Australian Journal of Basic and Applied Sciences, 5(2): 18-26, 2011
ISSN 1991-8178

Effect of Echinacea as Antioxidant on Markers of Aging

[1]Nematalla, Kh. M., [1]Sahar, A. Arafa, Ghada, [1]M. Yousef and [2]Zainb, A. Shabib

[1]Special Food and Nutrition Department,
[2]Experimental Kitchen Research Unit, Food Technology Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

Abstract: Aging is a multifactorial process that leads to loss of function and the inability to adequately respond to external stress. There has been a global trend toward the use of natural phytochemicals present in natural sources as antioxidants and functional foods. The herb, Echinacea, is one of the most popular herbal medicines and has become one of the top-selling herbs of all time with billions of dollars in worldwide sales annually. This study was aimed to examine the effect of ethanolic and water extracts of Echinacea purpurea roots as natural sources of antioxidants on markers of aging in rats. A total of 24 Sprague-Dawley female rats were obtained. 6 rats weighing 100 to 115 g considered as control young and the rest of rats weighing 260 to 275 g considered as aged rats. The rats were divided into 4 groups, each group contained 6 rats. The first and second groups fed on basal diet and considered as control young and control aged rats, respectively, while the other two groups administered ehtanolic and water extracts of Echinacea purpurea roots at doses of 6 mg for each rat once daily. Blood samples were taken from each rat and tested for markers of aging, e.g., the activity of superoxide dismutase (SOD) and glutathione-s-transferase (GST), liver functions, total cholesterol, HDL, LDL, VLDL and triglycerides levels. Blood hemoglobin and hematocrit counts were also measured. Aging resulted in a significant deterioration in all tested markers of aging including SOD, GST, total protein, albumin, globulin, HDL-cholesterol, hemoglobin concentration, white blood cells concentration and platelets count. Also, a significant increase in AST, ALT, triglycerides, total cholesterol, LDL and VLDL levels was observed during aging. Administring aged rats with Echinacea ethanolic and water extracts caused a significant improvement in the above mentioned markers and returned the abnormal markers back to the normal levels found in control young rats. So, it could be recommended that Echinacea extracts could be used to enhance the abnormal changes in health state of aging populations and return them to nearly the normal state found in yonger populations by using a natural source of antioxidants like E. purpurea roots.

Key words: Echinacea purpurea; Roots; Ethanolic extract; Water extract; Antioxidants; Aging.

INTRODUCTION

Aging in humans is associated with changes in physical characteristics and the decline of many physiologic functions. Observations accumulated in recent years have provided the basis for a modern concept of aging–the free radical theory, which relies on a time–dependent shift in the oxidant–antioxidant balance in the body that leads to increased oxidative stress, dysregulation of cellular function, and aging as manifested by phenotypic changes and functional deterioration in later life. Genetic, environmental and life style factors play important roles in the rate of changes in this balance and, thus, in the rate of aging and the development of age-associated diseases. (Meydani, 2000). Various medicinal plants, herbs, spices, tea, grains, fruits and vegetables are the main sources of natural antioxidants. There is evidence that phytochemicals may exert their antioxidant effects within the human body and it effectively prevents premature lipid oxidation. (Krings and Berger, 2001 and Lee *et al.*, 2002). Echinacea, also known as the purple coneflower is a member of the Asteraceae (daisy) family. It is one of the most popular herbal medicines with an estimated 1-4% of the general population using the herb in a given year. Echinacea has numerous claimed medicinal properties including: anti-viral, anti-bacterial, antifungal, anti-oxidant, anti-carcinogenic, anti-inflammatory and wound healing properties. It's popularity today is fuelled by claims that it has immune stimulating properties and can reduce the severity of symptoms and duration of the common cold and flu, especially if used in the early stages of infection. (Barrett, 2003). Although there are around nine species of Echinacea, only three of these are generally used medicinally:

Corresponding Author: Nematalla, Kh. M., Special Food and Nutrition Department, Food Technology Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

E. angustifolia DC, roots (underground parts); E. pallida (Nutt.), roots and E. purpurea (L.) Moench, roots and tops (aerial parts). Most clinical studies have focused on E. purpurea roots. Today preparations of Echinacea species are used as herbal drugs worldwide. In Germany alone, there are currently < 300 different Echinacea products on sale. These preparations contain different mixtures of various forms of Echinacea, both alone or in combination with other substances. (Lienert *et al.,* 1998 and Bauer, 1999). Four classes of active compounds have been identified within Echinacea, yielding different chemical profiles among its different species. It has been hypothesized that alkamides, caffeic acid derivatives, polysaccarides and glycoproteins are the main compounds responsible for the bioactivity of Echinacea. Echinacea purpurea contains alkamides, caffeic acid esters (cichoric acid), polysaccarides and polyacetylenes. Furthermore, levels of constituents vary during growth and across development. Studies are accumulating indicate that Echinacea may have anti-viral, anti-oxidant and anti-inflammatory properties, making it a very promising medicinal botanical. (Dalby-Brown *et al.,* 2005 and Chen *et al.,* 2005).

