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EVALUATION OF THE HEPATO-PROTECTIVE EFFECT OF GINGER EXTRACTS AGAINST HYDROGEN PEROXIDE IN ALBINO RAT

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ABSTRACT: Various oxidants deteriorate the functions of the body significantly, especially the functions of the liver, which is the main organ in most metabolic processes in the human body. Therefore, this study was designed to evaluate the effect of ethanolic and acetone extracts of ginger rhizomes (at concentrations 0.05 and .01% for each) against H_2O_2 (at a concentration of 0.05% in drinking water) in a one-month experiment. The results indicated a high content of both extracts (ethanolic and acetone) from total phenolics (654.8 and 563.3 mg/100g, respectively). The results of the fractionation of ethanolic and acetone extracts on the HPLC also showed that both extracts contained 16 compounds, the most important of which were zingerone and gingerols. The biological experiment showed that the treatment with H_2O_2 led to a significant increase in the levels of liver enzymes activities (ALT, AST and ALP) and a significant deterioration in the levels of both total protein and albumin in plasma. Whereas, treatment with the two extracts showed a significant improvement in liver functions, which supports the hypothesis of the positive effect of ethanol and acetone extracts for ginger rhizomes in combating free radicals that result from H_2O_2 and lead to the deterioration of liver functions.

Key words: Ginger rhizomes – Ethanolic extract – Acetone extract – Liver function

INTRODUCTION

Free radicals are atoms or molecules that have unpaired electrons, usually unstable and highly reactive (Finkel and Holbrook, 2000). Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Although oxidation reactions are crucial for life, they can also act as damaging agents; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases (Linster and Van Schaftingen, 2007).

Hydrogen peroxide (H_2O_2) is one of the most commonly used hepato-toxins in the experimental study of liver diseases; it has been proved that it induces oxidative stress in experimental animals.

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide ion radical, proxyl radicals, hydroxyl radicals, and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Halliwell and Gutteridge, 1990). Researchers have shown that the antioxidants of plant origin with free radical scavenging properties could have enormous importance as therapeutic agents in diseases caused due to oxidative stress (Ramchoun *et al.*, 2009). Phenolic compounds are widely distributed in plants. They can be divided into two main groups (phenolic acids and flavonoids). Flavonoids and phenolic acids are the most important groups of secondary metabolites and bioactive compounds in plants and that they are considered to as a good source of natural antioxidants in human diets (Ghasemzadeh and Jaafar, 2011and Kim *et al.*, 2011). Ginger *(Zingibiber officinale Rosc.)* is a natural dietary component, which has antioxidant and anticarcinogenic properties (Vaiyapuri and Namasivayam, 2005).

The aim of this study is evaluate the hepato-protective effect of ginger rhizomes extracts against hydrogen peroxide in experimental rats.

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MATERIAL AND METHODS

Materials

Plant material

Ginger rhizomes (*Zingiber Officinal*) were purchased from local market; identified by horticulture Department, Faculty of Agriculture Menoufia University.

Kits

All kits that used in blood analysis purchased by Spain React Company from Spain.

Methods

Preparation of ethanol and acetone extracts

500 grams of plant sample powders were steeped in 3000 ml of solvent (ethanol 96% and acetone 96%) and the mixture was then kept in shaker incubator for 24 hrs. at room temperature then filtered through filter paper and centrifuged at 3000 rpm at 15 min. The filtrate was placed in rotary vacuum evaporator to evaporate the solvents from it to obtain a dried powder.

Determination of total phenolic content

The amounts of total phenolics in the studies extracts were determined with the Folin-Ciocalteu reagent (Kim *et al*., 1993). Gallic acid was used as a standard and the total phenolics were expressed as mg gallic acid equivalents (GAE)/g dry weigh.10 ml of samples were extracted in methanol.0.5 ml of each sample and standard were introduced into test tubes and mixed with 2.5 ml of a 10 fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered tightly and allowed to stand for 30 min at room temperature before the absorbance which was read at 760 nm spectrometrically.

