Antioxidant Activity of Leek towards Free Radical Resulting from Consumption of Carbonated Meat in Rats

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Abstract: Forty -two albino male rats were randomly classified into six groups (7 rats each). The first group kept as control negative fed basal diet only. The other five groups fed on basal diet with 10% extreme grilled meat and classified into non treated group and treated groups with water leek extract, methanol leek extract, 2.5 % leek powder and 5% leek powder. Consumption of carbonyl meat only in non treated group decreased final weight, weight gain, food intake ,feed efficiency ratio(FER) , hemoglobin , packed cell volume ,blood glutathione peroxidase (GPX) , blood super oxide dismutase (SOD) , liver super oxide dismutase (SOD), liver glutathione peroxidase (GPX) and liver glutathione transferase (GST) but significant increase in AST enzyme, creatinine ,urea nitrogen blood free radical and serum lipid peroxide (LPX) and liver malondialdehyde (MDA) compared to normal control group. The treated groups with water ,methanol extracts, 2.5 % and 5% leak showed that the values of final weight, weight gain, food intake ,FER, hemoglobin, serum alanine amino transferase (ALT) enzyme, creatinine, urea nitrogen , blood GPX , blood SOD and blood GST around the values of normal control group. The treated groups with water ,methanol extracts, 2.5 % and 5% leak showed significant decrease in packed cell volume , liver SOD and GPX but significant increase in AST, blood free radical , LPX ,liver MDA compared to normal control group. The values of final weight, weight gain, food intake , FER hemoglobin , packed cell volume ,blood GPX , blood SOD, liver SOD, liver GPX and liver GST were increased but the values of AST enzyme, creatinine ,urea nitrogen, blood free radical, serum lipid peroxide and liver MDA were decreased in all treated groups compared to non treated group.

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Key wards: leek, free radical, antioxidant, carbonated meat& rats

1. Introduction:

The extreme grilled meat is usually consumed as (Cabab). Such food contains nitrated polycyclic aromatic hydrocarbons free radicals which have very high risk for body health (Solyakov and Skog 2002). Free radicals have unpaired electrons and so they try to steal them from other molecules in a process called oxidation. These oxidation damages the cell membranes, genetic material in cells (DNA), fatty acids and other body structures (Abd El-Ghany et al ., 2007). The antioxidative effects of natural phenolic compounds in pure forms or in their extracts from different plant sources such as vegetables, fruits and medicinal plants were studied in vitro using different model systems of oxidation. Antioxidants may be of great value in preventing the onset and/or the propagation of oxidative diseases (Pietta et al., 1998, and Garth and Rita 2006).

Fruits and vegetables contain different phytochemicals with biological activity that can be of valuable therapeutic index. Phytochemicals have an excellent antioxidant activity, including the ability to neutralise potentially harmful free radicals that helps prevent a number of chronic diseases (Franca and Harri 2001 and Liu 2003).

The allium group is one of the world's most widely cultivated vegetable groups, with their culinary and medicinal uses. Equally varied are their health benefits, for they contain a range of phytochemicals with an array of biological effects. Evidence shows they play an important role in protecting against major lifestyle chronic diseases as well as health problems associated with ageing. Their antimicrobial activity, long recognized in folk remedies, has also now been scientifically validated. The Allium genus includes approximately 500 species, the most widely used of which are onions, garlic, and leeks (Malairajan et al., 2006).

Leeks (Allium porrum or A. ampeloprasum var. porrum), sometimes called "the gourmet's onion" have flat leaves instead of tubular and relatively little bulb development and is native to Western Asia and the Mediterranean countries. The thick leaf bases and slightly developed bulb look like a giant green onion, and are eaten as a cooked vegetable. Leeks contain saponins and the major flavonoid in leeks is kaempferol, with only a small amount of quercetin, carotenoids and chlorophyll mainly in the green tops (Onyeagba et al., 2004 and Garth and Rita 2006).

The aim of this study was to investigate the antioxidant effect of leeks either powder or extract on free radical producing agents

2. Materials and Methods

A – Materials:

Carbonated meat and leek:

Meat was extremely grilled in hot oven until be carbonated then crushed and mixed in basal diet in 10 % all over the period of experiment. Leek was collected from the local market. Part of leek leaves was dried at 60°C and then crushed to powder. The leek powder added in 2.5 % and 5% to diet. The other part was used for preparation of water and methanol extract.