Pellati *et al.* (2005) reported that extraction of root samples by magnetic stirring with 80% methanol agueous solution at room temperature allowed the complete recovery of all compounds of interest. The total amount of phenolic compounds ranged from 23.23 to 33.95 mg/g of Echinacea roots. Cichoric acid was the main phenolic compound (2.27%) and the highest contents of caffeic acid derivatives were found in E. purpurea roots. Sloley *et al.* (2001) found that cichoric acid and verbascoside predominated in extracts of E. purpurea roots. Extracts of the roots and leaves were found to have antioxidant properties in a free radical scavenging assay and in a lipid peroxidation assay. The radical scavenging activity of Echinacea methanolic extracts was evaluated in vitro in the presence of a hydrogen donating antioxidant. The average EC50 value for E. purpurea was 134 micro g/ml. The radical scavenging activity of Echinacea root extracts reflected their phenolic composition. The results indicate that Echinacea roots and derivatives are a good source of natural antioxidants and could be used to prevent free radical-induced deleterious effects. (Pellati *et al.,* 2004).

Guiotto *et al.* (2008) and Spelman *et al.* (2009) found that at least 15 alkamides were identified in ethanolic extract of Echinacea purpurea roots. The important bioactive dodeca-2 E, 4E, 8Z, 10E/Z-tetraenoic acid isobutylamides are fully extracted from dry E. purpurea root in 2 days. Alkamides were found to be rapidly absorbed through the oral mucosa and measurable in plasma 10 min. after administration and remained detectable for more than 3 hrs. These results suggesting that the practice of macerating Echinacea extracts for weeks may be unnecessary for optimal alkamides extraction. Miller (2005) reported that, the combined action and balance of active constituents produces a more powerful effect together than would be expected from the addition of individual components. The active constituents of Echinacea work as a team, enhancing other effects–this phenomenon is known as synergy. Synergism has been reported between alkamides and caffeic acid derivatives and their ability to inhibit the oxidation of low-density lipoproteins, as an indicator of antioxidant activity. The aim of this study is to examine the effect of ethanolic and water extracts of Echinacea purpurea roots as natural sources of antioxidants on markers of aging in rats which could provide an effective means to improve or maintain healthy function with age.

MATERIALS AND METHODS

Materials:

Echinacea roots (Echinacea purpurea L. Moench), Asteraceae family were obtained from Medicinal Plants and Agricultural Seeds Breeding Section, Field Crops Department, Agricultural Research Centre, Giza, Egypt. Tops were cut off at 10 cm above soil level, and roots comprised the rest of the plant.

Methods:

Preparation of Echinacea Roots Extracts:

Roots were harvested and the plant material was drying for 8 days at 38°C in a forced–air dryer with constant humidity. The dried material was ground with a 40-mesh screen and stored at –20°C until extraction. Extracts were prepared by shaking the roots with 70% ethanol or water for 24 hrs. at room temperature to obtain ethanolic and water extracts. After complete drying of the extracts by evaporation the residues were stored at –30°C in the dark and used as stock solutions as described by Lalone *et al.* (2007).

Animals and Experimental Diets:

A total of 24 Sprague–Dawley female rats were obtaind from Food Technology Research Institute, Agricultural Research Centre, Giza, Egypt. 6 rats weighing 100 to 115 g considered as control young and the rest of rats weighing 260 to 275 g considered as aged rats were housed in plastic cages and fed on basal diet

and water for one week as an adaptation period. The basal diet composed of casein (12%), cellulose (5%), vitamins mixture (1%), salts mixture (4%), corn oil (5%) and corn starch (73%). The basal diet formulation was performed according to A.O.A.C (2006). The rats were divided into 4 groups, each group contained 6 rats.

The first group fed on basal diet and considered as a control young group. The second group fed on basal diet and considered as a control aged rats, while the other two groups administered the ethanolic and water extracts of Echinacea roots at doses of 6 mg for each rat once daily using a stomach tube before meal. Blood samples of rats were taken from orbital plexus venous by using fine capillary glass tubes. Blood samples were allowed to clot for one min. at 37°C, centrifuged at 1500 xg for 10 min. and the separated serum was kept frozen at –20°C until analysis.

Biochemical Analysis:

Serum total cholesterol, (high–density lipoprotein cholesterol and low–density lipoprotein cholesterol), very low-density lipoprotein cholesterol and triglycerides were determined according to the methods of Roeschlau *et al*. (1974); Assmann (1979); Hatch and Lees (1968) and Uwajima *et al.* (1984), respectively. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were colorimetrically determined according to the method of Bergmeyer and Harder (1986). Serum total protein content was estimated by the method of Lowry *et al*. (1951) using bovine serum albumin as standard. Albumin was determined by an enzyme-linked immunosorbent assay as described by Borcea *et al.* (1999). Globulin was calculated by subtracting albumin from serum total protein content. The activity of superoxide dismutase (SOD) and glutathione-s-transferase (GST) of rats blood serum were estimated according to Marklund and Marklund (1974) and Habig *et al.* (1974), respectively. Blood hemoglobin and hematocrit counts were measured according to Dacie and Lewis (1984).

Statistical Analysis:

The standard analysis of variance procedure in a completely rondomized design was applied for the present data according to Gomez and Gomez (1984). Least significant difference (LSD) test was done to compare a pair of group means. The level of statistical significance was set at $p< 0.05$.

