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INVESTIGATION OF THE EFFECT OF GINGER ON THE LIPID LEVELS AGAINST THE TOXIC EFFECT OF PHTHALATE IN MALE RABBITS

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Abstract:-

This study was designed to investigate the adverse effects of Di-(2ethylhexylphthalate (DEHP) the levels of total protein (TP), total lipids (TL), total cholesterol (TC), triglyceride (TG), high and low-density lipoprotein-cholesterol (HDL-c and LDL-c) in blood plasma in male New-Zealand white rabbits. Additionally, the study was extended to show the protective effect of ginger against DEHP toxicity. The levels of TL, TC, TG and LDL-c were significantly ($P<0.05$) increased, while HDL-c and TP were significantly ($P<0.05$) decreased in plasma of rabbits treated with DEHP alone as compared with control group. The levels of TL, TC, TG and LDL-c were significantly ($P<0.05$) decreased while HDL-c and TP was significantly ($P<0.05$) increased in rabbits treated with ginger alone as compared to control animals. The presence of ginger with DEHP caused significant ($P<0.05$) decrease in the induction in the levels of TL, TC, TG and HDL-c, and significant ($P<0.05$) increase in the reduction TP and LDL-c due to treatment with DEHP, and this means that ginger had protective effect against the toxicity of DEHP .

Key words:-*Ginger, phthalate, cholesterol, New-Zealand white rabbits*

INTRODUCTION

Humans are constantly exposed to a wide range of environmental contaminants from industrial processes, through air, food, water or contact with a variety of consumer products. One class of chemicals used as plasticizers called phthalates have attracted special attention from the scientific community and the general public due to their numerous applications and their high production volume that reach millions of tons annually [1]. Plasticizers, which are used to modify the properties of polymers, are the largest group of compounds used in the manufacture and processing of synthetic materials with an approximate 55% share of the global market [2]. Exposure to phthalate esters occurs through drinking water, food and personal care products. Some of these esters are also used as a coating for medications [3]. Metabolites of several phthalates are found in the blood and urine from biomonitoring studies in the United States and Europe at all age range from infants to adults, and in milk from lactating women [4]. [5] Showed an interaction between dietary fat and DEHP in lipid metabolism where presence of the phthalate in diet potentiated the growth-promoting effect of lipids in rats. Substantial reduction in serum cholesterol and proliferation of peroxisomes and elevation of catalase and carnitine acetyltransferase activity in livers of animals receiving DEHP via diet was reported by [6]. Induction of the fatty acyl-CoA oxidizing enzyme system located in peroxisomes by DEHP has been suggested by [7], and inhibition of lipid synthesis in rats has been shown by[8].

Reduced serum glucose and cholesterol and elevated serum free fatty acid have also been reported [9]. The rats receiving 0.5% of DEHP in a normal protein diet showed accumulation of phospholipids, decrease in cholesterol and triglyceride contents in liver and a rise in levels of plasma fatty acids. Such observations suggest the utilization of lipids for the energy source. An increase in the protein content in ver of DEHP-treated rats has been described by [10]. [11] Have shown that a DEHP-caused elevation in rat liver protein content is due to a decrease in protein breakdown.

Antioxidants are widely needed to prevent deterioration of otherr ox disable goods, such as cosmetics, pharmaceuticals and plastics. Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones. In addition, other biological properties such as anti-carcinogenicity, anti-mutagenicity, anti allergenicity and anti aging activity have been reported for natural and synthetic antioxidants [12]. Ginger (Zingiber officinale Roscoe, Zingiberacae) is one of the most commonly used spices around the world, especially in the South-Eastern Asian countries. Ginger is also a medicinal plant that has been widely used in Chinese, Ayurvedic and Unani-Tibb medicines, since antiquity, for a wide array of ailments that include arthritis, rheumatism, sprains, muscular throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis [13]. Ginger (Zingiber officinale) has been consumed since antiquityand is known to play diverse biological roles including anti oxidation, anti-inflammation, hypolipidemia, anti-carcinogenesis,anti-nausea, antithrombosis, and antibacterial process[14]. Therefore, the present study was conducted to examine the possible modifying effects of ginger on blood plasma biochemistry associated with DEHP exposure in male rabbit.

