Arab Journal of Nuclear Science and Applications, 46(1), (339-346) 2013

The role of antioxidant properties of Celery against lead acetate induced hepatotoxicity and oxidative stress in irradiated rats

Nadia, N. Osman
Food Irradiation Research Dep. National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

Received: 18/2/2012 **Accepted: 3/3/2012**

ABSTRACT

Man is exposed to natural radiations from cosmic or terrestrial origins. Furthermore the combined action of radiation with various chemicals is an inevitable feature of modern life. Radiation is known to cause cell death, mainly due to its ability to produce reactive oxygen species in cells. Lead is a serious public health problem in many parts of the world. As in the case of many chronic degenerative diseases, increased production of reactive oxygen species has been considered to play an important role, even in the pathogenesis of chronic lead toxicity. Celery is closely linked to its protective prope rties against free radicals attack. Therefore, the aim of this study was to evaluate the hepatoprotective effects of Celery juice (CJ) against the hazardous effect of lead acetate (1 mg/kg/day, i.p. for four weeks) and/ or whole body gamma irradiation at 4Gy.

Results showed that combined treatment of lead acetate and ?-radiation caused an elevation in liver TBARS and protein carbonyl content (PCC) associated with a reduction in activity of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). It also increased serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma - glutamyl transpeptidase (?-GT) as well as serum bilirubin. While serum total protein (TP) and albumin (Alb) were decreased. CJ administration improved the significant increase in TBARS, PCC and ameliorated serum liver enzymes as well as improved the decreased level of GSH content, CAT and SOD activities. It also could normalize serum billrubin, total protein and albumin. It is concluded that CJ has a protective effect against lead acetate and gamma-irradiation induced hepatotoxicity through antagonizing the free radicals generation beside enhancement of the antioxidant defense mechanisms.

***Key words*: *lead acetate, hepatotoxicity, Celery, irradiated rats*.**

INTRODUCTION

Study of the effects of ionizing radiation on humans in the era of intensive use of nuclear energy and ionizing radiation in medical diagnostics and information technologies is an important activity aiming to protect the health of human population[1]. Excessive levels of trace metals may occur naturally as a result of geological phenomena such as ore formation, weathering of rocks and leaching which may make these metals available to the biosphere. Man releases more of these metals by burning fossil fuels, mining, smelting, and discharging industrial, agricultural and domestic waste and by deliberate environmental application of pesticides[2]. Lead is a pervasive and persistent environmental pollutant that can be detected in almost all phases of environment and biological systems. Lead was found naturally in earth and present in food, air, water, dust, soil, paint and tissues of living organisms including human[3]. Lead is one of the most toxic metals, producing severe organ damage in animals and humans[4]. Several studies have reported that lead toxicity is associated with impaired functioning of brain, liver, kidney, testes and the hematopoietic system[5,6]. Combined action

of ionizing radiation and other agents is of potentially great importance, because there are many occasions when interactions might occur in our environment. The combination of ionizing radiation and lead exposure can potentially be extremely toxic to tissues due to heightened oxidative stress. Lead and/or radiation exposure induce the production of reactive oxygen species (ROS) [7,8].

To overcome this problem of scavenging free radicals produced by heavy metal intoxication, antioxidant is the best suggested therapy. Treatment of antioxidants of natural origin is the best recommended way to conquer the problem of lead toxicity. Celery *(Apium graveolens* L., Apiaceae) is a medicinal herb used as a food, and also in traditional medicine. It contains aromatic substances in the roots, stem and leaves. The healing properties of celery are due to the essential oils and flavonoids, mostly apiin and apigenin [9]. Celery can lower blood pressure and regulate heart function. It can be used also to slow down and treat complications caused by diabetes because it influences the blood glucose level by stimulating the pancreas to insulin secretion [9]. Among many other effects, Celery is particularly known for its anti-cancer [10] and antioxidant effects [11]. Phytochemical investigations of Celery seeds revealed the presence of terpenes like limonene, flavonoids like apigenin and phthalide glycosides. Apigenin is an antioxidant that was documented as one of the major Celery's active principals in *Apium graveolens* [12]. The efficacy of Celery as an anti-cancer remedy may then be attributed to the presence of flavonoids, particularily apigenin in its extract [13]. Based on the above mentioned information, the goal of the present study was to throw the light on the possibilities of Celery extract in preventing or minimizing the oxidative stress and hepatotoxic effects induced by lead-acetate and/or gamma-radiation exposure.