RESULTS AND DISCUSSION

In the present study, we chose to use ethanolic and water extracts of Echinacea roots due to the work conducted by Bone (2004) and Barnes *et al.* (2005), which supports the use of ethanol extractions of Echinacea to allow for the enrichment of lipophilic compounds including the ethanol–soluble alkamides. The quercetin–pentahydroxy flavone, found in E. purpurea are know to exert anti-inflammatory as well as antioxidant effects were completely extracted by ethanolic extraction**.** Addition of water to ground Echinacea purpurea roots prior to adding ethanol resulted in the extract going brown within seconds due to enzymatic browning forming polymeric brown pigments. Extraction with 70% ethanol serves to denature this enzymatic reaction. (Kreis *et al.,* 2000). We selected to use daily doses of 6 mg of Echinacea purpurea roots extracts administered orally to rats on the basis derived from the average recommended dose for adult humans which ranged from 3 to 4.5 g dried Echinacea roots/day according to WHO (1999) and JC (2008).

Effect of Echinacea Roots Extracts on Body Weight in Aged Rats:

Data presented in Table (1) show the effect of Echinacea purpurea roots extracts on body weight in aged rats from zero time to the end of the experiment (30 days). Body weight was recorded at three time intervals at zero time, after 15 days and at the end of the experiment. Body weight of control young rats was not significantly different during the experimental period, but it was significantly different compared with body weight of control aged rats at zero time and during the experimental period. There was insignificant difference in body weight of control aged rats at zero time and the decrease in body weight at the end of the experiment was not statistically significant. Administring Echinacea ethanolic and water extracts orally to rats caused a significant decrease in body weight from zero time till the end of the experiment. The reduction in body weight was 10.37% and 14.89% for water extract and ethanolic extract of Echinacea roots, respectively, compared with control aged rats at the end of the experiment. The decrease in body weight was gradually but not suddenly during the three time intervals. Echinacea ethanolic extract was more effective in weight loss compared with Echinacea water extract. The present results were different compared with the results reported by Sun *et al.* (1999) and Hermann *et al.* (2003) who found that, normal mice on Echinacea containing diets were clinically no different from littermates and cagemats consuming untreated chow, with respect to body

weight, coat texture and level of activity. It was found that feeding Echinacea at either 2 or 4% of the total diet did not affect body weight or gain:feed ratio compared to control animals not supplemented with dietary Echinacea.

Effect of Echinacea Roots Extracts on Antioxidant Enzymes in Aged Rats:

Table (2) shows the effect of Echinacea purpurea roots extracts on antioxidant enzymes, superoxide dismutase (SOD) and glutathione–s-transferase (GST) activities as markers of oxidative stress in aged rats. Serum superoxide dismutase levels were significantly decreased in aged rats compared with control young rats at zero time and during the experimental period. The decrease reached about 24.75% at the end of the experiment compared with control young rats as a result of oxidative stress associated with aging. SOD levels of control aged rats for all groups were not significantly different at zero time and during the experimental period. Aged rats groups fed on basal diet and administered Echinacea ethanolic and water extracts resulted in an increase in SOD levels by about 65.22% and 48.95%, respectively, compared with control aged rats at the end of the experiment. The increase in SOD levels was statistically significant ($p<0.05$). Echinacea ethanolic extract caused significant increase in SOD levels compared with Echinacea water extract and control young rats. From Table (2) it could be observed that, glutathione-s-transferase levels were reduced in aged rats compared with young rats as a result of aging which increase oxidative stress. Administring Echinacea ethanolic and water extracts orally to aged rats caused significant increase in GST levels compared with control aged rats at the end of the experiment and the increase reached about 29.33% and 25.09%, respectively. GST levels in serum of rats dosed with Echinacea extracts were not statistically different compared with control young rats.

During aging, mitochondria decay, rates of oxidant production increase and oxidative damage to important biomolecules increase and may in part be responsible for aging as well as age–associated degenerative diseases. The cellular distribution and bioavailability of key antioxidants have become altered with age. A shift in the oxidant:antioxidant balance because of increased production of free radicals is observed during aging. (Hagen *et al.*, 1997). Sarban *et al.* (2005) found that superoxide dismutase (SOD) constitutes the first line of defences against superoxide anion mediated oxidative injury. Superoxide dismutase enzyme activity was estimated based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. Glutathione–s-transferase (GST) is a major contributor to the eukaryotic cell`s defences against chemical and oxidative stress. Tissue–specific expression of GST subunits may occur at or prior to RNA processing. The cellular uptake of isothiocyanates is predominantly dependent on glutathione reduced conjugation reactions promoted by glutathione-s-transferase. (Zhang, 2001 and Kazi and Ellis, 2002). When sulforaphane is absorbed, the major isothiocyanate derived from glucosinolate, it is conjugated with glutathione and metabolized via the mercapturic acid pathway to be excreted as N-acetylcysteine conjugates. Although this conjugation can occur nonenzymatically, it is likely that this reaction occurs via the activity of glutathione–s–transferase (GST) *in vivo*. (Talalay and Fahey, 2001). The marker compounds commonly standardized for in Echinacea extracts are phenolic (caffeic) acid derivatives. These compounds have antioxidant and anti-in flammatory properties. The standardized extracts of Echinacea were superior to E. purpurea herb powder for these functional activities. However, the antioxidant activity from standardized Echinacea extracts was highly variable, with EC50 values ranging from 23 to 137 µg/ml. Quantitative analysis of E. purpurea extracts indicate that they contain caftaric acid, cichoric acid and undeca–2Z,4E-diene –8,10-diynoic acid isobutylamide at concentrations of 0.70, 0.71 and 2.0 mg/ml, respectively. (Rininger *et al.*, 2000 and Cech *et al.*, 2006).