Basal diet:

The experimental diet composed of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg) , cellulose (30 g/kg) ,corn oil (50g/kg), mineral mixture (100g/kg) , vitamin mixture (20g/kg) and DL-methionine (3g/kg).The standard diet was performed according to NRC (1995).

Experimental animals:

42 adult male of white albino rats (Sprague dawley strain) weighing 115 ±5g were provided from of National Research Center, Cairo, Egypt.

Biochemical kits:

BioMeriuex Kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt.

B- Methods

1-Preparation of water and methanol leek extracts

270 g of fresh leek were peeled and then was chopped with both 300 mL distilled water and ethanol by using a blender for 1 min at average speed. The mixture were macerated during 24h at the + 4ºC. After that, resulting extracts were filtered using a 0.45 µm pore size cellulose acetate membrane filter. The extracts were used directly (Irkin and Korukluoglu 2007).

2-Experimental design:

Rats were housed in wire cages under the normal laboratory conditions and fed on basal diet for a week as adaptation period. Food and water were provided ad-libitum. The rats were randomly classified into six groups (7 rats each). The first group kept as normal control fed basal diet only. The other five groups fed on basal diet with 10% of extreme grilled meat and classified into

1-Non treated group.

2- Treated group with water leek extract.

3- Treated group with methanol leek extract.

4- Treated group with 2.5 % leek powder.

5- Treated group with 5% leek powder.

The experiment continued for eight weeks. The food intake was calculated daily and the body weight gain was recorded weekly. Feed efficiency ratio (FER) was determined by Chapman et al., (1950) as following: FER = weight gain (g)/ feed intake (g).

3-Collection of blood and liver samples:

At the end of experiment, rats were anesthetized, blood sample were collected from hepatic portal vein in clean centrifuge tubes. The liver of sacrificing rats was removed by careful dissection, blotted frees of adhering blood, washed with cold saline solution, and dried between two filter papers. Livers perfuse with 50 to 100 of ice cold 0.9%NaCL solution for some analyses.

4- Blood analysis:

Part of blood was heparinized for estimation of hemoglobin and packed cell volume (Drabkin, 1949 and Mc Inory, 1954) .The rest of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum biochemical analyses were estimated colorometriclly . Serum alanine and aspertate aminotransferase (ALT, AST) enzymes activity, creatinine and urea nitrogen were estimated colorometriclly according to Reitman and Frankel (1957), Husdan and Rapoport (1968) and Fawcett and Scott (1960), respectively. Blood glutathione peroxidase (GPX), superoxide dismutase (SOD) and also serum lipid peroxide (LPX) were estimated by BioMeriuex Kits according to Beuther et al., (1987), Beuchamp and Fridovich, (1971), and Botsoglou et al., (1994), respectively. Blood free radical was estimated according to Borg, (1976) by an Electron Spin Resonance spectroscopy National Research Center.

5- Liver analysis:

Liver superoxide dismutase (SOD), glutathione peroxidase (GPX) , glutathione S-transferase (GST) and malondialdehyde (MDA) were estimated according to Beuchamp and Fridovich,(1971) , Weiss et al., (1980) , Habig et al.,(1974) and Uchiyama and Mihara (1978), respectively.

8-Statisticl analysis:

Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to Artimage and Berry (1987).

3. Results and Disscusion

Consumption of carbonyl meat only in non treated group decreased final weight, weight gain, food intake and FER at $p<0.05$, 0.01 &0.001 compared to normal control group. The treated groups with water, methanol extracts, 2.5 % and 5% leak showed the values of final weight, weight gain, food intake and FER around the values of normal control group. The values of final weight, weight gain, food intake and FER of all treated groups were increased compared to non treated group as shown in table (1).

