Ain Shams University
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Evaluation of the influence of ginger (*Zingiber officinale*) extract on some metabolic disorders in male albino rats.

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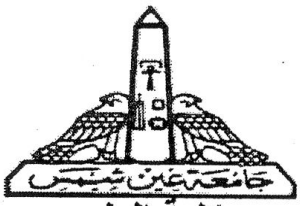

كلية العلوم
قسم علم الحيوان

تقييم تأثيرمستخلص الزنجبيل (*زنجيبر اوفيشينال*) على بعض الإضطرابات الأيضية فى ذكور الجرذان البيضاء.

رسالة كجزء مكمل للحصول على درجة الماجستير فى العلوم

(علم الحيوان)

مقدمة من

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ABSTRACT

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Evaluation of the influence of ginger (*Zingiber officinale*) extract on some metabolic disorders in male albino Wistar rats.

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Key words: Ginger, *Zingiber officinale*, Acetaminophen, Hepatotoxicity, Cystine, Atherosclerosis, Antioxidant, Glutathione metabolizing enzymes.

The present study investigated the hepatoprotective and antihyperlipidemic effect of ginger water suspension (GS) or ginger ethanol extract (GE) at a dose 150 mg/kg body weight (b.w) on hepatotoxicity induced by acetaminophen (APAP) or hyperlipidemia and atherosclerosis induced by cystine. Oral Treatment of rats with APAP either at a single dose at 2.5 g/kg b.w or at three doses at 500 mg/kg b.w or treatment with diet enriched with cystine 5% resulted in significant elevations in activities of serum alanine aminotransferase (ALAT), alkaline phosphatase (ALP), catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G-6-PDH), serum and hepatic malondialdhyde

(MDA), total cholesterol (T. chol.), triacylglycerol (TAG), cholesterol in low density lipoprotein, total lipids and significant reductions in serum cholesterol in high density lipoprotein (HDL-chol), serum total protein, alpha-1 and alpha-2 globulins, beta-globulin and albumin, hepatic reduced glutathione (GSH) and activities of hepatic glutathione peroxidase (GPx), superoxide dismutase (SOD), CAT, GR, GST and G-6-PDH as compared to control. On treating animals with GS or GE and challenging them with either APAP or cystine at the different dosing regimens mentioned before, both ginger extracts were able to significantly modulate the oxidative insults caused by APAP or cystine ameliorating most of the parameters measured. Treatment of healthy rats with GS or GE resulted in significant elevations in the activities of hepatic GPx and serum and hepatic G-6-PDH, hepatic GSH and significant reductions in level of T. chol., TAG, LDL-chol. and serum MDA level as compared to control. GE elevated the activities of hepatic CAT, GST and SOD as compared to control. In conclusion, GS or GE has been successful in decreasing the adverse effects resulting from treatment with APAP and cystine. GE is superior than GS indicating that the non-polar lipophilic constituents of ginger are most likely responsible for these protective and therapeutic actions of ginger.

INTRODUCTION

Herbal medicine has recently attracted much attention as alternative medicine useful for treating or preventing lifestyle-related disorders and relatively very little knowledge is available about the mode of action. However, most of the beneficial effects of herbs are attributed to the high antioxidant content in most of these plants. Liver is the key organ in metabolism, excretion and detoxification of xenobiotics. Liver is also responsible for detoxification of environmental pollutants and chemotherapeutic agents. Therefore, liver is subjected to a variety of diseases and disorders. Several hundred plants have been examined for use in a wide variety of liver disorders. Antioxidants in plants play an important role in inhibiting and scavenging the free radicals and thus providing protection against many liver diseases **(Fraghaly and Hussein, 2010)**.

The rhizome of the plant *Zingiber officinale*, commonly known as ginger, has been widely studied for its pharmacological activities and has been reported to exhibit antithrombotic, antinflammatory, antioxidative, antitumor, antimicrobial, antifungal, antipyretic, analagesic, hypoglycemic, antimigraine, antischistosomal, hepatoprotective, diuretic, hypocholesterolemic and antihypertensive activities **(Stoilova *et al*., 2007; Habib *et al*.,**

2008; Heeba and Abd-Elghany, 2010). Phytochemical studies showed the presence of pungent principles, such as gingerol, shogaol, Zingerone, and paradol, while the main aroma defining component is zingiberol **(Ghosh *et al.,* 2011)**. Two common metabolic disorders were induced in a rat model; hepatotoxicity induced by acetaminophen (APAP) and hyperlipidemia and hypercholesterolemia induced by cystine. APAP, a pain-reliever, is one of the most widely used medications in the world. APAP at a normal dosage is considered non-toxic drug for therapeutic applications, but when taken at overdose levels, it produces liver damage in human and various animal species **(Ajith *et al*., 2007)**. Hypercholesterolemia in humans is related to an increased risk of atherosclerosis. Among 18 individual L-amino acids tested, L-cystine is one of the most severely toxic. Excess dietary cystine has caused hyperlipidemia in rats after 2-4 months due to increased cholesterogenesis in the liver **(Guochun and Aoyama, 2003)**.