Phytochemical analysis of Echinacea extracts revealed that, the 60% ethanol extract contained 48.9% polysaccarides and 2.3% alkamides, and the 20% ethanol extract contained 42.1% polysaccarides and only 0.1% alkamides. Assays for caffeic acid derivatives showed that the 60% ethanol extract contained 0.16 mg per milliliter of cynarine. Repeated analysis of the study treatments over the course of the study ensured that the composition of the treatments remained constant. HPLC analysis of E. purpurea extracts documented that a series of caffeic acid derivatives and other polyphenolics including cichoric acid were present and may contain glycosylated flavanoids. Aqueous extracts of Echinacea contain unusual polysaccarides reported to be responsible for certain anti-inflammatory and antioxidant activitives. (Turner *et al*., 2005 and Birt *et al*., 2008).

Effect of Echinacea Roots Extracts on Liver Functions in Aged Rats:

Effect of Echinacea purpurea roots extracts on liver functions in aged rats was shown in Table (3). Total protein, albumin, globulin, AST and ALT activities were determined in order to show the effect of ethanolic

and water extracts of Echinacea on these hepatic parameters.Total protein, albumin and glboulin levels are performed to evaluate the toxicological nature of various chemicals. Aging caused a significant decrease in total protein, albumin and globulin levels in serum of control aged rats compared with control young rats as a result of oxidative stress associated with aging which reduced liver functions. Administring aged rats with Echinacea ethanolic and water extracts caused a significant increase in total protein and albumin levels compared with control aged rats. The increase in total protein and albumin levels in serum of aged rats was not statistically significant compared with control young rats. The significant decrease in total protein and albumin with aging is indication of compromized liver excretory function and impairment of the liver synthetic function, which improved by treating with Echinacea extracts. Ethanolic extract of Echinacea caused more effective improvement in total protein, albumin and glboulin levels compared with water extract.

Albumin is the most abundant protein in human plasma, representing 55-65% of total protein. It is synthesized in the liver at a rate that is dependent on protein intake. Little albumin is filtered through the kidney and most of that is reabsorbed by erythropoietin. (Chun *et al.,* 2000). From Table (3), it could be noticed that aging resulted in a significant increase in liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities as a result of oxidative stress which reduced liver functions compared with control young rats at zero time and during the experiment. A significant decrease in AST and ALT activities was found as a result of administring Echinacea ethanolic and water extracts orally to aged rats at the end of the experimental period compared with control aged rats. AST and ALT enzymes may be released into blood plasma and serum levels of these enzymes may increase due to cellular damage in the liver. Liver enzymes activities were decreased to the normal levels found in control young rats with non statistical significance after administration of Echinacea ethanolic and water extracts. From the present results, it could be found that serum total protein, albumin, globulin, AST and ALT levels were improved by administring Echinacea ethanolic extract followed by Echinacea water extract orally to aged rats which indicate an improvement in liver functions. The increase of the activity of liver enzymes in serum is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream as mentioned by Mansour *et al*. (2002). *In vivo*, ethanolic root extracts of Echinacea purpurea significantly increased the phagocytic activity of liver and spleen in mice treated 3 times daily for 2 days by gavage. (O'Hara *et al.,* 1998).

Effect of Echinacea Roots Extracts on Lipid Profiles and Triglycerides Levels in Aged Rats:

Effect of Echinacea purpurea roots extracts on lipid profiles and triglycerides levels in aged rats is mentioned in Table (4). Aging resulted in a significant increase in serum triglycerides levels compared with control young rats. There was insignificant difference in triglycerides between aged rats at zero time and during the experimental period. Administring aged rats with Echinacea ethanolic and water extracts showed a significant reduction in triglycerides levels compared with control aged rats. Serum triglycerides levels appeared to be still elevated compared with control young rats although they are not statistically different. Aged rats recieved Echinacea ethanolic extract lowered their triglycerides levels from 273.47 mg/dl to 153.60 mg/dl (43.83%), while Echinacea water extract caused 39.10% reduction in triglycerides levels compared with control aged rats not recieving Echinacea. From data in Table (4), it could be observed that total cholesterol, LDL-cholesterol and VLDL-cholesterol levels significantly increased in aged rats compared with control young rats. When aged rats dosed with 6 mg Echinacea ethanolic and water extracts, the levels of lipid profiles (total cholesterol, LDL and VLDL) were significantly reduced compared with control aged rats. The reduction was statistically significant for total cholesterol and LDL levels and insignificant for VLDL levels compared with control young rats. Ethanolic extract of Echinacea roots caused the highest reduction in lipid profiles followed by water extract. On the opposite, HDL-cholesterol level in aged rats significantly decreased compared with control young rats because of aging. Administring aged rats with Echinacea extracts caused a significant increase in the levels of HDL-cholesterol compared with control aged rats and the increase was not significantly different by using Echinacea ethanolic extract compared with control young rats. It was found that Echinacea root extracts suppressed the oxidation of human low–density lipoprotein (LDL), as evaluated by reduced agarose electrophoretic mobility following oxidative modification by Cu (2+). The mechanisms of antioxidant activity of extracts derived from Echinacea roots included free radical scavenging and transition metal chelating. Antioxidant activity was found to delay the formation of conjugated diene hydroperoxide and extend the lag phase of peroxidation of soybean liposomes. (Hu and Kitts, 2000).