Common cooking heat processing of protein-rich foods such as boiling, frying, flame-grilling, induce the formation of potent mutagenic and carcinogenic hydrocarbons HCAs .The grilled meat contain heterocyclic amines as phenylimidazo [4,5] pyridine which as potent mutagens (Solyakov and Skog 2002). Vegetables are an important source of mineral and phenolics which play an important role in nutritive value. Allium vegetables and related organosulfur compounds inhibit of mutagenesis, modulation of enzyme activities, inhibit of DNA adduct formation, scavenge of free-radical, and effect on cell proliferation and tumor growth (Garth and Rita 2006).Leeks are a good source of dietary fiber, folic acid, calcium, potassium, and vitamin C. Leeks are easy to digest and have laxative, antiseptic, diuretic, and anti-arthritic properties. Leeks support healthy digestion by promoting the growth of usefull bacteria in the gut due to contain prebiotics carbohydrates that serve as fuel for good bacteria in the digestive tract. These probiotic bacteria fortify the immune systems and keep digestive processes running smoothly. Leeks fiber energizes the human body to perform many types of biological functions like digestion and metabolism (Dorant et al., 1996 and Riley et al., 2001).

Consumption of carbonyl meat only in non treated group decreased hemoglobin and packed cell volume at $p<0.01$ compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed non significant decrease in hemoglobin at $p>0.05$ but significant decrease in packed cell volume at $p<0.05$ compared to normal control group. The treated groups with water, methanol extracts, 2.5 % and 5% leak showed increase in hemoglobin and packed cell volume compared to non treated group as shown in table (2).

Leeks are a good source of allyl sulfides and also rich in the flavonoid especially kaempferol. Leeks contain excellent amounts of vitamin C, as well as folate, and some useful amounts of B vitamins, vitamin E, copper, potassium and iron. These vitamins and minerals work together to help stabilize blood. Calcium in leeks is also used for the proper clotting of blood in the human body. Allium species also have immune enhancing actions that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer-cell activity (Merchant, 2003 and Fortin, 2004)

Consumption of carbonyl meat only in non treated group increased serum aspartate amino transferase (AST) enzyme, creatinine and urea at $p<0.01$&0.001 compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed non significant increase in serum alanine amino transferase (ALT) enzyme ,creatinine and urea nitrogen at $p>0.05$ but significant increase in AST at $p<0.05$ compared to normal control group. The treated groups with water,methanol extracts ,2.5 % and 5% leak showed decrease in AST enzyme, creatinine and urea nitrogen compared to non treated group as shown in table (3).

These results were in agreement with the fact that ALT was widely distributed in cells throughout the body and found predominantly in the cytoplasm of hepatic parenchymal cells. ALT is widely considered to be specifically for the liver increase in blood and indicative for liver damage as hepatitis, cirrhosis or hepatic tumors. If cells were damage, ALT will excreted into the blood (Thoma, 2000).Creatinine was a waste product in the blood created by the normal breakdown of muscle during activity. Healthy kidneys take creatinine out of the blood and put it in the urine to leave the body. Creatinine builds up in the blood in kidney disease. The significant increase in creatinine and urea in the present study may be attributed to renal damage due to administration of carbonated meat. Phenylimidazo [4,5] pyridine (PhIP) was naturally formed in meats during the cooking process at least in part due to heat dependent condensation of creatinine and phenylalonine which are the two natural components of muscle meats (Sinha et al., 1995). Dietary onions partailly reversed the abnormalities in blood urea and creatinine in streptozotocin induced diabetes mellitus rats. The significant decrease in creatinine and uric acid in the present study may be due to the higher antioxidants activities of leek. The non-nutrient constituents influence biotransformation enzymes involved in activation and detoxification of xenobiotic compounds (Block 1999 and Bordia, et al., 2002).

Consumption of carbonyl meat only in non treated group decreased blood glutathione peroxidase (GPX) and superoxide dismutase (SOD) at $p<0.01$ but increased blood free radical and serum lipid peroxide (LPX) at $p< 0.001$ compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed non

significant decrease in blood glutathione peroxidase and super oxide dismutase at p>0.05 but significant increase in blood free radical and serum lipid peroxide at p< 0.01 compared to normal control group.

The treated groups with water, methanol extracts, 2.5 % and 5% leak showed increase in blood glutathione peroxidase (GPX) and super oxide dismutase but decrease in blood free radical and serum lipid peroxide compared to non treated group as shown in table (4).

Free radicals are highly reactive compounds that are created in the body during normal metabolic functions or introduced from the environment. Free radicals are inherently unstable since they contain "extra" energy. To reduce their energy load, free radicals react with certain chemicals in the body and in the process interfere with the cells ability to function normally. Free radicals are believed to play a vital role in more than sixty different health conditions, including the aging process, cancer, and atherosclerosis. Fatty acyl side chains peroxidation in biological membranes by reactive oxygen species and transition metal ions in a free radical chain reaction can be deleterious for membrane permeability as it can produce toxic compounds, such as malonaldehyde and acetaldehyde, which in turn produce abnormal adducts with biological substances, including DNA and RNA (Wei and Lee 2002).