MATERIALS AND METHODS

Materials:

In this study di-(2-ethylhexyl) phthalate (DEHP) and ginger were used. Di-(2-ethylhexyl) phthalate (purity 99.0%) was purchased from Sigma–Aldrich (USA) and ginger was obtained from Superior Nutrition and Formulation by Jarrow Formulas, Los Angeles, USA. All other chemicals used in the experiment were of analytical grade.

Mature male New Zealand White rabbits (age of 7 months and initial weight of (2.917 ± 28.9 Kg) were used. Twenty mature male rabbits were randomly divided into four equal groups (each five rabbits)**: Group I:** Rabbits were used as control and received an equivalent volume of the vehicle (corn oil) alone by oral gavage daily for 12 successive weeks.

Group II: Rabbits were treated with ginger. Ginger was given ginger daily by gavage at a dose of 100 mg/kg B.W,[15] which dissolved in corn oil for 12 successive weeks. **Group III:** Rabbits were treated daily with di-(2-ethylhexyl) phthalate (DEHP) by gavage at a dose of 500 mg/kg B.W/day (1/50 of DEHP lethal dose [16]. **Group IV:** Rabbits were given with DEHP daily at a dose of 500 mg/kg B.W/day by savage like group III and given the ginger concurrently daily at a dose of 100 mg/kg B.W/day by gavage like group II for 12 successive weeks.

Methods:

At the end of the experimental period, all rabbits were weighed then sacrificed under ether anesthesia. Blood samples were collected in clean dry centrifuge tubes. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and then quickly frozen at -20°c for biochemical analysis.

Biochemical analysis:

The lipids were extracted according to the method of [17]. This method involved two successive steps of extraction. In the first one, the lipids were extracted with a solvent system (chloroform-methanol, 2:1 v/v) through homogenization. The homogenate was centrifuged. In the second step, the supernatant which contains the total lipids is mixed with one fifth its volume of distilled water. Certain mineral salts may be used for washing the inorganic phase for extraction of any free fatty acids. Total cholesterol was determined according to the method of [18]. This method was based on the hydrolysis of cholesterol esters in sample by cholesterolester hydrolase (ChEH). The cholesterol oxidase (ChOD) then oxidizes free cholesterol in presence of oxygen to 4-Cholesten-3-one and H_2O_2. Then the peroxidase enzyme catalyzes the formation of red quinonimine derivatives from hydrogen peroxide (H_2O_2) and 4aminoantipyrine in the presence of phenol. The

intensity of this red color is proportional to the cholesterol concentration in the sample. Triglyceride (TG) was determined according to the method of [19]**.** The triglyceride was hydrolyzed in the sample by lipoprotein lipase into glycerol and fatty acids. Then, glyceol was phosphorylated by glycerol linase (GK) in the presence of ATP and Mg** ions. Glycerol -3-P was oxidized by glycerol-3-phosphate oxidase (GPO) in the presence of molecular oxygen (O_2) to dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2). A colored product was formed from hydrogen peroxide, 4-aminoantipyrine and phenol-derivative in the presence of the peroxidase (POD).

High-density lipoprotein -cholesterol was determined according to the method of [20]**.** This method was based on the precipitation of other lipoprotein fractions (LDL) with a mixture of phosphotungstic acid and magnesium chloride solutions and removed by centrifugation. Then the concentration of cholesterol in the HDL of the clear supernatant can be measured. The low density lipoprotein cholesterol (LDL-c) content was calculated according to the following formula of **[21]:** LDL-c (mg/g tissue) = Total cholesterol – (TG/5) – HDL-c