Low density lipoprotein cholesterol (LDL) is the most critical factor that many health disorders are connected with. Liver longevity may represent an integral response to its level in blood. Therefore, dietary therapy with antioxidants are helpful for health promotion at this direction. Total cholesterol is a risk factor for the progression of inflammations in the body. Omitting cholesterol from the diet lowered plasma VLDL-cholesterol and prevented the development of inflammations (Colin *et al.,* 2008 and Wouters *et al.,* 2008).

Effect of Echinacea Roots Extracts on Hemoglobin Concentration, White Blood Cells Concentration and Platelets Count in Aged Rats:

Data presented in Table (5) show effect of Echinacea roots extracts on hemoglobin concentration, white blood cells concentration and platelets count in aged rats. Oxidative stress associated with aging caused a significant decrease in hemoglobin concentration, white blood cells concentration and platelets count in aged rats compared with control young rats. There was insignificant difference for the three parameters in control aged rats at zero time and during the experiment. Administring aged rats with ethanolic and water extracts of Echinacea roots caused a significant increase in hemoglobin concentration, white blood cells concentration and platelets count compared with control aged rats at zero time and during the experimental period. Aged rats dosed with Echinacea extracts were not significantly different compared with control young rats for hemoglobin concentration, while the difference was statistically significant for white blood cells concentration and platelets count. The highest increase in hemoglobin concentration, white blood cells concentration and platelets count was achieved by using ethanolic extract of Echinacea roots.

Cundell *et al*. (2003) conducted a double–blind, placebo–controlled study to investigate whether Echinacea could affect total and differential white blood cell counts in old, healthy male rats. Echinacea (50 mg/kg of aerial parts) significantly increased circulating total white blood cell counts during the first 2 weeks of administration. No such changes were observed in animals given peanut butter alone. Echinacea purpurea administration had a suppressive effect on radiation–induced leukopenia, especially on lymphocytes and monocytes, and resulted in a faster recovery of blood cell counts. Mouse peripheral blood antioxidant activity was increased by E. purpurea. (Mishima *et al.,* 2004). The results mentioned by Melanie and Miller (2005) provided concrete evidence that chronic (long–term) intake of Echinacea was not only not detrimental but also distinctly prophylactic. Mice in control cages eating untreated chow had a 79% survival by 10 months of age, while mice living under identical conditions with the one variable being Echinacea in the daily chow, were still 100% alive by 10 months of age. By 13 months of age, control mice were 46% still alive, while those consuming Echinacea were 74% alive. At cell level, Echinacea extract also enhanced cell viability from 100 to 1000 ng/ml in association with growth factors, epidermal growth factor or insulin up to 37% against control. (Cucuzza *et al.,* 2008). It was found that alkamides have become a major focus for researchers studying Echinacea due to their abundance in both aboveground and underground parts of the plant in most species. Studies link this class of compounds to a vast repertoire of immuno–modulatory activities including antiviral, antimicrobial, antibacterial, antioxidant as well as anti-inflammatory properties. Alkamides can play important roles in the bioactivity of Echinacea speacies and shown to be readily bioavailable through the Caco–2 cell monolayer, more than other active compounds, such as the caffeic acid derivatives found in Echinacea. (Mathias *et al.,* 2004). It is obviously of fundamental importance that Echinacea itself, as with any agent given either prophylactically or therapeutically, is not deleterious to the host. In the case of Echinacea, there appears to be no *in vivo* toxic level, i.e., over dose level as defined by several assays and criteria. Moreover, there is very little available information concerning the potential for deterimental interactions of Echinacea with either other herbs or pharmaceuticals. E. purpurea extracts or isolated polysaccarides were neither toxic nor mutagenic when tested *in vitro* and *in vivo*, in mice as well as in humans. Overall, Echinacea is a safe and well–tolerated medicinal botanical with few adverse effects including headache, nausea and fatigue. (Mullins and Heddle, 1998 and Sparreboom *et al.,* 2004). From the above mentioned results, it could be observed that aging caused a significant deterioration in all tested parameters and markers of aging including superoxide dismutase and glutathione-s-transferase enzymes activities, liver functions (total protein, albumin, globulin, AST and ALT levels), lipid profiles (total cholesterol, HDL, LDL, VLDL and triglycerides), hemoglobin concentration, white blood cells concentration and platelets count. A significant reduction was observed in SOD, GST, total protein, albumin, globulin, HDL-cholesterol, hemoglobin, white blood cells and platelets count, while a significant increase was found in AST and ALT activities, triglycerides levels, total cholesterol, LDL and VLDL levels as a result of oxidative stress associated with aging. Administring aged rats with Echinacea ethanolic and water extracts at dose of 6 mg/daily caused a significant improvement in the above mentioned markers and Echinacea extracts were found to regulate the increase or the decrease in the disorder parameters which changed during aging compared with young rats. Echinacea extracts returned the abnormal markers back to the normal levels found in control young rats or nearst to them with the highest improvement achieved by using ethanolic extract. So, it could be recommended that Echinacea extracts could be used to enhance the abnormal changes in health state of aging populations and return them to nearly the normal state found in younger populations by using a natural source of antioxidants like Echinacea purpurea roots.

Table 1: Effect of Echinacea roots extracts on body weight in aged rats.

	Body weight (g)		
Groups	At zero time	After 15 days	At the end
Control young rats	107.33 ^{A}	111.66^{A}	113.39^{A}
Control aged rats	273.20^{B}	268.00^{B}	262.55^{B}
Echinacea ethanolic extract	269.33^{B}	247.44^{C}	223.45^{D}
Echinacea water extract	266.64^{B}	255.32^{C}	235.33^{D}
L.S.D.	7.53	8.33	12.44

* Numbers in the same column followed by the same letter are not significant different at $p<0.05$.
* L.S.D. for interaction between time and treatments= 11.66

Table 2: Effect of Echinacea roots extracts on antioxidant enzymes in aged rats.