MATERIALS AND METHODS

***Chemicals*:** Pure lead acetate was purchased from Sigma Chemical (St. Louis, Mo).
Plant Material: Celery leaves were obtained from a local market of Herbs and Medicinal plants, Cairo, Egypt. Fresh leaves were ground in a blender and 5% solution (v/v) was prepared by diluting pure juice with distilled water (Kolarovic et al., 2009).

***Animals*:** Male albino rats weighing (125-150g) purchased from the Egyptian Organization for Biological Product and Vaccines in Cairo, Egypt, were housed in cages under good ventilation and illumination condition and fed on a balanced standard diet and received water *ad-libitum*. All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85– 23, revised 1985).

Radiation treatment: Irradiation of rats was performed by the use of a Canadian Gamma Cell-40 (137Cs) at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. The irradiation dose rate was 0.5 Gy/minute calculated by Dosimetry Department in our NCRRT.

Experimental design: The experimental animals were divided into 8 groups (each group contains 6 rats) as follows:
Group1 (Control): Rats didn't receive any treatment.
Group2 (CJ): Rats received Celery leaves juice in drinking water (5% solution v/v) for four weeks[14].
Group3 (IRR): Rats were whole body gamma irradiated (4Gy) administered as one shot dose on the first day of experiment.
Group4 (Lead acetate): Rats received lead acetate at a dose of 1 mg/kg/day, (i.p.) for four weeks [15].
Group5 (IRR+ Lead acetate): Animals received the first dose of lead acetate and after one hour they exposed to gamma irradiation at dose 4Gy and lead acetate treatment continued for four weeks.
Group6 (CJ + IRR): Rats received CJ for four weeks and exposed to gamma irradiation at dose 4Gy on the first day of experiment.

Group7 (CJ +lead acetate): Rats given CJ along with lead acetate (1 mg/kg/day, i.p.) for four weeks.
Group8 (CJ + IRR+ lead acetate): Rats supplemented with CJ for 4 weeks and injected with lead acetate and whole body gamma irradiation as group 5.

Biochemical analysis: At the end of experimental period rats were sacrificed and blood samples were collected in sterile heparinized tubes by heart puncture. Liver was quickly removed.
The quantitative determination of serum ALT and AST were done using the method of Reitman and Frankle [16]. ALP activity was estimated according to the method of Kind and King ([17], ?-glutamate transpeptidase (?- GT) was determined using the method of Szasz [18]. Total bilirubin, total protein, and Albumin concentrations were measured using the methods of Malloy and Evelyn [19], Lowry *et al.*, [20] and Doumas *et al* [21], respectively.

The liver was homogenated in saline solution. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for the biochemical analysis. The extent of lipid peroxidation was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) according to Yoshioka *et al*,[22] . Protein carbonyl content (PCC) was measured according to the procedure of Levine *et al.* [23] . Superoxide dismutase (SOD) and catalase (CAT) activities were determined according to Minami and Yoshikawa *et al* [24], and Aebi [25], respectively. The content of reduced glutathione (GSH) was determined according to Beutler *et al* [26].

Statistical analysis: Data were analyzed using one way analysis of variance (ANOVA). Posthoc Duncan test was used to determine significant differences between means. Values were expressed as mean ±SE. (n=6). Differences between means were considered significant at P= 0.05.