Aim of the work:

The present study investigated the influence of ginger ethanol extract and ginger water suspension at 150 mg/kg b.w on acute and chronic hepatotoxicity induced by administration of APAP and hyperlipidemia induced by L-cystine in male albino rats. Many parameters have been measured including

the activities of some liver marker enzymes such as alanine aminotransferase (ALAT) and alkaline phosphatase (ALP) and many antioxidant and glutathione metabolizing enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and glucose-6-phosphate dehydrogenase (G-6-PDH). Reduced glutathione content (GSH), malondialdyde (MDA) as a marker of lipid peroxidation level, total cholesterol (T. chol.), triacylglycerol (TAG), cholesterol in high density lipoprotein (HDL-chol.), cholesterol in low density lipoprotein (LDL-chol.), T. chol./HDL-cholesterol ratio, LDL- chol. /HDL-chol. ratio and total protein were also measured. Helena serum protein electrophoresis was also used to determine the concentration of protein fractions such as albumin, alpha-1globulin, alpha-2 globulin, beta-globulin and gamma globulin.

REVIEW OF LITERATURE

Zingiber officinale **(ginger):**

The genus *Zingiber* (family: Zingiberaceae) includes many species that are distributed all over the world. The rhizomes of the plant are used in folk and traditional medicine in the treatment of many conditions such as abdominal pain, anorexia (loss of appetite), arthritis, dyspepsia (indigestion), bleeding, cancer, chest congestion, chicken pox, cholera, chronic bronchitis, cold extremities, colitis, common cold, cough, cystic fibrosis, diarrhea, difficulty in breathing, dropsy, fever, flatulence, disorders of gall-bladder, hyperacidity, hypercholesterolemia, hyperglycemia, morning sickness, nausea, irritable bowel syndrome, rheumatism, sore throat, stomach ache, vomiting, microbial infections and cardiovascular diseases **(Afzal *et al*., 2001)**. Other parts of the plant **(**stem, leaves, flower, seeds, and fruit**)** were not monitored for their pharmacological effects **(Muhammad *et al.*, 2005)**.

The rhizomes of *Zingiber officinale* are known in Arabic as zanjabil and in English as ginger, and are also known under different names in many countries; Gingebre in Spain and Vietnam, Gingembre in France, Ginger in Tanzania, Brazil, China, Greece, Guyana, India, Jamaica, Japan, Nicaragua,

Philippines, Sri Lanka, Taiwan and United States, Halia bara, Halia and Haliya merah in Malaysia, Haliya in Indonesia, Janzabeil in Sudan, Jenegibre in Venezuela, Jengibre in Cuba, Zanjabeel in Arabic countries and Iran, Zazvor in Czech Republic and Slovakia, Zencebil in Turkey, Zencefil in Turkey, Zenzero and Zenzevero in Italy, Skenjabil and Skenjbir in Morocco.

Scientific classification of *Zingiber officinale*:

Kingdom: Plantae.

Division: Angiosperme.

Class: Monocotyledoneae.

Order: Scitaminaea.

Family: Zingiberaceae.

Genus: *Zingiber*.

Species: *officinale*.

Morphology:

The ginger is an erect perennial plant growing from one to three feet in height. Rhizomes are 7-15 cm long and 1-1.5 cm broad and laterally compressed. The branches arise obliquely from the rhizome are about 1-3 cm long and terminate in depress scars or in undeveloped buds **(figure I)**. The outer surface is buff coloured and longitudinally striated or fibrous **(Ghosh *et al*., 2011)**.

Fig. (I): The dried rhizomes of *Zingiber officinale* (Ghosh *et al*., 2011).