Groups	Superoxide dismutase (U/mL)		Glutathione-s-transferase (U/L)	
	At zero time	At the end	At zero time	At the end
Control young rats	83.77^{A}	85.33^{A}	2484.03^{A}	2598.06^{A}
Control aged rats	73.14^{B}	64.21^{B}	2155.72^{B}	2140.66^{B}
Echinacea ethanolic extract	69.56^{B}	106.09^{C}	2146.83^{B}	2768.52^{A}
Echinacea water extract	72.19^{B}	95.64^{D}	2153.64^{B}	2677.88^{A}
L.S.D.	9.63		286.31	

Numbers in the same column followed by the same letter are not significant different at $p<0.05$.

Table 3: Effect of Echinacea roots extracts on liver functions in aged rats.

Groups	Total protein(g/dl)		Albumin (g/dl)		Globulin (g/dl)		AST (IU/L)		ALT (IU/L)	
	Zero	End	Zero	End	Zero	End	Zero	End	Zero	End
Control young rats	6.84^{A}	6.88^{A}	3.24^{A}	3.26^{A}	3.60^{A}	3.62^{A}	24.63^{A}	24.90^{A}	19.30^{A}	19.68^{A}
Control aged rats	6.29^{B}	6.20^{B}	2.79^{B}	2.72^{B}	3.50^{B}	3.48^{B}	29.17^{B}	31.56^{B}	24.55^{B}	24.93^{B}
Echinacea ethanolic extract	6.25^{B}	6.84^{A}	2.75^{B}	3.64^{A}	3.50^{B}	3.20^{C}	28.66^{B}	23.45^{A}	24.48^{B}	18.89^{A}
Echinacea water extract	6.22^{B}	6.69^{A}	2.73^{B}	3.55^{A}	3.49^{B}	3.14^{C}	30.47^{B}	25.00^{A}	24.63^{B}	20.42^{A}
L.S.D.	0.53		0.44		0.09		3.4		2.53	

Numbers in the same column followed by the same letter are not significant different at $p<0.05$.

Table (4): Effect of Echinacea roots extracts on lipid profiles and triglycerides levels in aged rats (mg/dl).

Groups	Triglycerides		Total cholesterol		HDL-cholesterol		LDL-cholesterol		VLDL-cholesterol	
	Zero	End	Zero	End	Zero	End	Zero	End	Zero	End
Control young rats	120.83^{A}	123.45^{A}	83.55^{A}	85.67^{A}	35.32^{A}	36.45^{A}	24.06^{A}	24.53^{A}	24.17^{A}	24.69^{A}
Control aged rats	272.35^{B}	274.67^{B}	116.66^{B}	116.12^{B}	26.47^{B}	25.53^{B}	35.72^{B}	35.66^{B}	54.47^{B}	54.93^{B}
Echinacea ethanolic extract	273.47^{B}	153.60^{A}	116.46^{B}	87.43^{AC}	26.63^{B}	36.24^{A}	35.14^{B}	20.47^{A}	54.69^{B}	30.72^{C}
Echinacea water extract	272.89^{B}	166.25^{AC}	115.73^{B}	88.68^{AC}	26.78^{B}	32.65^{C}	34.38^{B}	22.78^{A}	54.57^{B}	33.25^{C}
L.S.D.	35.4		3.12		2.18		4.7		2.94	

Numbers in the same column followed by the same letter are not significant different at $p<0.05$.

Table 5: Effect of Echinacea roots extracts on hemoglobin concentration, white blood cells concentration and platelets count in aged rats.

Group	Hemoglobin conecentration (%)		White blood cells conecentration (%)		Platelets count	
	At zero time	At the end	At zero time	At the end	At zero time	At the end
Control young rats	12.43^{A}	13.06^{A}	8.66^{A}	8.83^{A}	722.66^{A}	725.72^{A}
Control aged rats	11.56^{B}	11.36^{B}	7.36^{B}	7.18^{B}	455.33^{B}	450.66B
Echinacea ethanolic extract	11.63^{B}	13.37^{AC}	7.45^{B}	12.87^{C}	454.63^{B}	673.33^{C}
Echinacea water extract	11.50^{B}	12.53^{AD}	7.28^{B}	12.54^{C}	453.18^{B}	584.68^{D}
L.S.D.	0.74		1.06		30.46	

Numbers in the same column followed by the same letter are not significant different at $p<0.05$.

REFERENCES

A.O.A.C., 2006. Official Methods of Analysis. 18th Edition, Association of Official Agricultural Chemists. Washington D.C., USA.

Assmann, G., 1979. Cholesterol determination in high density lipoproteins separated by three different methods. Internist, 20: 559-604.

Barnes, J.L., S. Anderson, Gibbons and J. Phillipson, 2005. Echinacea species: A review of their chemistry, pharmacology and clinical properties. J. Pharm. Pharmacol., 57: 929-954.

Barrett, B., 2003. Medicinal properties of Echinacea: A critical review. Phytomedicine, 10: 66-86.

Bauer, R., 1999. Standardization of *Echinacea purpurea* expressed juice with reference to cichoric acid and alkamides. J. Herbs, Spices Med. Plants, 6: 51-62.