RESULTS AND DISCUSSION

Lead is a common environmental occupational toxic heavy metal, known to have direct and indirect effects on biological systems and cells. It is an ubiquitous toxic agent, and its toxicity remains an important public health problem because of the great amount of sources in any household and environment [27].

Liver is considered to be the principal target organ for lead toxicity [28]. In the present study, results indicated that lead acetate and/or gamma-irradiation of rats induced positive effect on serum ALT, AST, ALP and ?- GT activities (Table 1). These results agreed with those found by many investigators who studied the effect of lead on liver function [29, 30].

Table 1: Effect of Celery juice administration to rats on hepatic serum markers levels due to exposure to gamma radiation and/or lead acetate.

Groups	Control	CJ	IRR	Lead acetate	IRR +lead acetate	CJ +IRR	CJ +lead acetate	CJ +IRR +lead acetate
ALT (IU/L)	31.51± 4.15^{a}	32.50± 3.24^{a}	55.23± 3.31^{c}	66.92± 3.13^{d}	70.30± 3.31^{d}	35.22± 3.50^{a}	37.05± 3.24^{b}	42.61± 4.10^{b}
AST (IU/L)	73.24± 5.45^{a}	72.63± 4.98^{a}	123.52± 11.25^{c}	134.04± 13.09^{c}	151.07± 10.01^{d}	83.34± 5.64 ^{ab}	81.43± 5.78^{ab}	88.83± 6.11^{b}
ALP (IU/L)	69.79± 7.72^{a}	70.17± 3.10^{a}	101.92± 5.40^{b}	105.21± 2.68^{b}	173.93± 6.27^{c}	75.47± 4.38^{a}	70.12± 2.61^{a}	100.40± 3.52^{b}
?-GT (IU/L)	2.04± 0.19^{a}	1.93± 0.12^{a}	4.48± 0.49^{c}	4.40± 0.38^{c}	5.25± 0.41^{d}	2.38± 0.28^{a}	2.21± 0.20^{a}	3.53± 0.33^{b}

Values are given as mean ±SE for 6 rats. Values not sharing a common superscript letter differ significantly at P= 0.05.

The elevation of serum AST and ALT level might be due to the release of these enzymes from the cytoplasm, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage. ALP is an ecto-enzyme of hepatocyte plasma membrane; an increase in serum ALP reflects the pathological alteration in biliary flow and damage to the liver cell membrane [31]. High concentrations of serum transaminases are considered to be an index of hepatic injury where elevation of ALT is regarded as a more sensitive indicator and is usually accompanied by a rise in AST [32] . ?-glutamyl transferase (?- GT) is a microsomal enzyme, which is widely distributed in tissues including liver. Serum ?- GT is most useful in diagnosis of liver diseases [33]. Changes in ?-GT is parallel to those of aminotransferases.

Data presented in Table (2), show that lead acetate induced a significant decrease in serum total protein and albumin among the tested rat groups, while total bilirubin level showed a significant increase, compared with normal control rats. Determination of serum total proteins and albumin level, to assess the ability of synthetic function of the liver is well documented [34]. Bilirubin is regarded as a member of the antioxidant family, even though it is known to have toxic effects at high concentrations [35]. A decease of serum total protein and albumin levels in rats receiving lead acetate is in agreement with previous studies [36]. The decreased level of protein may indicate protein catabolism dysfunction [37]. Moreover, Adeyemi *et al* [34] noted a significant increase in the bilirubin level of rats treated with Pb compared to control, while serum globulin and albumin concentrations was significantly lower ($p < 0.05$) than those of control. The elevation of plasma bilirubin value by lead acetate treatment may be due to the induction of heme oxygenase. The catabolism of heme from all heme proteins is carried out in the microsomal fraction of cells by a complex enzyme system and heme oxygenase is an enzyme which can convert heme to bilirubin [38]. They reported that bilirubin formed in different tissues is transported to liver as a complex with serum bilirubin. Bilirubin is conjugated with glucuronide in the smooth endoplasmic reticulum of liver, but under the effects of lead toxicity, the conjugation of bilirubin with glucouronoid will become inactive. This may be due to the peroxidation of membrane lipids of smooth endoplasmic reticulum. Bilirubin has a protective role against oxidative damage of cell membrane induced by metals [39].