Quantitative analysis of phenolic compounds by HPLC

Phenolic compounds were fractionated and determined by using HPLC at the Department of food science, Faculty of Agriculture, Cairo University, according to the method of (Goupy *et al.,* 1999). five gram of samples were extracted by ethanol and centrifuged at 10000 × g for 10 min and supernatant was filtered through a 0.2 µm Millipore membrane filter then 1 ml was collected in a vial and use 200 µl for injection in HPLC Hewilet Pckared (series 1050) equipped with auto-sampling injection, solvent degasser ultraviolet (UV) detector set at 280nm and quaternary HP pump (series 1050). Hewlett Packard using a column Alltima C18,5mm (150 mm×4.6mm Allech). The column temperature was maintained at 35^{o}C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/ min. Phenolics standards (from sigma Company) were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate phenolic compounds concentration by the data of Hewllet packared software.

Biological experiment

The mature Wister rats were obtained from the "Agriculture Research Center, Giza, Egypt. The present studies were performed on 60 male albino rats having an average weight of 150 ±10gm (1.5 – 2 months old). Rats were allowed to acclimatize to laboratory conditions for a minimum period of 2 weeks prior to the experiment. Animals were kept on a balanced diet throughout the experimental period. Rats were fed throughout the experiment on a diet containing carbohydrate as starch, protein as casein, fat as corn oil, salt mixture and vitamins mixture in the percentages of 80, 10, 5, 4 and 1% respectively.

Experimental design

Randomized groups of rats were housed in cages containing wood shaving as bedding and were allocated into six groups, each having 10 male rats. All groups fed on normal diet during the experiment (30 days), while the all treatments added in drinking water. Groups treatments described in Table (1).

Blood sampling

Blood samples were collected from orbital sinus veins technique using heparinized capillary tubes into clean, dry, and labeled ependorf tubes

(1.5 ml). The tubes contained heparin as anticoagulant according to Schalm, (1986). Samples were centrifuged at 3600 rpm for 15 min in a centrifuge (under cooling) to separate plasma. Then plasma samples kept in a deep freeze at (-20 °C) till the different assays were carried out.

Liver functions biomarkers

Alanine amino transferees (ALT) and Aspartate amino transferees (AST) were determined calorimetrically according to the method of Reitman and Frankle, (1957), and alkaline phosphatase (ALP) activity was determined according to the method of Weisshaar, (1975). While total protein was determined according the method of Bradford, (1976) and albumin content was determined by the method of (Doumas *et al.*, 1971).

RESULT AND DISCUSSION

Total phenolic content of ginger rhizomes extracts

Total phenolics in ethanolic extract of ginger rhizomes was 654.8 mg/100g while it was 563.3 mg/100g in acetone extract of ginger rhizomes. These results indicate a high content of total phenolics in both extracts, with a clear distinction in favor of the ethanolic extract, which is agreement with Rababah *et al.*, (2004) and Hinneburg *et al.*, (2006) who have conducted studies on these extracts in ginger rhizomes and found that the levels of the ethanolic extract content of total phenolics are the highest compared to the rest of the extracts.

Identification of phenolic compounds in ginger extracts by HPLC

Phenolic compounds in ethanolic and acetone extracts of ginger rhizomes were analyzed by high performance liquid chromatography (HPLC).

From data in Table (2) it was found that ethanolic extract of ginger rhizome contain 17 phenolic compounds from which gingerols was the main one (8.77%) followed by zingerone (6.39%), while shogaols, vanillin, taxifolin, ferulic acid and cinnamic acid were in a moderate amounts (4.82%, 3.7%, 3.4%, 3.08% and 2.0%), respectively. on the same approach acetone extract of ginger rhizome contain 16 phenolic compounds, the results of HPLC analysis for acetone extract were as follow: gingerols (9.6%), zingeroene (7.0%), kaempferol (5.52%), shogaols (5.46%), vanillin (4.63%), taxifolin (4.53%), gallic acid (4.45%), ferulic acid (4.2%), cinnamic acid (2.63%) and syringic acid (1.96%).