RESULTS

As shown in Table 1 the data recorded plasma levels of total protein (TP), total lipids (TL) and total cholesterol (TC) in control and different treated rabbit groups. In group that treatment with DEHP was caused significant (P<0.05) decrease in the levels of plasma of total TP, TL and TC in comparing with control rabbits. While, administration of ginger alone was caused significant (P<0.05) increase in three of them if compared to control. However, intake of ginger with DEHP caused significant (P<0.05) amelioration in the reduction of total of TP, TL and TC as well as an improvement in the level of theses biochemical compounds as compared to control. The data in Table 2 represents plasma levels of triglyceride (TG), high and low-density lipoprotein-cholesterol (HDL-c and LDL-c) in control and different treated rabbit groups. The treatment with DEHP was caused significant (P<0.05) increase plasma levels of TG. HDL-c and LDL-c while, ginger caused significant (P<0.05) decrease in the levels of TG. HDL-c and LDL-c compared to control. The presence of ginger with DEHP caused decrease in the levels of TG. HDL-c and LDL-c as compared to control. These indicate that ginger counteracted the toxic effects of DEHP.

Table 1: Plasma levels of total protein (TP), total lipids (TL) and total cholesterol (TC) in control and different treated rabbit groups

Parameters	Animal Groups			
	Control	ginger	DEHP	Ginger+DEHP
Total protein (TP; g/dl)	6.83 ± 0.021^{b}	6.98 ± 0.043^{a}	6.59 ± 0.047^{d}	6.72 ± 0.028^{c}
Total lipids (TL; mg/dl)	524 ± 7.9^{b}	490 ± 9.9^{c}	565 ± 6.8^{a}	$541 \pm 11.1^{a b}$
Total cholesterol (TC; mg/dl)	120.4 ± 0.49^{b}	118.3 ± 0.50^{c}	122.1 ± 0.36^{a}	120.6 ± 0.52^{a}

Values are means ± SE of 5 rabbits in each group Mean with different letters **(a- d)** are significantly difference ($p \leq 0.05$). Mean with the same letters (a-d) are non-significantly difference ($p \geq 0.05$).

Table 2: Plasma levels of triglyceride (TG), high and low-density lipoprotein-cholesterol (HDL-c and LDL-c) in control and different treated rabbit groups

Parameter	Animal Groups			
	Control	ginger	DEHP	Ginger+DEHP
Triglyceride (TG; mg/dl)	0.783 ± 0.006^{cb}	0.769 ± 0.006^{c}	0.807 ± 0.007^{a}	0.796 ± 0.004^{a}
High density lipoprotein cholesterol (HDL-c; mg/dl)	57.5 ± 0.62^{b}	60.6 ± 0.53^{c}	55.8 ± 0.55^{a}	58.8 ± 0.64^{b}
LOW density lipoprotein cholesterol (LDL-c; mg/dl)	59.3 ± 0.84^{b}	56.6 ± 0.63^{c}	61.9 ± 0.63^{a}	58.6 ± 0.79^{b}

Values are means ± SE of 5 rabbits in each group
Mean with different letters (a- d) are significantly difference ($p \leq 0.05$).
Mean with the same letters (a_d) are non-significantly difference ($p \geq 0.05$).

DISCUSSION

[22] Reported that total protein is done as a routine test to evaluate the toxicological nature of various chemicals. The effect of di-ethylhexylphthalate on serum biochemical parameters observed in this study (Table 1and 2) is in agreement with previous results obtained on rabbits [23]. The reduction in serum protein in animals exposed to environmental pollutants could be attributed to the changes in protein and free amino acid metabolism and their synthesis in the liver [24]. The

protein depression in the blood was also reported to be mainly due to excessive loss through nephrosis [25]. Additionally, the decrease in blood protein may be due to loss of protein either by reduced protein synthesis or degradation [26]. Changes in the level of total protein reflect disorders in the synthesis and metabolism of proteins [27]. The present study showed that ginger caused an increase in protein, and this is inagreement with the obtained results by [22] who reported that total protein increased in rats treated with ginger.

There are many studies showed that exposure to some environmental pollutants caused alterations in the concentrations in plasma lipids profile [28- 31]. Abnormal lipid levels contribute significantly to the risk of coronary heart disease, a major cardiovascular disease and a serious health problem. Changes in blood cholesterol and triglyceride levels are indicative of disorders of the lipid metabolism [27]. The present study showed that DEHP caused changes in lipid profile in plasma (Tables 1 and 2). [32]found that DEHP, DnHP, di-noctyl phthalate all affect triglyceride synthesis and fatty acid oxidation in freshly isolated hepatocytes and that the effect of the straight-chain phthalates is greater than that of DEHP. Accordingly, it is clear that the phthalate esters may interfere in the regulation of lipid metabolism.