Table 2: Effect of Celery juice administration to rats on serum total protein, albumin and bilrubin levels due to exposure to gamma radiation and/or lead acetate.

Groups	Control	CJ	IRR	Lead acetate	IRR +lead acetate	CJ +IRR	CJ +lead acetate	CJ +IRR +lead acetate
T.P (g/dl)	10.14± 0.80^{a}	9.11± 0.21^{a}	7.34± 0.33^{c}	7.54± 0.20^{c}	7.00± 0.24^{c}	8.44± 0.11^{b}	8.14± 0.32^{b}	8.00± 0.22^{b}
Alb (g/dl)	6.53± 0.13^{a}	6.11± 0.12^{b}	4.54± 0.15^{d}	4.52± 0.14^{d}	4.00± 0.13^{e}	6.24± 0.13^{b}	6.00± 0.12^{b}	5.14± 0.12^{c}
Bilrubin mg/dl	0.43± 0.06^{a}	0.45± 0.04^{a}	0.86± 0.09^{c}	0.83± 0.07^{c}	0.97± 0.07^{d}	0.65± 0.04^{b}	0.63± 0.05^{b}	0.83± 0.07^{c}

Values are given as mean ±SE for 6 rats. Values not sharing a common superscript letter differ significantly at P= 0.05.

The present study showed that exposure of rats to lead acetate resulted in severe oxidative stress (Tables 3-4). There was an increase in lipid peroxidation (TBARS), protein carbonyl content (PCC) as well as a significant decrease in GSH content and antioxidant enzymes (SOD and CAT activities) in liver tissues. These observations are consistent with the findings of several studies, which reported alterations in antioxidant enzymes activity in lead exposed animals [6] and suggestion of a possible involvement of oxidative stress in the pathophysiology of lead toxicity.

The stimulation of lipid peroxidation, presumably caused by lead treatment, could be due to the formation of free radicals [40] through exhaustion of antioxidants [28] and subsequently to oxidative stress [41]. Protein modifications elicited by direct oxidative attack lead to the formation of protein carbonyl derivatives and protein carbonyl content (PCC) is the most commonly used biomarker for protein oxidation [42]. The observed increase in protein carbonyl content in lead exposed rats confirms the oocurrance of oxidative stress induced by lead acetate in hepatocytes.

Table 3: Effect of Celery juice administration to rats on liver lipid peroxides (measured as TBARS) and liver protein carbonyl content (PCC) due to exposure to gamma radiation and/or lead acetate.

Groups	Control	CJ	IRR	Lead acetate	IRR +lead acetate	CJ +IRR	CJ +lead acetate	CJ +IRR +lead acetate
TBARS (µmol/g wet tissue)	6.81± 1.93^{a}	5.24± 1.71^{a}	26.91± 1.30^{c}	27.34± 1.23^{c}	29.03± 1.89^{c}	11.56± 1.26^{b}	11.98± 1.17^{b}	12.40± 2.77^{b}
PCC (n mol/ mg protein)	10.71± 2.04^{a}	10.00± 2.12^{a}	26.62± 3.57^{c}	29.93± 3.15^{c}	32.91± 2.28^{d}	16.71± 2.35^{b}	18.43± 1.76^{b}	22.91± 1.17^{b}

Values are given as mean ±SE for 6 rats. Values not sharing a common superscript letter differ significantly at P= 0.05.