Table (1): Group treatments in biological experiment

Group No.	Group name	Treatments
1	Negative control (NC)	Normal drinking water
2	Positive control (PC)	0.5% H_2O_2 in drinking water
3	Ethanolic ginger extract 1 (EG1)	0.5% H_2O_2 in drinking water + 0.05% ethanolic extract of ginger
4	Ethanolic ginger extract 2 (EG2)	0.5% H_2O_2 in drinking water + 0.1% ethanolic extract of ginger
5	Acetone ginger extract 1 (AG1)	0.5% H_2O_2 in drinking water + 0.05% acetone extract of ginger
6	Acetone ginger extract 2 (AG2)	0.5% H_2O_2 in drinking water + 0.1% acetone extract of ginger

Table (2): HPLC analysis of ethanolic and acetone extracts of ginger rhizomes

Phenolic compounds	Ethanolic extract		Acetone extract	
	RT	Area %	RT	Area %
Gallic acid	3.31	1.55	11.18	4.45
Cholorogenic acid	4.10	0.70	3.44	1.07
Catechins	4.43	0.29	4.09	0.51
Methyl gallate	5.77	0.63	8.08	00
Coffic acid	6.06	0.86	6.50	1.24
Syringic acid	6.50	1.34	12.38	1.96
Coumaric acid	9.05	0.66	4.78	1.05
Vanillin	9.82	3.76	10.05	4.63
Ferulic acid	10.05	3.01	11.79	4.18
Naringenin	10.36	0.83	6.50	1.37
Taxifolin	12.39	3.42	12.59	4.52
Cinnamic acid	14.28	2.00	14.63	2.63
Keamferl	14.38	1.37	4.101	5.51
Shogaols	12.59	4.81	14.28	5.45
Zingerone	11.81	6.39	5.63	7.01
Gingerols	13.37	8.76	5.73	9.61
Un known	--	59.53	--	44.73

These results are in accordance with Morakinyo and Adeniyi, (2008) and Sahdeo and Amit, (2015), who studied the contents of extracts of ginger rhizomes and their results indicated the presence of many phenolic compounds as the presence of both gingerol and zingerone in large quantities.

Biological experiment

Hydrogen peroxide was used as a catalyst to raise the oxidative stress of experimental animals. Ginger rhizomes extracts were given daily and the blood samples were taken through the experimental period for assaying liver functions

Liver functions biomarkers

The results in Table (3) indicate that the positive control group recorded the highest levels in the activity of estimated liver enzymes (ALT = 132, AST=149 and ALP=170 u/l), with very high significant differences compared to the other treatments as well as the control group. The other treatments (with ginger rhizomes extracts) caused a significant improvement in these indicators. It was noticed that there is the higher the concentration of the extract, the greater its effect in improving the estimated liver enzymes activities (ALT, AST and ALP). The ethanolic extract with the highest concentration (EG2) gave the best effect in all treatments (39, 39 and 87, respectively).

The results of Table (4) indicate the significant negative effect of hydrogen peroxide treatment on the levels of total protein as well as albumin in the positive control group when compared with the rest of the groups (Total protein =5g/dl and albumin =3g/dl). Whereas, treatment with ethanolic and acetone extracts of ginger led to a significant improvement in both protein and albumin levels.

Table (3): The effect of different ginger extracts on plasma ALT, AST and ALP activities in rats treated with hydrogen peroxide.

Groups	Activities of of ALT, AST and ALP in plasma (U/L)		
	ALT	AST	ALP
NC	23±2 e	25±2 e	33±3 d
PC	132±3 a	149±3 a	170±4 a
EG1	48±3 b	51±3 b	90±2 b
EG2	39±2 d	39±2 d	85±2 bc
AG1	44±3 c	48±3 c	88±3 bc
AG2	37±2 d	39±2 d	87±4 c

NC: normal drinking water, PC: 0.5% H_2O_2 in drinking water, EG1: 0.5% H_2O_2 in drinking water + 0.05% ethanolic extract of ginger, EG2: 0.5% H_2O_2 in drinking water + 0.1% ethanolic extract of ginger, AG1: 0.5% H_2O_2 in drinking water + 0.05% acetone extract of ginger and AG2: 0.5% H_2O_2 in drinking water + 0.1% acetone extract of ginger. Values represent means ± S.D obtained from 6 rats, means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$)

Table (4): The effect of different ginger extracts on plasma total Protein levels in rats treated with hydrogen peroxide.