Some phthalates have been shown to disrupt several gene pathways, including cholesterol transport and steroidogenesis, as well as pathways involved in intracellular lipid and cholesterol homeostasis, insulin signaling, transcriptional regulation, and oxidative stress [33]. [34]who found subchronic administration of DEHP (1000 mg/kg b.wt./day) for 6 weeks in rats caused increase fatty acid and phospholipids synthesis and enlargement of liver due to increase in cell proliferation. [10]suggested that intake of 1% di (2-ethyl hexylphthalate in rats induces hyper phospholipids of the liver tissue but attenuates TG and cholesterol levels. A decrease in circulating cholesterol and triglyceride levels is also associated with DEHP exposure in rats [35]. Increased fatty acid catabolism decreases the concentration of free fatty acids available for export from the liver as circulating triglycerides. This provides a rationale for the lowered triglyceride values. The lowered serum cholesterol concentration apparently results from inhibition of cholesterol synthesis and stimulation of the conversion of cholesterol to bile acids in the liver [36]. For example, feeding female rats DEHP at an estimated dose of 500 mg/kg/day for 13 days significantly inhibited sterologenesis from ^{14}C-mevalonate in liver and adrenal minces [37]. DEHP also inhibited cholesterol synthesis in the liver from male rats and rabbits as well as in rats' testes [38]. In a subsequent study,[39] demonstrated that the inhibition of cholesterol synthesis in the liver was due to a reduction in the activity of microsomal acylCoA:cholesterol acyltransferase, an enzyme responsible for the esterification of cholesterol
The decrease in lipid profiles and increase in HDL-c of rabbits treated with ginger (Tables 1and 2) are in accordance with the results obtained by[40] who found that Zingiber officinale (200 mg/kg) fed orally for 20 days produced significant ($P < 0.01$) reduction in serum total cholesterol, triglycerides and increased the HDL-cholesterol levels. Also,[41] found that ginger has been shown to reduce plasma lipids in cholesterol-fed hyperlipidaemic rabbits and in streptozotocin induced diabetic rats and were also found to inhibit LDL oxidation in atherosclerotic mice. Besides, the aqueous extract of ginger has also been shown to reduce serum cholesterol and triglycerides in normal rats.

[42] Reported that a significant reduction in levels of cholesterol was observed in the rats given ginger. This finding is in consistent with previous reports demonstrating the hypocholesterolemic and anti-atherosclerotic effects of ginger [40]. [43] Reported that ginger significantly lowered serum total cholesterol, LDL, VLDL, and triglycerides, and raised HDL. It was found that ginger acted on the liver to reduce cholesterol biosynthesis and may stimulate cholesterol's conversion to bile acids and increase its faecal excretion.[44] has been reported that ginger stimulates the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body. As mentioned previously, the activity of hepatic cholesterol-7 alpha-hydroxylase, the rate-limiting enzyme of bile acid biosynthesis from cholesterol, was significantly elevated in ginger-treated animals.

[45] Who found a significant decrease in blood serum glucose and cholesterol when feeding chicks up to 6% ginger? The supplementation of ginger reduced cholesterol levels in blood serum because of its antioxidative action which also a mechanism could be used as anti-stress approach [46]. The hypocholesterol action may be done by ginger acting as a potential inhibitor of cholesterol synthesis [47]. The hypolipidemic effect of ginger has been shown by other investigators [48]. It is likely that the hypochlosterolemic effects of ginger stem from the inhibition of cellular cholesterol synthesis. Attenuation of cholesterol synthesis results in augmentation of LDL receptor activity, which leads to elimination of LDL from plasma [49]. It is well established that elevation of LDL oxidation induces oxidative stress and resultant damage. The alleviation in most investigated biochemical parameters in ginger group presented in the present work could be attributed to the antioxidant properties of ginger. Because ginger contains phenolic compounds such as shogaols and gingerols, zingiberene, zingiberol, curcurmene, zingerone, geraniol and neral in zingiber have antioxidative properties [50].

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