The obtained results displayed that treatment of rats with lead caused a reduction of liver superoxide dismutase (SOD), catalase (CAT) and reduced glutathione content (GSH) (Table 4). The recorded data are on the same line of those observed previously by Patra *et al.,* [15]. They reported that lead enhances lipid peroxidation level in the liver and brain and decreased in the activity of SOD and CAT. Moreover, recent studies showed that lead inhibit the activities of antioxidant enzymes, including glutathione peroxidase, catalase and superoxide dismutase [43] .

Table 4. Effect of Celery juice administration to rats on hepatic oxidative stress- related parameters due to exposure to gamma radiation and/or lead acetate.

Groups	Control	CJ	IRR	Lead acetate	IRR +lead acetate	CJ +IRR	CJ +lead acetate	CJ +IRR +lead acetate
CAT (U/g wet tissue)	17.32± 0.43^{a}	18.18± 0.53^{a}	12.67± 0.88^{c}	12.44 ± 0.64^{c}	9.40± 0.42^{d}	14.44± 0.41^{b}	15.52± 0.22^{b}	11.82± 0.30^{c}
SOD (U/ mg wet Tissue)	10.17± 0.28^{a}	10.27± 0.33^{a}	6.65± 0.17^{c}	6.47± 0.20^{c}	5.55± 0.18^{d}	8.75± 0.22^{b}	8.12± 0.20^{b}	6.61± 0.26^{c}
GSH (mg/g wet tissue)	18.86± 0.61^{a}	19.70± 0.88^{a}	14.60± 0.40^{c}	14.31± 0.45^{c}	12.34± 0.43^{d}	16.40± 0.31^{b}	16.23± 0.37^{b}	14.50± 0.50^{c}

Values are given as mean ±SE for 6 rats. Values not sharing a common superscript letter differ significantly at P= 0.05.

Ionizing radiations are known to induce oxidative stress through the generation of reactive oxygen species resulting in an imbalance in the pro-oxidant, antioxidant status in the cells [44]. It was observed that exposure of rats to ?-radiation resulted in elevated levels of serum AST, ALT, ALP and ?-GT when compared to the control group, (Table 1). These results stand in well agreement with those of El-Gawish *et al,* [45]. The increased levels of AST and ALT could be referred to the drastic dysfunction of the liver cells induced by radiation interaction with cellular membranes and also related to extensive breakdown of liver parenchyma [46].

Table (2) shows a significant decrease ($P<0.05$) in serum total protein and albumin levels in ? -irradiated rats as compared with the corresponding control rats. This marked decrease in total protein may be attributed to the damage of vital biological processes or due to change in the permeability of liver, kidney and other tissues resulting in leakage of protein via the kidney [47]. The decline in the level of albumin concentration could be due to enhanced degradation as well as enhanced loss of albumin through the gastrointestinal tract [48]. Exposure of rats to ?-radiation resulted in a signi?cant increase in lipid peroxidation, as measured by the formation of TBARS in the liver (Table 3). Such an increase occurred in protein carbonyl (PCC), a major product of protein oxidation (Table 3). The increased concentration of TBARS and protein carbonyl in the rat liver, indicating high level of oxidative stress. Similar observations were reported on radiation-induced oxidative damage in several organs [49,50]. Ionizing radiation generates ROS as a result of water radiolysis. In actively metabolizing cells, there is considerable water apart from the target macromolecules. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes [51]. Products of lipid peroxidation such as MDA and 4NHE (4-hydroxynonenal) have the ability to interact with and alter macromolecules, possibly resulting in diseases [52]. Oxidative damage to proteins, as assessed by formation of carbonyl groups is a highly damaging event, and may occur in the absence of lipid peroxidation [53].