Groups	Levels of total protein and albumin in plasma (g/dl)	
	Total protein	Albumin
NC	6.7 ±0.2 a	4.6 ±0.2 a
PC	5 ±0.2 d	3 ±0.2 d
EG1	6.2 ±0.1 c	4 ±0.1 bc
EG2	6.3 ±0.2 b	4.1 ±0.2 b
AG1	6.0 ±0.2 c	3.8 ±0.2 c
AG2	6.4 ±0.1 b	4.2 ±0.1 b

NC: normal drinking water, PC: 0.5% H_2O_2 in drinking water, EG1: 0.5% H_2O_2 in drinking water + 0.05% ethanolic extract of ginger, EG2: 0.5% H_2O_2 in drinking water + 0.1% ethanolic extract of ginger, AG1: 0.5% H_2O_2 in drinking water + 0.05% acetone extract of ginger and AG2: 0.5% H_2O_2 in drinking water + 0.1% acetone extract of ginger. Values represent means ± S.D obtained from 6 rats, means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$)

The results of this study are similar to many studies conducted on the effect of ginger extracts on improving liver function in experimental animals (Bishai *et al.,* 1994; Sakr *et al.,* 2010; Bak and Jun, 2012; Abd-Allah *et al.,* 2016).

The significant positive effect of ginger extracts on liver function in rat treated with hydrogen peroxide can be explained by the fact that these extracts contain good amounts of gingerol and zingerone, which act as powerful antioxidants in combating the free radicals that

result from hydrogen peroxide and destroy liver cells (Abozid and El-Sayed, 2013).

Conclusion

This study shows that both ethanol and acetone extracts of ginger rhizomes led to a significant improvement in liver function in experimental rat treated with H_2O_2, which may be attributed to the fact that these extracts contain large amounts of phenolic compounds that act as natural antioxidants in combating the free radicals, which makes this plant promising in preventing the negative effects of oxidants to which humans are exposed from different sources.

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تقييم التأثير الواقي للكبد لمستخلصات الزنجبيل في مواجهة فوق أكسيد الهيدروجين في الجرذان البيضاء

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الملخص العربى

تؤدي المؤكسدات المختلفة إلى تدهور وظائف الجسم بشكل كبير ، وخاصة وظائف الكبد ، وهو العضو الرئيسي في معظم عمليات التمثيل الغذائي في جسم الإنسان. لذلك صُممت هذه الدراسة لتقييم تأثير المستخلصات الإيثانولية والأسيتون لريزومات الزنجبيل (بتركيزات ٠,٠٥ و .٠١٪ لكل منهما) مقابل فوق أكسيد الهيدروجين (بتركيز ٠,٠٥٪ في مياه الشرب) في تجربة مدتها شهر واحد. أشارت النتائج إلى وجود نسبة عالية من كلا المستخلصين (الإيثانول والأسيتون) من إجمالي الفينولات (٦٥٤,٨ و ٥٦٣,٣ ملجم / ١٠٠ جم على التوالي). كما أظهرت نتائج تفريد المستخلصات الإيثانولية والأسيتون على جهاز HPLC أن كلا المستخلصين يحتويان على ١٦ مركبًا أهمها الزنجرون والجنجيرول. أما بالنسبة للتجربة البيولوجية فقد أوضحت أن المعاملة بـفوق أكسيد الهيدروجين أدت إلى زيادة معنوية عالية في مستويات أنشطة إنزيمات الكبد المقدرة في التجربة (ALT و AST و ALP) وفي نفس الوقت أدى ذلك إلى تدهور كبير في مستويات كلا من البروتين الكلي والألبيومين في البلازما. في حين أظهرت المعاملة بالمستخلصين تحسنًا معنويًا كبيرًا في جميع مؤشرات وظائف الكبد المقدرة في التجربة ، مما يدعم فرضية التأثير الإيجابي لمستخلصات الإيثانول والأسيتون لريزومات الزنجبيل في مكافحة الشقوق الحرة الناتجة عن المعاملة بفوق أكسيد الهيدروجين والتي تؤدي إلى تدهور وظائف الكبد.