Assessment of Antioxidant Changes of Aged Rats Treated With Sumac Extract

M. M. Abbass[1], A. H. Mahmoud[1], Mohamed M. A. Hussein[2] and Salah A. Gabr[1]

Biological Applications Dept., Isotopes Applications Division, Nuclear Research Center, Atomic Energy Authority[1],
Biochemistry Dept., Faculty of Veterinary Medicine, Zagazig University[2]
manalmounir71@yahoo.com

Abstract: It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of free radicals. Many synthetic drugs are said to protect from oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicine. Sumac extract is a widely used herbal plant that affects the biological activities. The present study has focused on the potential role of sumac extracts on age- related changes in senile rats. Thirty male albino rats were used for this purpose. They were divided into three groups (10 animals in each), 1st group (6 months old) served as adult or control group, 2nd group (24 months old) as senile group, 3rd group (22months old as senile rats received 200mg / kg b.w. of sumac extract orally three times a week for two months) as treated group. Blood glutathione (GSH), catalase (CAT), malondialdehyde (MDA), hematological parameters (Hb, RBCs, WBCs and hematocrit value), some liver enzymes (AST and ALT), total protein, albumin, globulins and hormones (testosterone, triiodothyronine (T_3) and thyroxine (T_4) were measured. The sumac extract induced a significant increase in GSH concentration and CAT activity and decrease in the concentration of MDA of senile rats. The plant extract, raised the Hb concentration, RBCs count and hematocrit value. Also, the level of total protein and albumin in treated rats as compared with senile rats was significantly elevated. However, it made no significant change in ALT, AST, T3 and T4. Meanwhile, testosterone level increased significantly in treated rats in comparison with senile rats. These findings demonstrated that sumac extract had a positive effect on antioxidant status through minimize free radical-induced damage which is a main cause of aging.

[M. M. Abbass, A. H. Mahmoud, Mohamed M. A. Hussein, and Salah A. Gabr. **Assessment of Antioxidant Changes of Aged Rats Treated With Sumac Extract.** *Journal of American Science*. 2012; 8(4): 553-558]. (ISSN: 1545-1003). http://www.americanscience.org. 73

Keywords: Sumac extract, antioxidant status, hematological parameters, senile rats.

1. Introduction

Medicinal plants can be promising sources of natural products with beneficial activity **(Mothana *et al*., 2009; Kroliczewska *et al*., 2011).** Sumac (*Rhus coriaria* L., family Anacardiaceae) is the common name for genus Rhus that contains over 250 individual species **(Rayne and Mazza, 2007).** It is widely used as a spice **(Chakraborty *et al*., 2009)** , also as a herbal remedy in traditional medicine owing to its antifibrogenic, antifungal, anti-inflammatory, antimalarial, antimicrobial, antimutagenic, antioxidant, antithrombin, antitumorigenic, antiviral, cytotoxic hypoglycaemic, leukopaenic and atheroprotective effects**(Rayne and Mazza, 2007)**. Sumac is known to be a rich source of tannins **(Zargham and Zargham, 2008).** *In vitro* and *in vivo* studies have shown that tannins have anticarcinogenic effect **(Ram *et al*., 1997).**

Sumac extract was reported to be a source of natural antioxidants **(Ozcan, 2003).** The methanolic extracts of Rhus showed a remarkable radical scavenging effect even at low concentrations **(Mothana *et al*., 2009)**. A crude extract of *R. coriaria* exhibits interesting antioxidant properties, expressed by the capacity to either scavenge superoxide radical or uncompetitively inhibit xanthine oxidase **(candan, 2003)**. One of the main constituents of sumac is gallic acid **(Rayne and Mazza, 2007)**, which possesses potent antioxidant properties **(Rozek *et al*., 2007).**

Aging process is accompanied by progressing modifications of most physiological functions and the cells exhibit lower abilities to react with exogenic factors and to stress formation **(Jurivich *et al*., 1997).** Numerous studies have reported correlations between age and the accumulation of oxidative damage in cellular macromolecules and this damage could result from a multitude of factors including a decrease in antioxidant defenses **(Wolford *et al*., 1987; stadman, 2001; Posadas *et al*., 2009).**