Superoxide dismutase, catalase and glutathione peroxidase constitute the major enzymatic antioxidant defenses which convert active oxygen molecules into nontoxic compounds. Exposure of rats to ?-radiation decreased the activity of these antioxidant enzymes in the tissues which are indicative of oxidative stress occured in the liver (Table 4). The decline in these enzymes in the present study could be explained by the assumption that excess superoxide radicals may inactivate H_2O_2 scavengers, thus resulting in inactivation of superoxide dismutase [54]. Excessive liver damage and oxidative stress caused by ?-irradiation might be responsible for the depletion of GSH. Oxidative stress induced by ?-irradiation of animals resulted in an increased utilization of GSH and subsequently a decreased level of GSH was observed in the liver tissues. Depletion of GSH is known to cause an inhibition of the glutathione peroxidase activity and has been shown to increase lipid peroxidation [55]. A similar correlation between the depletion of GSH and increase of lipid peroxidation was remarked observed in the present investigation.

The changes recorded in the group of rats exposed to lead acetate +IRR were more pronounced than those observed for irradiated rats or exposed to lead acetate (Tables 1-4). It is well documented that free radical scavengers and antioxidants are useful in protecting against oxidative stress toxicity [56]. In the present study, Celery *(Apium graveolens)* administration has ameliorated the increase of ALT, AST, ALP and ?-GT activities as well as the concentration of billirubin in the serum of lead treated rats, irradiated rats, as well as, rats subjected to both treatment. Furthermore, oral administration of celery was able to improve the levels of endogenous antioxidants (SOD, CAT and GSH) in the liver and to decrease the level of TBARS and PCC in liver tissues of Pb-treated, irradiated and Pb -treated irradiated rats. In accordance, the administrations of methanolic extract of *A. graveolens* seeds (Celery) showed hepatoprotective effect through significant prevention the rise in serum ALT,AST, ALP, and bilirubin levels [57]. It probably does this action by free radical scavenging capacity which has been established in an in- vitro studies [58].

Therapeutic activities of celery depend mainly on the presence of ß-carotene, lutein and the flavones, luteolin and apigenin [59]. López-Lázaro [60] had found that luteolin possesses a variety of pharmacological activities, including antioxidant and anti-inflammatory activities. Different Celery leaf extracts are scavengers of OH and DPHH radicals and reduce liposomal peroxidation, which points to their antioxidant activity. The antioxidant activity of Celery leaf extracts may be due to the presence of flavonoids [58]. Manju *et al.* [61], observed enhancement of plasma and hepatic antioxidant status (glutathione GSH, pyrogallol peroxidase PPx, glutathione-S-transferase GST,glutathione reductase GR,SOD, CAT, Vitamin C, Vitamin A and ß-carotene) in rats with 1,2-dimethylhydrazine induced colon cancer upon intragastric administration of 0.2 mg/kg luteolin(which is a flavone contained in celery). Moreover, Jain *et al.* [62], reported that co-administration of methanolic extract of A. graveolens seeds along with Di-(2- ethylhexyl) phthalate (DEHP) induced hepatotoxicity in rats, signifycantly prevented the rise in TBARS level with a concomitant elevation in the concentration of hepatic glutathione and ascorbic acid suggesting alleviation of oxidative stress and restoration of antioxidant defense system resulting in membrane stabilization.

CONCLUSION

According to the results obtained in the present study, it appears that CJ administration to rats would decrease the toxicity associated with oxidative stress and thereby reducing the damage induced by exposure to lead acetate and/or radiation.

