparison study on the effect of thymoquinone and nano-thymoquinone on lipid profile of diazinon induced toxicity in male rats.

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Received: 18/9/2022
Accepted: 18/10/2022

Abstract: The objective of the current study was to compare between the effect of thymoquinone (TQ) and nano-thymoquinone (N-TQ) on lipid profile on diazinon (DZN) induced toxicity in male rats. Rats were haphazardly divided into seven sets, six for each: Group 1 (control): rats receiving distilled water; group 2 (corn oil): rats receiving corn oil by gastric tube daily for 21 days; group 3 (TQ): rats receiving TQ (40 mg / kg bw) daily oral administration for 21 days; group 4 (N-TQ): rats receiving N-TQ (40 mg/kg bw) oral administration for 21 days; group 5 (DZN): Rats are given DZN (15 mg/kg bw) orally for 21 days; group 6 (TQ +DZN) rats of this group orally received TQ then after one hour DZN for 21 days and group 7 (N-TQ +DZN) rats of this group orally received N-TQ then after one hour DZN for 21 days. Our results showed significant increases TC, TG, LDL-C, as well as major decreases in HDL-C in serum. On the other hand, TQ and N-TQ administration significantly improved the deviation resulting from DZN in the tested parameters. Ultimately, it could be concluded that to boost the hepatic damage caused by DZN, TQ and N-TQ should be taken before DZN. Although, the effect of N-TQ was more effective.

keywords: thymoquinone, Nano-thymoquinone, diazinon, toxicity, lipid.

Abbreviations: TQ, thymoquinone; N-TQ, nano -thymoquinone; DZN, Diazinon; CO, corn oil; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride.

1. Introduction

Nowadays, there is an increment in the using of pesticides. These Pesticides which are occasionally used indiscriminately in large amounts causing environmental pollution and therefore, are a cause of concern [1]. Pesticide residues has a major interest as environmental pollution due to their wide use in agriculture and in public health life [2].

Nigella sativa, a highly potent medicinal plant of Ranunculaceae family, is an annual flowering herb which usually grows 20-90 cm tall. *Nigella sativa* locally referred to black seed or black cumin is natively found in the regions of North Africa, Southern Europe and Southwest Asia. Currently it is grown in numerous countries across the world [3, 4]. Seeds contain a lot of terpene alcohols and esters of unsaturated fatty acids; moreover, presence of alkaloids as:

i. Nigellimin is a representative of isochinoline alkaloids.

ii. Nigellimin-N-oxide and the pyrazol alkaloids nigellidin and nigellicin are examples of this.

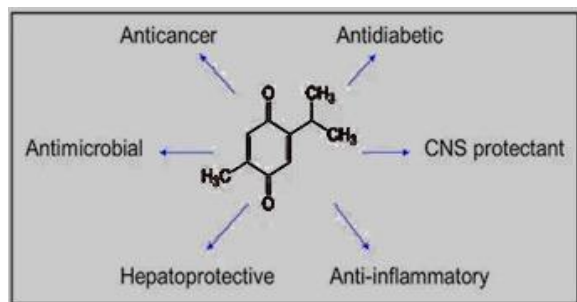
For the essential oil, thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone) Besides p-cymene, pinene, dithymoquinone, and thymohydroquinone, was determined to be the primary constituent. Only trace levels of other terpene compounds were discovered.

Additionally, the seeds have:

i. Unsaturated fatty acids, primarily dihomolinoleic acid, linoleic acid, oleic acid, and eicodadienoic acid.

ii. Palmitic and stearic acid, two types of saturated fatty acids. [5].

The *Nigella sativa* black seeds also contain volatile oil, fatty oil, protein, fat, carbs, crude fibre, and total ash. [6], moisture and cellulose [7]. The seeds are also loaded with minerals and a variety of vitamins, particularly A, B1, B2, B3, and C. (Ca, K, Se, Cu, P, Zn, Fe). Its seeds and roots have also been shown to contain carotene and vanillic acid. [8].



Biological activities of thymoquinone

2. Materials and methods

Diazinon (DZN) was purchased from American Sigma Company code 454258-250MG, it was dissolved in corn oil and was given orally at a dosage (15 mg/kg bw) to male albino rats. TQ (99% pure analytical standard, Sigma Aldrich, Germany), Nano-thymoquinone was prepared from TQ according to [10]

Animals: 42 White male albino rats (*Rattus norvegicus*) 90-100g used for this study. Rats were purchased from Egyptian Institute for Vaccine and Serological manufacture, Helwan, Egypt, and were kept in the faculty of veterinary medicine's department of physiology's animal house at Kafrelsheikh University. Rats located in stainless steel cages containing wood-chip for bedding and renewed daily. The rats maintained a 24 h cycle in a controlled temperature setting. Two weeks before the start of the experiment, all rats were acclimatized to the location. During the experiment time, the animals were provided with normal diet and water *ad libitum*.