Most living organisms possess enzymatic and nonenzymatic defence systems against excess production of reactive oxygen species. However, different external factors such as smoke, diet, alcohol and some drugs and aging could decrease the capability of such protective systems resulting in disturbances of the redox equilibrium that is established in healthy conditions. Superoxide dismutase (SOD) is, an enzyme, found in the cytosol and mitochondria. SOD is responsible for decreasing superoxide levels. Glutathione peroxidase occurs in the mitochondria and cytosol and reduces organic hydroperoxides and hydrogen peroxide in reaction that involves glutathione (Kanno et al., 2004).Reduced glutathione (GSH) is not only a cofactor for GST but also serves as a reductant for glutathione peroxidase (GPX), an enzyme involved in natural protection by free radicals, in addition to superoxide dismutase and catalase. Reducing exposure to free radicals and increasing intake of antioxidants nutrients has the potential to reduce the risk of free radical-related health problems. Phytochemicals are molecules of plant origin (such as carotenoids, flavenoids, phytosterols, chlorophylls, terpenoids, indoles and allylic compounds) consumed in the diet that are not true vitamins but can be very potent antioxidant nutrients (Ames et al., 2002 and Garth and Rita 2006).

Consumption of carbonyl meat only in non treated group decreased liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione transferase (GST) at p<0.001 but showed significant increase in liver malondialdehyde (MDA) at p<0.001 compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed significant decrease in liver superoxide dismutase (SOD) and glutathione peroxidase (GPX) at p<0.05 but significant increase in liver malondialdehyde (MDA) at p< 0.05 and non significant decrease in glutathione transferase (GST) compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed increase in liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione transferase (GST) but decrease in liver malondialdehyde (MDA) compared to non treated group as shown in table (5).

Organosulfur compounds enhance glutathione-S-transferase enzyme system, which are biochemical pathways involved in the liver's detoxification of carcinogenic substances. *Allium* vegetables and related organosulfur compounds inhibit mutagenesis modulate of enzyme activities, inhibit of DNA adduct formation, scavenge free-radical, and effect on cell proliferation and tumor growth (Takahashi et al., 1992 and Franca and Harri 2001). The antioxidant properties of *Allium* vegetables result from the contributions of various sulfur components. Allyl derivatives of leek oils stimulate the activity of GPX and inhibited the decreased ratio of reduced to oxidized glutathione produced by 12-*O*-tetradecanoylphorbol-13- acetate in epidermal cells. Diallyldisulfide increase GPX activity in animal tissues with increased the activity of glutathione reductase, and superoxide dismutase (Jin and Baillie 1997 and Riley et al., 2001).

It is recommended to consume leek as a good source of antioxidant especially with grilled meat for scavenger of free radicals.

Table (1): Mean values ± SD of body weight gain, food intake and feed efficiency ratio (FER) of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanolic extract	Leek powder (2.5%)	Leek powder (5%)
Initial weight	117.33 ± 3.71[a]	118.55 ± 3.60[a]	115.99 ± 4.25[a]	119.41 ± 4.14[a]	118.75 ± 3.18[a]	117.39 ± 3.61[a]
Final Weight (g)	201.41 ± 8.61[a]	160.71 ± 7.11[b***]	200.41 ± 9.21[a]	202.35 ± 13.78[a]	199.91 ± 9.30[a]	198.89 ± 10.22[a]
Weight Gain (g)	84.08 ± 6.11[a]	42.16 ± 4.36[b***]	84.42 ± 5.61[a]	82.94 ± 6.14[a]	81.16 ± 6.18[a]	81.50 ± 5.19[a]
Food Intake (g/d))	16.38 ± 1.45[a]	14.33 ± 1.39[b*]	16.24 ± 1.25[a]	16.57 ± 1.14[a]	16.01 ± 1.31[a]	16.11 ± 1.29[a]
FER	0.085 ± 0.001[a]	0.049 ± 0.001[b**]	0.086 ± 0.003[a]	0.083 ± 0.004[a]	0.084 ± 0.002[a]	0.084 ± 0.001[a]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (2): Mean values ± SD of blood hemoglobin (HB) and packed cell volume (PCV) of the experimental ratgroups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek powder (5%)
HB (gm/dl)	**13.01 ± 2.11[a]**	**8.11 ± 1.21[b**]**	**11.34 ± 1.51[a]**	**11.81 ± 1.17[a]**	**10.91 ± 1.98[a]**	**11.45 ± 2.20[a]**
PCV %	**39.40 ± 6.17[a]**	**25.98 ± 5.11[c**]**	**34.61 ± 7.01[b*]**	**33.81 ± 6.14[b*]**	**32.11 ± 6.38[b*]**	**35. 19 ± 5.18[b*]**