REFERENCES

1- Danová D., Kafka, I., Kalenicová, Z., and Nováková J. .Acta Vet. Brno.; 79 (2010).
2- Purohit, R.K., Chakrawarti, A. and Bhartiya, K.M.. Iran J Radiat Res.; 5(2007).
3- Mohamed, N. A. and Saleh, S. M. Egypt. J. Histol.; 33(2) (2010).
4- Spivey, A. Environ. Health Perspect.; 115(2007).
5- Bellinger, D.C. Curr. Opin. Pediatr.; 20(2008).
6- El-Nekeety, A.A., El-Kady, A.A., Soliman, M.S., Hassan, N.S. and Abdel-Wahhab, M.A. Food Chem. Toxicol.; 47(2009).
7- Borek, C. J. Nutr.; 134(2004).
8- Ercal, N., Gurer-Orhan, H. and Aykin-Burns, N..Curr. Top. Med. Chem., 1(2001).
9- Mimica-Duki c, N., Popovic, M. In *Recent Progress in Medicinal Plant Species*; Govil, J.N., Singh, V.K., Eds.; Phytopharmacology and Therapeutic Values III, LLC: Houston, TX, USA, 21, (2007)
10- Sultana, S., Ahmed, S., Jahangir, T. and Sharma, S. Cancer Lett., 221(2005).
11- Momin, R.A. and Nair, M.G. Phytomedicine. 9(2002).
12- Miean, K. H. and Mohammed, S. J. Agric. Food Chem., 49(2001).
13- Hamza, A. and Amin, A.. J. Exper. Zoology 307A(2007).
14- Kolarovic, J., Popovic, M., Mikov, M., Mitic, R. and Gvozdenovic, L. Molecules, 14(2009).
15- Patra, R.C., Swarup, D. and Dwivedi, S. Toxicol., 162(2001).
16- Reitman, S. and Frankel, S. Am. J. Clin. Pathol.28(1) (1957).
17- Kind, D. P. and King,E. G. Practical Clinical Biochemistry 1: H. Varley, AH Gownlock and M Bell, eds, 5thedition, William Heinmann Medical Books Ltd, London, 892(1954).
18- Szasz, G. Clin.Chem.,22(1969).
19- Malloy, H.T. and Evelyn, K. A. J. Biol. Chem. 2(1937).
20- Lowry,O.H., Rosebrough, N.J. , Farr, A.L. and Randall, R. J. J. Bio. Chem. 193(1951).
21- Doumas, B.T., Watson, W.A. and Biggs, H.G. Clin. Chem. Acta. 31(1977).
22- Yoshioka,T., Kawada, K., Shimada, T. and Mori, M. Am. J. Obstet. Gynecol.135 (1979).
23- Levine, R. L., Garland, D., Oliver,C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S. and Stadtman, E. R. Methods Enzymol. 186 (1990).
24- Minami, M. and Yoshikawa, H. Clinica Acta.; 92(1979).