Bergmeyer, H.U. And M. Harder, 1986. A colorimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Clin. Biochem., 24: 458-481.

Birt, D.F., M.P. Widrlechner; C.A. Lalone, L.Wu, J. Bae; A.K. Solco, S.C. Hebert and J.P. Price, 2008. Echinacea in infection. Am. J. Clin. Nutr., 87(2): 488S-492S.

Bone, K., 2004. Echinacea: Quality, uses and immunomodulating activities from a phytotherapist's perspective. In Echinacea The Genus Echinacea; Miller, S., ed., CRC Press: Boca Raton, FL, pp: 39.

Borcea, V.J., S.P. Nourooz-Zadeh; M. Wolff, H. Slevesath, Urich and P.P. Nawroth, 1999. Alpha-lipoic acid decreases oxidative stress even in diabetic patients with poor glycemic control and albuminuria. Free Radic. Biol. Med., 26: 1495-1500.

Cech, N.B., M.S. Eleazer; M.R. Crosswhite; A.C. Davis and A.M. Mortenson, 2006. HPLC and electrospray ionization mass spectrometry for simultanous analysis of alkamides and caffeic acid derivatives from *Echinacea purpurea* extracts. J. Chromatography A, 27: 1103(2): 219-228.

Chen, Y., T. Fu, T. Tao, J. Yang, Y. Chang, L. Kim, L. Qu and R. Scalzo, 2005. Macrophage activiating effects of new alkamides from the roots of Echinacea species. J. Nat. Prod., 68: 773-776.

Chun, Y.S., E. Choi, G.T. Kim, H. Choi, C.H. Kim, S. Kim and J.W. Park, 2000. Blocks of hypoxia-inducible factor mediated response to hypoxia by stimulating the proteasome-groups in tisue with ellmans reagent. Anal. M. J. Biochem., 25: 293-298.

Colin, S., E. Bourguignon; A.B. Boullay, J.J. Tousaint, S. Hunt; J.M. Lobaccaro and P. Delerive, 2008. Intestine-specific regulation of PPAR (alpha) gene transcription by liver X receptors. Endocrinology, 19: 210-214.

Cucuzza, L.S., M. Motta; P. Accornero and M. Baratta, 2008. Effect of *Echinacea angustifolia* extract on cell viability and differentiation in mammary epithelial cells. Phytomedicine, 15(8): 555-562.

Cundell, D.R., M.A. Matrone, P. Ratajczak and J.D. Pierce, 2003. The effect of aerial parts of Echinacea on the circulating white cell levels and selected immune functions of the aging male Sprague-Dawley rat. Inter. Immunopharmacol., 3(7): 1041-1048.

Dacie, J.V. and S.M. Lewis, 1984. Practical Haematology. A collaborative analysis of an improved procedure.

Dalby-Brown. L., H. Barsett, A. Landbo, A. Mayer and P. Molgaard, 2005. Synergistic antioxidative effects of alkamides, caffeic acid derivatives and polysaccaride fractions from Echinacea purpurea on *in vitro* oxidation of human low-density lipoproteins. J. Agric. Food Chem., 53: 9413-9423.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons, Inc., USA.

Guiotto, P., K. Woelkart; I. Grabnar, M. Granzotto and R. Bauer, 2008. Pharmacokinetics and immunomodulatory effects of phytotherapeutic iozenges with *Echinacea purpurea* extract. Phytomedicine, 15(8): 547-554.

Habig, W.B., M.J. Pabst and W.P. Jakoby, 1974. Glutathione-s- transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Hagen, T.M., D.L. Yowe, J.C. Bartholomew, C.M. Wehr, J.Y. Park and B.N. Ames, 1997. Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogenicity and oxidants increase. Proc.Natl. Acad. Sci., USA 94: 3064-3069.

Hatch, F.T. and R.S. Lees, 1968. Practical methods for plasma lipoprotein analysis. Adv. Lipid Res., 6: 1-6.

Hermann, J.R., M.S. Honeyman, J.J. Zimmerman, P.J. Holden and C.C. Chang, 2003. Effect of dietary *Echinacea purpurea* on viremia and performance in porcine reproductive and respiratory syndrome virus-infected nursery pigs. J. Anim. Sci., 81: 2139-2144.

Hu, C. and D.D. Kitts, 2000. Studies on the antioxidant activity of Echinacea root extract. J. Agric. Food Chem., 48(5): 1466-1472.

Justice Canada. J.C., 2008. Natural Health Products Regulations. Ottawa (on): Justice Canada: 196.

Kazi, S. and E.M. Ellis, 2002. Expression of rat liver glutathione-s-transferase GSTA5 in cell lines provides increased resistance to alkylating agents and toxic aldehydes. Chem. Biol. Interactions, 140(2): 121-135.

Kreis, W., U. Sussner and B. Nusslein, 2000. Purification and characterization of a polyphenol oxidase from the herbal drug *Echinacea purpurea*. J. Appl. Bot., 74: 106-112.

Krings, U. and R.G. Burger, 2001. Antioxidant activity of some roasted foods. Food Chem., 72: 223-229.

Lalone, C.A., K.D. Hammer, L. Wu, N. Leyva, A.K. Solco, G.A. Kraus, K. Kim, K. Seo and D.F. Birt, 2007. Echinacea species and alkamides inhibit prostaglandin E2 production in RAW264.7 mouse macrophage cells. J. Agric. Food Chem., 55(18): 7314-7322.