Physiological aging affects all normal cells, tissues and organ systems as hematological parameters are highly differentiated **(Videan *et al*., 2008).** Fibrosis of various organs, including heart, Kidney and Liver is a hallmark of aging **(Sum *et al*., 1998).** Moreover, the function of many endocrine glands alters by aging **(Correa *et al*., 2001).** In relation to these findings an extensive range of antioxidants, whether synthetic or natural have been presented for the treatment or prophylaxis of disorders attributed to free radical oxidative damages **(Maxwell, 1995; Halliwell, 2000).**

Restriction on the use of synthetic antioxidants due to their probable side effects has increased the contribution of natural antioxidants **(Velioglu *et al*., 1998).** Based on this knowledge, the aim of the present work is to determine the effect of sumac extract (*Rhus coriaria*) on biochemical and hematological parameters of aged rats.

2. Material and Methods

Sumac extract via supercritical fluid extraction

Supercritical fluid extraction was performed at central laboratories for Radioisotopes Applications Division, Nuclear Research center, Egyptian Atomic Energy Authority on an ISCO supercritical fluid extractor, model SFX 220 equipped with two ISCO 260 D syringe pumps (lincoin, NE, USA).The pressure and temperature were 5000 psi and 80°C respectively. Every 2 gm of plant material was loaded into an extraction cell. The remaining volume was filled with glass wool. The flow rate through the extraction vessel was kept at 1.0 ml/ min during the extraction time (30 minutes). All sumac extracts was collected in 20 ml vials containing 5 ml methanol **(Fadel *et al*., 1999).**

Experimental design:

Thirty male (160-220gm) albino rats (bred under specific pathogen- free conditions at the animal Farm of Faculty of Veterinary Medicine, Zagazig University, Egypt) with light/dark cycle:12hrs and transferred to the Animal House of the Nuclear Research Center in Anshas Area. They were kept in metal cages during the whole experiment period under hygienic condition, fed on balanced ration and provided with water *ad-libtum* through the experiment.

The rats were divided into three equal groups of 10 each; 1st group (6 months old rats) as adult or control group, 2nd group (24 months old rats) as senile group and 3rd group (22 months old) as senescent rats are received 200 mg/kg b. w. of sumac extract according to **Mohammadi *et al*. (2010)** three times a week for 2 months through oral gavage, served as treated group.

At the end of the experimental period, the blood was collected and divided into two parts. The first, on EDTA anticoagulant while the second without anticoagulant to separate serum and centrifuged at 4000rpm and kept at -20°C till used for biochemical analysis.

Heamatological parameters; Hemoglobin content (Hb), erythrocytic count (RBCs), leucocytic count (WBCs) and hematocrit were determined using Sysmex (KX-21)–cell counter, with a Kit manufactured by (Diamond, Philadelphia, USA). On the other hand, (MDA) was measured spectrophotometrically by the method described by **Draper and Hadley (1980)**. CAT activity and GSH concentration were determined according to **Aebi (1984) and Beutler *et al.* (1963)** respectively.

The serum was used for biochemical analysis, the concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured according to **Reitman and Frankel (1957)**, total protein, albumin were determined according to **Doumas *et al.* (1981)** and **Drupt (1974)** respectively while plasma globulins were calculated by subtracting the albumin obtained from total protein. All of these parameters were performed on the Hitachi 902 biochemical analyzer.

Testosterone, triiodthyronine (T_3) and thyroxine (T_4) was estimated by radioimmunoassay techniques (RIA) using commercial kits purchased from Siemens Medical Solutions Diagnostics, Los Anglos, USA.

Statistical analysis:

Data were expressed as mean ± standard error of values. Comparison across groups were performed by Anova (one-way classification F- test), following by Duncan's multiple range test according to **Lind and Masson (1996)**. Statistical analyses of data were done using the spss (Statistical package for social sciences) for windows.