25- Aebi,H. Methods Enzymol.; 105(1984).
26- Beutler, E., Duron, O. and Kelly, B.M. Lab. Clin. Med. 61(5) (1963).
27- El-Nahal, D. M. J. Am. Sci. 6 (12) (2010).
28- Abdel-Wahhab, M.A., Omara, E.A., Abdel-Galil, M.M., Hassan, N.S., Nada, S.A.,Saeed, A.and Elsayed, M.M. Afr. J. Tradit. Complement. Altern. Med. 4(3) (2007).
29- Shalana, M.G.; M.S. Mostafa; M.M. Hassouna; S.E. El-Nabi and A. El- Refaie Toxicology 206 (1) (2005).
30- Kilikdar, D., Mukherjee, D., Mitra, E., Ghosh, K. A., Basu, A., Chandra, A. M. and Bandyoapdhyay, D. Indian J. Exp. Bio.,49 (2011).
31- Dunsford, H. A., Karnasuta, C., Hunt, J. M.and Sell, S. Cancer Research, 49(1989).
32- Ha, W. S., Kim, C. K., Song, S. H., Kang, C. B. J. Vet. Sci., 2(2001).
33- Kanchana, N. and Sadiq, A. M. Int. J. Pharm.Sci. 3(1) (2011).
34- Adeyemi, O., Ajayi, J.O., Olajuyin, A.M., Oloyede, O.B., Oladiji, A.T., Oluba, O.M., Ololade, I.A. and Adebayo, E.A.. Food and Chemical Toxicology 47(2009).
35- Hagymàsi, K., Kocsis, I., Lengyel, G., Sipos, P., Fehér, J. and Blàzovics, A. Acta. Biol. Szeged. 47 (1–4) (2003).
36- Swarup, D., Ram, V., Naresh, P., Varshney, M., Balagangatharathilagar, P., Kumar, D., Nandi, D. and Patra, R.C. Res. Vet. Sci., 82(2007).
37- Abdel-Wahhab, M.A., Abdel-Azim, S.H. and. El-Nekeety, A.A Toxicon. 52 (4) (2008).
38- Seddik, L., Bah, T.M., Aoues, A., Brnderdour, M. and Silmani, M. Eur. J. Sci. Res., 42(1) (2010).
39- Noriega, G.O., Tomaro, M.L. and Battle, A. M. Biochim. Biophys. Acta. 1638(2) (2003).
40- Abdel-Wahhab, M.A. and Aly, S.E. J. Appl. Toxicol. 25(3) (2005).
41- Shabani, A. and Rabbani, A. Toxicology, 149(2-3) (2000).
42- Sailaja, R.O.H. and Setty, O.H. *J. Ethnopharmacol*, 105 **(**2006).
43- Bolin, C.M., Basha, R., Cox, D., Zawia, N.H., Maloney, B., Lahiri, D.K. and Cardozo-Pelaez, F. Toxicology, 223(2006).
44- Bhosle, S. M., Huilgol, N. G. and Mishra, K.P. Clinica. Chimica. Acta. 359, (1-2) (2005).
45- El-Gawish, M. A., Ebrahim, A. T. and Eid F. A. Egypt. J. Hospital Med., 23(2006).
46- Kafafy, Y. Egypt. J. Rad. Sci. Applic., 13 (2) (2000).
47- Roushdy, H.M., Ibrahim, H.A. and Edress, G.M.F. J. Agric. Food Chem., 14(1984).
48- Abbady, M.M. Ph.D. Thesis, Fac. of Science, Ain Shams Univ. (1994).
49- Bhatia, A.L., Manda, K., Environ. Toxicol. Pharmacol., 18 (2004).
50- Sener, G., Jahovic, N., Tosun, O., Atasoy, B.M. and Yegen, B. Life Sci., 74(2003).
51- Cadet, J., Bellon, S., Douki, T., Frelon, S., Gasparutto, D., Muller, E., Pouget, J.P., Ravanat, J.L., Romieu, A. and Sauvaigo, S. J. Environ. Pathol. Toxicol. Oncol., 23 (1) (2004) .
52- Petersen, D.R. and Doorn, J. A. Free Radical Biol. Med., 37 (7) (2004).
53- Dean, R.T., Fu, S., Stocker, R. and Davies, M.J., J. Biochem., 324 (1997).
54- Blech, D. M. and Borders,C.L. Arch. Biochem. Biophys.;224 (1983).
55- Jagetia, G.C. and Reddy, T. K. Life Sci.; 77 (2005).
56- Soares, J.R., Dinis, T.C.P., Cunha, A.P. and Almeida, L.M. Free Radical Res.; 26 (1997).
57- Ahmed, B., Alam, T., Varshnev, M. and Khan, S. A. J. Ethnopharmacol.; 79 (2002).
58- Popovic, M., Kaurinovic, B., Trivic, S., Mimica-Dukic, N. and Bursac, M. Phytother. Res., 20 (2006).
59- Lugasi, A., Hovari, J. Sagi, K. V. and Biro, L. Acta. Biol. Szegediensis.; 47 (2003).
60- López-Lázaro, M. Mini Rev. Med. Chem.; 9 (2009).
61- Manju, V. and Nalini, N. Ital. J. Biochem.; 54 (2005).
62- Jain,G. C., Pareek, H., Khajja, B. S., Jain, K., J. halani, S., Agarwal, S. and Sharma, S. African J. Biochem.Res.; 3 (5) (2009).