Lee, J.C., H.R. Kim, J. Kim and Y.S. Jang, 2002. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus*-indica. J. Agric. Food Chem., 50: 6490-6496.

Lienert, D., E. Anklam and U. Panne, 1998. Gas chromatography-mass spectral analysis of roots of Echinacea species and classification by multivariate data analysis. Phytochem. Anal., 88-98.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mansour, H.A., A.S. Newairy, M.I. Yousef and S.A. Sheweita, 2002. Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Toxicology, 170: 221-228.

Marklund, S. and C. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

Mathias, A., J. Blanchfield, K. Penman, I. Toth, C. Lang and R. Lehmann, 2004. Permeability studies of alkylamides and caffeic acid conjugates from Echinacea using a Caco-2 cell monolayer model. J. Clin. Pharm. Ther., 29: 7-13.

Melanine, B. And S.C. Miller, 2005. Enhancement of natural killer cells and increased survival of aging mice fed daily Echinacea root extract from youth. Biogerontology, 6(3): 157-163.

Meydani, M., 2000. Effect of functional food ingredients: vitamin E modulation of cardiovascular diseases and immune status in the elderly. Am. J. Clin. Nutr., 71(6): 1665S-1668S.

Miller, S.C., 2005. Echinacea: A miracle herb against aging and cancer. Evidence *in vivo*. Evid. Based Compl. and Altern. Med., 3: 304-314.

Mishima, S., K. Saito, H. Maruyama, M. Inoue, T. Yamashita and T. Ishida, 2004. Antioxidant and immuno-enhancing effects of *Echinacea purpurea*. Biol. Pharm. Bull., 27(7): 1004-1009.

Mullins, R.J. and R. Heddle, 1998. Adverse reactions associated with Echinacea. J. Biol. Rhythms, 13: 245-252.

O'Hara, M.A., D. Kiefer; K. Farrell and K. Kemper, 1998. A review of 12 commonly used medicinal herbs. Arch. Fam. Med., 7: 523-536.

Pellati, F., S. Benvenuti; L. Margo, M. Melegari and F. Soragni, 2004. Analysis of phenolic compounds and radical scavenging activity of Echinacea species. J. Pharm. Biomed. Anal., 35(2): 289-301.

Pellati, F., S. Benvenuti, M. Melegari and T. Lasseigne, 2005. Variability in the composition of antioxidant compounds in Echinacea species by HPLC. Phytochem. Anal., 16(2): 77-85.

Rininger, J.A., S. Kichner, P. Chigurupati, A. Mclean and Z. Frank, 2000. Immunopharmacological activity of Echinacea preparations following simulated digestion on murine macrophages and human peripheral blood mononuclear cells. J. Leuk. Biol., 68: 503-510.

Roeschlau, P., E. Bernt and W.J. Gruber, 1974. Kinetics of the cholesterol-sulfuric acid reaction. A fast kinetic method for serum cholesterol. Clin. Chem. Clin. Biochem., 12: 403-408.

Sarban, S., A. Kocyigit, M. Yazar and U.E. Isikan, 2005. Plasma total antioxidant capacity, lipid peroxidation and erythrocyte antioxidant enzyme activities in patients with rheumatoid arthritis and osteoarthritis. Clin. Biochem., 38: 981-986.

Sparreboom, A., M.C. Cox, m. r. Acharya and W.D. Figg, 2004. Herbal remedies in the US: potential adverse interactions with anticancer agents. J. Clin. Oncol., 22: 2489-2503.

Spelman, K., M.H. Wetschler and N.B. Cech, 2009. Comparison of alkamide yield in ethanolic extracts prepared from fresh versus dry *Echinacea purpurea* utilizing HPLC-ESI-MS. J. Pharm. Biomed. Anal., 49(5): 1141-1149.

Sloley, B.D., C. Tywin, R.T. Coutts, P.K. Pang and J.J. Shan, 2001. Comparison of chemical components and antioxidants capacity of different Echinacea species. J. Pharm. Pharmacol., 53(6): 849-857.

Sun, L.Y., N.L. Currier and S.C. Miller, 1999. The American coneflower: a prophylactic role involving nonspecific immunity. J. Altern. Compl. Med., 5: 437-446.

Talalay, P. and J.D. Fahey, 2001. Phytochemicals from cruciferious plants protect against cancer by modulating carcinogen metabolism. J. Nutr., 131: 3027S-3033S.

Turner, R.B., R. Bauer, K. Woelkart, T.C. Hulsey and G.D. Gangemi, 2005. An evaluation of *Echinacea angustifolia* in experimental rhinovirus infections. The New Engl. J. Med., 353(4): 341-348.

Uwajima, T., Y. Shimizu and O. Ferda, 1984. Glycerol oxidase, a novel copper hemoprotein from Aspergillus japonicus. J. Biol. Chem., 259: 2748-2753.

WHO., 1999. Doe J. Radix Echinacea. In: WHO monographs on selected medicinal plants. Vol.1, Geneva: World Health Organization: 125-135.

Wouters, K., P.J. vanGorp., V. Bieghs, M.J. Gijbels, H. Duimel, B. Staels and M.H. Hofker, 2008. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. Hepatology, 48(2):474-486.

Zhang, Y., 2001. Molecular mechanism of rapid cellular accumulation of anticarcinogenic isothiocyanates. Carcinogenesis, 22: 425-431.