Animal grouping

After two weeks of acclimatization period, rats were randomly divided into seven groups, each containing six animals as follows: control group: this group's rats were not treated. Corn oil treated group: this group was administered corn oil daily for 21 days. Thymoquinone (TQ) treated group: Rats of this group were given TQ (40 mg/kg bw) daily for 21 days. Nanothymoquinone (N-TQ) treated group: rats

of this group were given N-TQ (40 mg/kg bw) daily for 21 days. Diazinon (DZN) treated group: rats from this group were given DZN (15 mg/kg bw) daily for 21 days. Thymoquinone and Diazinon (TQ + DZN) treated group: rats of this group were given TQ and after one hour were given DZN. Nanothymoquinone and Diazinon treated group (N-TQ+DZN): rats of this group were given N-TQ, and after one hour were given DZN.

Sample collection:

After 21 days fasted rats were sacrificed overnight (24 hours after the last treatment) and Blood samples were drawn into centrifuge-clean glass tubes, allowed to clot, and then spun at a speed of 4000 rpm for 15 minutes. Quickly extracted and placed in labelled Eppendorf tubes, the clear non-hemolyzed sera were frozen at -20°C for various biochemical analyses.

Used kits:

TC, TG, HDL-C were estimated according to the method of [11-15] using TC Biodiagnostic kit from Biodiagnostic Co. Dokki, Giza, Egypt. LDL-C concentration was determined using the equation provided by [16].

Statistical analysis:

Using GraphPad Prism 5.0, data are analyzed. A standard error mean (SEM) was used to express the experiment's results ($n = 6$). Results were examined using one-way analysis of variance (ANOVA), multiple correlation testing, and Newman-Keuls.

$P < 0.05$ values are considered statistically significant.

3. Results and Discussion

The findings revealed that the concentration of serum TC, TG, LDL-C in diazinon (DZN) treated group was significantly higher than its concentration in control group. While in thymoquinone (TQ) and Nano-thymoquinone (N-TQ) treated groups, TC, TG, LDL-C concentration has significantly improved compared to that in DZN treated groups. The improvement was more marked in N-TQ group than TQ treated group. However, the results indicated that there were insignificant changes in the groups treated with corn oil (CO), TQ

and N-TQ when compared to control and HDL-C was significantly decreased in DZN treated

group and increased in TQ and N-TQ treated group.

Table (1): Serum total cholesterol (mg/dl)

Rat groups	TC	C	CO	TQ	N-TQ	DZN	TQ+DZN	N-TQ+DZN
Mean		68.5	67.75	64	60.5	246.8 ^a	100.7 ^{ab}	97.93 ^{ab}
±SE		±3.18	2.69	±1.87	±1.71	±6.66	±4.47	±2.47
% of change	*		-1.09	-6.57	-11.68	+260.29	+47.01	+42.96
	**						-59.20	-60.32

Table (2): Serum triglycerides (mg/dl)

Rat groups	TG	C	CO	TQ	N-TQ	DZN	TQ+DZN	N-TQ+DZN
Mean		97.25	96.35	95.33	92.4	219.5 ^a	150.3 ^{ab}	145.5 ^{ab}
±SE		±1.931	±1.836	±2.083	±3.774	±13.02	±6.775	±6.85
% of change	*		-0.93	-1.97	-4.99	+125.71	+54.55	+49.61
	**						-31.53	-33.71

Table (3): Serum low density lipoprotein cholesterol (mg/dl)

Rat groups	LDL-C	C	CO	TQ	NCS	DZN	TQ+DZN	NCS+DZN
Mean		14.58	12.60	8.85	5.28	180.40 ^a	42.98 ^{ab}	38.82 ^{ab}
±SE		1.30	0.91	1.05	1.11	3.44	2.24	0.25
% of change	*		-13.58	-39.33	-63.77	+1137.31	+194.79	+166.26
	**						-76.18	-78.48

Table (4): Serum high density lipoprotein cholesterol (mg/dl)

Rat groups	HDL-C	C	CO	TQ	N-TQ	DZN	TQ+DZN	N-TQ+DZN
Mean		33.51	35.91	36.09	36.74	22.45 ^a	27.7 ^b	30.02 ^b
±SE		1.55	2.07	1.93	1.86	1.38	1.02	0.96
% of change	*		+7.16	+7.70	+9.64	33.01	-17.34	-10.41
	**						+23.39	+33.72

Results were presented as means ±SE and % of change for 6 rats in each group.