3. Results:

The results are presented in table (1) showed significant decrease ($p \leq 0.05$) in GSH concentration and CAT activity in senile rats (2nd group) as compared with adult rats (1st control group). Meanwhile, MDA was significantly increased in senile rats.

Treating the senile rats with sumac extract for 2 months lessened the alteration happened in the antioxidant status of aging rats. The results were optimistic since it showed a significant increase ($p \leq 0.05$) in GSH concentration and CAT activity and decline in the concentration of MDA in treated group (3rd group) as compared with senile rats (2nd group).

The hematological parameters of the experimental groups were studied and represented in table (2). In the senile rats (2nd group), hemoglobin content, RBCs count and hematocrit were significantly decreased ($p \leq 0.05$) in comparison with adult rats (1st control group). After oral administration of sumac extract to senile rats (treated or 3rd group), Hb concentration, RBCs count and hematocrit were significantly increased ($p \leq 0.05$) as compared with senile rats (2nd group).

Biochemical parameters of rats are presented in Table (3). Results showed no changes in AST and ALT activities between senile rats (2nd group) and adult rats (1st control group). Meanwhile, Total protein, Albumin and globulins decreased in senile rats.

After treatment with sumac, statistical evaluation showed significant increase ($p \leq 0.05$) in total protein, albumin (3rd group) and restored nearly to their levels in adult rats (1st control group).

The pessimistic effect of aging was extended to some endocrine gland functions represented here in T3, T4 and testosterone hormones that demonstrated a significant decrease ($p \leq 0.05$) in senile rats (2^{nd} group) as compared with adult rats (1^{st} or control group) as shown in table (4).

Sumac administration had slight or no effect on T3 and T4 levels. Meanwhile, testosterone level increased significantly ($p \leq 0.05$) in treated rats (3^{rd} group) in comparison with senile rats (2^{nd} group).

Table (1). Blood glutathione (GSH) concentration, catalase activity (CAT) and malondialdehyde (MDA) level in experimental groups (means±SE).

Groups / Parameters	Group 1	Group 2	Group 3
GSH (mg/dl)	74.3±7.2[a]	52.3±6.4[b]	72.8±6.7[a]
CAT (U/ml)	30.7±2.8[a]	20.2±3.1[b]	29.5±2.6[a]
MDA (µmol/L)	67.5±7.3[c]	95.7±8.5[a]	79.4±7.6[b]

Means within the same row have different superscripts are significantly different at ($p \leq 0.05$).

Table (2). Hematological parameters in experimental groups (means±SE).

Groups / Parameters	Group 1	Group 2	Group 3
Hb (g/dl)	12.9±0.6[a]	10.3±0.4[b]	11.8±0.5[a]
RBCs (N x 10^6/ µl)	7.20±0.3[a]	5.5±0.2[b]	6.7±0.2[a]
WBCs (N x 10^3/ µl)	9.8±0.4[a]	9.5±0.3[a]	9.7±0.4[a]
Hematocrit (%)	37.6±0.3[a]	33.4±0.4[c]	34.8±0.3[b]

Means within the same row have different superscripts are significantly different at ($p \leq 0.05$).

Table (3). Some Liver enzymes (AST, ALT), total protein, albumin and globulins in experimental groups (means±SE).

Groups / Parameters	Group 1	Group 2	Group 3
AST (U/L)	194.3±13.5[a]	210.6±12.8[a]	202.5±13.6[a]
ALT (U/L)	79.2±6.7[a]	85.1±5.9[a]	71.5±5.3[a]
Total protein (g/dl)	6.9±0.32[a]	5.8±2.4[b]	6.4±0.41[a]
Albumin (g/dl)	4.1±0.10[a]	3.3±0.20[b]	3.9±0.10[a]
Globulins (g/dl)	2.8±0.09[a]	2.5±0.04[b]	2.5±0.06[b]

Means within the same row have different superscripts are significantly different at ($p \leq 0.05$).

Table (4). Testosterone, triiodothyronine and thyroxine in experimental groups (means±SE).