Chemical constituents of *Zingiber officinale*:

Crude ginger contains about (5-8%) oleoresin. The non-volatile pungent compounds accounting for 25% of the oleoresin are mainly gingerols. [6]-Gingerol, the main gingerol is, more pungent than [8]-gingerol or [10]-gingerol. Other gingerols include methylgingerol and gingerdiol, dehydrogingerdione, [10]- dehydrogingerdione, gingerdiones, diarylheptanoids equivalent to curcuminoids such as hexahydrocurcumin, diterpenlactones and galanolactone. Ginger contains up to 3% essential oil, accounting for (20-25%) of the oleoresin. Gas chromatography/mass spectrometry

analyses identified 66 compounds in the essential oil of ginger, of which the major compounds are camphene, ß-phellandrene and 1,8-cineol. Other constituents include (-)-α-zingiberen, (-)-ß-bisabolen, (+)-ar-curcumen, (-)-ß-sesquiphellandren and acyclic afarnesen. The ratio of (+)-ar-curcumen increases with the storage time and (-)-α-zingiberen plus (-)-ß-sesquiphellandren decreases with the storage time and the viscosity of the essential oil increases with the storage time. The taste of ginger is mainly affected by monoterpenes (camphen, limonen, myrcen, ß-phellandren and α-pinen, borneol, 1,8-cineol, citronellol, geranial, geraniol, geranylacetate, linalool, neral and others). Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% lipids or glycolipids, 1.2% minerals, 2.4% fibre and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. Ginger also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. The composition varies with the type, variety, agronomic conditions, drying and storage conditions **(Chrubasik *et al*., 2005; James, 2005; Badreldin *et al*., 2008).**

Physicochemical characteristics of the essential oils from *Zingiber officinale* rhizomes:

Table (1) showed the physicochemical characteristics of essential oils from ginger root. The high iodine value indicates that ginger oil has a high content of unsaturated fatty acids suggesting that the oil may be used in the manufacture of cosmetics, oil paints and vanishes and for cooking or manufacture of margarine. The high iodine value of ginger oil also indicates that the oil takes longer time to undergo oxidative deterioration. The high saponification value suggests that the ginger oil contains high molecular weight fatty acids and low level of impurities **(Akanni *et al*., 2005; Dawodu, 2009)**.

Table (I): Physiochemical properties of essential oils of *Zingiber officinale* (Ginger).

Parameters characterized	Ginger oil
State at room temperature	Liquid
Colour	Brownish yellow
Specific gravity	0.866
Acid value (mg KOH/g)	2.1
Peroxide value (mg/O_2/g)	2.63
Iodine value	151.95
Saponification value (mg KOH/g)	188.13
Free fatty acid (%)	1.47

A. Oxidative stress:

Oxidative stress is defined as a disturbance in the pro-oxidant/antioxidant balance within the tissues. Free radicals are molecules with one or more unpaired electrons. The main free radicals existing in the biological systems include five types; oxygen-centered, carbon-centered, sulfur-centered, nitrogen-centered, and transitional metal ions. Among these free radicals, oxygen centered is the most reactive towards various cellular and molecular targets **(Halliwell, 1994)**. The term Reactive Oxygen Species (ROS) is generally used in the literature for oxygen-centered free radicals. It includes superoxide, hydroxyl, alkoxy, and peroxy radicals. In addition, it also includes hydrogen peroxide (H_2O_2), singlet oxygen and hypochlorous (HOCl) which are chemically not free radicals but which are functionally related to oxygen-centered free radicals **(Halliwell and Aruoma, 1997)**. Free radical-mediated oxidative stress has been implicated in the pathogenesis of a variety of diseases such as atherosclerosis, diabetes, hypertension, cancer, and AIDS.

B- Cellular source of ROS generation:

The generation of free radicals *in vivo* is a constant phenomenon due to either physiological metabolism or pathological alterations. Four endogenous sources appear to

account for most of the oxidants produced by the cells. Because of normal aerobic respiration occurring in the mitochondria, it is known that about 2% of total mitochondrial oxygen consumption goes towards the production of ROS. Therefore, the mitochondria have been considered a major source of intracellular ROS generation **(Ames *et al*., 1993)**. There are mainly two pathways in the oxidation of the microsomal CYP450 system involved in ROS generation: (A) oxidation of relatively inert substances by monooxygenase enzymes, which requires the donation of electrons from NADPH to produce partially reduced oxygen species; (B) the metabolism of xenobiotics by microsomal enzyme systems in which ROS can be directly formed **(Trush and Kensler, 1991)**. Although ROS formation from microsomes is not a major source under normal conditions, it may constitute an important mechanism in the oxidative damage caused by xenobiotics. Peroxisomes, which are responsible for degrading fatty acids and other molecules, produce H_2O_2 as a by-product. Normally, H_2O_2 is localized in peroxisomes and catalyzed by catalase (CAT). There is evidence now showing that under certain conditions, some H_2O_2 escape the effect of CAT and get released into other cellular compartments causing oxidative damage **(Halliwell, 1991)**. Moreover, some studies show that peroxisomes also produce superoxide radical ($O_2^{\cdot -}$) and