C: Control, CO: corn oil, TQ: Thymoquinone, N-TQ: Nano-thymoquinone, DZN, diazinon

(*): % of change related to control group.

(**): % of change related to diazinon group.

(a,b) indicate significant change at $P \leq 0.05$, compared to control and diazinon groups respectively.

Diazinon (DZN) is one of the widely used organophosphorous compounds in agriculture [17]. It is a synthetic chemical substance with broad spectrum insecticide activity [18]. It is a colourless fluid that is mostly absorbed by the skin, inhalation, and ingestion. Chronic symptoms include increased salivation, nausea, headaches, confusion, irritability, alertness, and diarrhea [19].

In various organs, including the kidney and liver, DZN is broken down and quickly eliminated by oxidation and hydrolysis mechanisms [20]. Also, it acts as an acetylcholinesterase (AChE) inhibitor, an enzyme needed for proper nervous system function [21]. Acetylcholine builds up in the

synaptic cleft when AChE is inhibited. Furthermore, when it enters the body, In the liver microsomal enzyme system, it can be oxidatively degraded into diazoxon utilising NADH and O₂ [22].

Diazoxon, an active oxygen metabolite of DZN, causes predictably higher levels of AChE inhibition than the parent compound owing to the fact that the parent compound does not inhibit AChE directly [23, 24].

In addition, earlier studies have indicated that DZN could increase the production of reactive oxygen species (ROS), damage of mitochondrial membrane, oxidative stress (OS), lipid peroxidation (LPO), oxidative modifications and fragmentation of DNA in the content of genomic DNA of the tissues/cells to induce genotoxicity, neurotoxicity, cardiotoxicity or cytotoxicity and apoptosis [25-27]. It also has direct effect on biochemical and haematological biochemical parameters of mice, rats and rabbits [28, 29]. Other systems that could be affected are pancreas, reproductive system, immune system, urinary system and liver [21].

Hepatocellular carcinoma (HCC), which is one of the primary causes of death worldwide,

is often brought on by liver injury [30, 31]. Hepatitis, which is characterised by inflammatory cell infiltration, clogged blood vessels, and cellular deterioration alterations, is one of the earliest symptoms of liver injury. Viral infections, microbial metabolites, autoimmune and metabolic illnesses, environmental toxins, drugs and alcohol addiction can all cause hepatitis [32, 33]. If hepatitis is not treated properly, additional difficult liver conditions including cirrhosis and fibrosis can develop and, in severe situations, become risk factors for HCC. [34].

Traditional methods of treating cancer and liver damage with natural remedies include alternative therapeutic approaches. [35]. For thousands of year's natural products have played a very important role in health care and prevention of diseases. The ancient civilizations of the Chinese, Indians, north Africans and Egyptian provide written evidence for the use of natural sources for curing various diseases [36]. Among these medicinal natural products, *Nigella sativa* and the primary phytochemical thymoquinone (TQ) it contains have a number of health-promoting properties, including antioxidant, anti-inflammatory, immune-stimulating, antibacterial, hypoglycemic, and anti-arthritis activities as well as hepatoprotective properties [37, 38]. TQ reduced hepatotoxicity due to its strong antioxidant and anti-inflammatory effects [39]. It is used as natural food additive [40]. Therefore, the present study aimed to manifest the effect of TQ and nano-thymoquinone (N-TQ) on DZN induced hepatic injury.

The creation of nanosized delivery systems that can transport medications to the target location is one of the most alluring areas of research in the oral drug delivery system [41]. There are many nano delivery techniques as polymeric nanoparticle and lipid nanoparticle [42, 43]. Regarding ROS susceptibility, lipids are regarded to be among the most sensitive biological substances. specially, unsaturated fatty acids, which are located in tissues, cellular membranes and blood, are prone to ROS attack [44].