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (3): Mean values ± SD of serum amino transferase enzymes (ALT & AST), creatinine and urea nitrogen of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek Powder (5%)
ALT (µ /ml)	18.53 ± 5.81[a]	23.79 ± 5.45[a]	22.14 ± 4.33[a]	21.16 ± 3.22[a]	22.81 ± 3.69[a]	21.91 ± 4.01[a]
AST (µ /ml)	38.41 ± 6.13[c]	51.31 ± 6.22[a***]	45.32 ± 4.15[b*]	44.14 ± 5.22[b*]	48.32 ± 5.38[b*]	46.34 ± 4.87[b*]
Creatinine (mg/dl)	0.88 ± 0.02[b]	1.21 ± 0.33[a**]	0.98 ± 0.12[b]	0.89 ± 0.11[b]	0.97 ± 0.03[b]	0.87 ± 0.04[b]
Urea nitrogen (mg/dl)	35.41 ± 3.24[b]	55.32 ± 6.71[a**]	39.14 ± 4.12[b*]	40.21 ± 4.91[b]	38.33 ± 3.77[b]	41.32 ± 4.81[b]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (4): Mean values ± SD of blood glutathione peroxidase (GPX), superoxid dismutase (SOD) enzymes and free radical and serum lipid peroxide (LPX) of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek powder (5%)
GPX (µ /ml)	7.13 ± 1.10[a]	3.31 ± 0.44 [b**]	6.66 ± 1.14 [a]	6.17 ± 1.13 [a]	5.49 ± 1.12 [a]	5.79 ± 1.01 [a]
SOD (µ /l)	20.55 ± 2.34 [a]	10.88 ± 1.67 [b**]	18.49 ± 1.73[a]	17.99 ± 2.21 [a]	18.41 ± 2.34 [a]	18.91± 1.98 [a]
free radical	20266.45± 3230.57 [c]	73464.67 ± 4532041 [a***]	41320.87 ± 3357.22 [b**]	39421.77 ± 3567.22 [b**]	45425.11 ± 4034.67 [b**]	43334.35± 2947.41 [b**]
LPX (mg/dl)	1.30 ± 0.22[c]	5.45 ± 1.71 [a***]	2.99 ± 0.55[b**]	1.89 ± 0.93 [b*]	2.61 ± 0.65 [b**]	2.85 ± 0.67 [b**]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (5): Mean values ± SD of liver superoxid dismutase (SOD), glutathione peroxidase (GPX), glutathione transferase (GST) and malondialdehyde (MDA) of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek Powder (5%)
SOD (µ /mg)	115.71 ± 10.25[a]	45.11 ± 5.14 [b***]	85.17 ± 7.34 [c*]	89.36 ± 6.99 [c*]	86.33 ± 8.23 [c*]	88.34 ± 8.13 [c*]
GPX (µ /mg)	110.33 ± 11.21 [a]	52.18 ± 6.13 [b***]	87.75 ± 7.81[c*]	90.12 ± 10.10 [c*]	88.66 ± 9.54 [c*]	86.32 ± 8.22 [c*]
GST (µ /mg)	1.22 ± 0.13 [a]	0.44 ± 0.02 [b***]	1.12 ± 0.06 [a]	o.99 ± o.13 [a]	1.01 ± 0.33 [a]	1.21 ± 0.52 [a]
MDA (nmol/g)	13.14 ± 2.21[c]	31.21 ± 3.71 [a***]	19.81 ± 2.14[b*]	18.31 ± 1.41 [b*]	17.99 ± 1.49 [b*]	19.33 ± 2.01 [b*]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

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