The present study indicated that administration of DZN (15 mg/kg) for 21 days by stomach tube to male albino rats resulting in

a substantial increase in lipid profile parameters as total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) in contrast to significant decrease in high density lipoprotein cholesterol (HDL-C). These results are in agreement with [45-48]. The marked increase in the serum TC level may be assigned to an increment in the level of cholesterol synthesis in the liver or it may be a mark of liver damage that can be referred to the effect of DZN pesticides on the hepatocellular membrane permeability. Moreover, the increase in serum TC level may be assigned to the blockage of liver bile ducts that cause cessation or reduction of its secretion to the duodenum [49].

Furthermore, DZN increased the activity of the target gene 5-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol production. [46]. The observed hypercholesterolemia may be brought on by hepatocyte injury brought on by OS-mediated cell membrane rupturing that results in cholesterol leakage into the blood. Additionally, impaired cholesterol secretion into the bile led to stoppage of bile flow in bile ducts as a result of periportal cell death (confirmed by higher alkaline phosphatase), which ultimately resulted in an increase in total blood cholesterol in DZN-treated rats. [50].

Regarding to HDL-C it is mostly synthesized in intestinal cells and liver. It plays an important role in cholesterol efflux from tissues and transport it back to the liver for elimination of bile acids [51]. Generally, the esterification of free plasma TC takes place in plasma HDL, where the reaction is catalyzed by the enzyme lecithin-cholesterol acyltransferase (LCAT), which uses HDL as a substrate [52]. Previous studies have indicated that the LCAT enzyme plays a crucial role in the formation of HDL. Organophosphorous compounds (OPCs) can inhibit the action of this enzyme [48]. Therefore, the inhibition of LCAT activity may be an explanation for decreasing serum HDL-C levels in DZN exposure. LDL-C and HDL-C are diagnostic lipoproteins. LDL-C carries cholesterol to various tissues such as adipose tissue, and HDL-C which transports endogenous cholesterol from the tissues to the liver for disposal [53, 54].

In addition, the increase in serum TG may be attributed to an inhibition of lipase enzyme activity of both hepatic TG and plasma lipoproteins [55], so hypertriglyceridemia could occur through single or dual inhibition of the fatty acid amide hydrolase and/or monoacylglycerol lipase. [56]. Moreover, the elevated TG can be explained by increasing the lipolysis of adipocyte as DZN – enhanced the inhibition or resistance of insulin of plasma hepatic lipase (HL) and lipoprotein lipase (LPL) [47].

It was documented that the liver is an important organ in the metabolism of lipids since it is where lipoproteins are taken up, made, and exported into the bloodstream. The primary lipid carriers from the liver to the peripheral cells are LDL-C and VLDL-C, while HDL-C carries extra cholesterol from the peripheral cells to the liver. Therefore, It may be argued that the elevated levels of serum VLDL-C and LDL-C in DZN-treated rats were caused by the inhibition of LPL and HL activity as well as decreased hepatic absorption because of hepatocellular injury. [47].

However, the levels of HDL-C and TG in the blood are inversely correlated. As a result, a lower HDL-C blood concentration could be linked to hyperlipidemia and/or decreased HDL-C synthesis by the liver (as demonstrated by the concurrent reduction in total protein (TP) concentration), which led to atherosclerosis [47,57, 58]. On the other hand, the results of this study also revealed that oral administration of TQ and nano-thymoquinone (N-TQ) (40 mg/kg) for 21 days ameliorated the lipid profile parameters altered by DZN as (TC, TG and LDL-C) and increased the level of HDL-C concentration in the serum. These results are in agreement with [59-62]. The decrease in the concentration of TC, TG and LDL-C may be attributed to the presence of linoleic acid and palmitic acid that show important effects of TQ to get rid of lipid by inhibition of HMG-CoA reductase activity and the increased numbers of the receptors of LDL [59]. Also, [63] documented that TQ improved metabolic processes by bringing these endpoints toward control. Evidently, histopathological examinations of liver also supported TQ therapy as it helped in the improvement of hepatocellular architecture. This clearly

indicated stabilizing effect of TQ on the membrane by scavenging free radicals.

Furthermore, ameliorating effect of TQ on dyslipidemia induced by DZN may be assigned to its promoting effect on regulatory effects on cholesterol metabolism-influencing genes, hepatic arylesterase activity and its potent antioxidant properties [64]. However, *Nigella sativa* has a large number of pharmacological effects, such as hypotension, hypoglycaemia and protective effects on liver tissues that can improve the metabolism of the body. In this line, it has also been reported that plants with antioxidant properties as it reduces high blood lipid and improves the body metabolism [65].

Conclusion

In order to reduce the effect of Diazinon on fat metabolism in liver cells, the results confirmed that the use of NTQ gives more effective results

4. Reference

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