Groups / Parameters	Group 1	Group 2	Group 3
Testostrone (ng/ml)	2.5±0.13[a]	1.8±0.09[c]	2.1±0.08[b]
T_3 (ng/ml)	2.4±0.13[a]	1.6±0.08[b]	1.9±0.11[b]
T_4 (µg/dl)	6.5±0.31[a]	3.6±0.32[b]	3.5±0.24[b]

Means within the same row have different superscripts are significantly different at ($p \leq 0.05$).

4. Discussion

A central tent of green chemistry is the ability to obtain a commercially viable product with desirable properties from a widely renewable feed stock **(Rayne and Mazza, 2007)**. In particular there is significant interest in obtaining extracts with particular biological activities from plants.

Sumac is a well known, popular spice and has been utilized extensively for medicinal and other purposes. Phytochemical studies of this plant reported that its leaves contained phenolic acids **(Mavlyanov *et al.*, 1995)**, anthocyanins, hydrolysable tannis and gallic acid, condesed tannis and several flavonoids **(Mavlyanov *et al.*, 1995; Zalacain *et al.*, 2003)**. These substances have gained interest because may reduce the risk of chronic diseases, reinforcing the defences against free radical species.

Aging is characterized by declining ability of the individual to adapt environmental stress. It is a physiological process involving many biological systems.

The role of antioxidant in aging is of critical importance as normal aging is accompanied by a decline in antioxidant defenses reflected in GSH in blood and different tissues of animals and humans **(Muthuswamy *et al.*, 2006)**. In this study, these findings were confirmed since GSH level and catalase activity were significantly decreased in senile rats (2^{nd} group) as compared to their levels in adult rats (1^{st}group or control) Table (1). This decrease may be due to oxidation of sulfhydryl group and diminished level of GSH **(Sarker *et al.*, 1998)**. Moreover, there is a gradual increase in the level of MDA in senile rats as compared with control group. This increase may be also referred to the failure of antioxidant system to combat oxygen radicals and/or the positive relation between age and oxidative stress. As the increase in age related oxidative damage could result from a multitude of factors including imbalance between antioxidant defenses and oxidative stress **(Posadas *et al.*, 2009).** Based on the experimental data in this study, the oral administration of sumac extract for two months resulted in a significant increase in the level of

GSH and CAT activity while MDA level was decreased in treated group as compared with senile rats. This result may be due to the presence of various substances mainly tannin and gallic acids that may be responsible for the antioxidant ability of this plant. It was reported that the antioxidant activity of gallotannins from *R. coriaria* extract was stronger than that of gallic acid **(Beretta *et al*., 2009).** Tannic acid extracted from *Rhus chinensis* Mill could effectively scavenge the O^{2-} **(Fu, 1991).** Glycoprotein isolated from *R. verniciflua* Stokes fruit improved the antioxidant levels in mice **(Oh *et al*., 2006).**

The values of hematological parameters are affected by a number of factors even in apparently healthy populations. These factors include age, sex, nutritional and environmental factors. The present study showed that, the senile rats (2^{nd} group) had hematological disorders manifested by drop in hemoglobin content, RBCs count and hematocrit value as compared with their values in control group (1^{st} group). This is consistent with data from **Videan *et al*. (2008)** who found a significant age related increase in anemia risk, based on significant decreases in hemoglobin and hematocrit. Also, **Wolford *et al. (1987)*** reported that hematological parameters changes with aging. These hematological changes are reflected by a decline in marrow cellularity, increased risk of myeloproliferative diseases and a decline in adaptive immunity **(Prabhakar, 2009).**

Our results reported that the sumac extract induced an increase in Hb content, RBCs and hematocrit of treated group as compared with the senile rats (Table 2).

The present study demonstrated insignificant increase in some liver enzyme activities (AST, ALT) in senile rats as compared with control (Table 3). Meanwhile, there are decreases in their values after oral administration of sumac extract but they didn´t reach to the normal range.

Albumins represent a very abundant and important circulating antioxidant **(Roche *et al*., 2008).** Study of **Bourdon *et al*. (1999)** confirmed and extended the idea that serum albumin is an important protein that presents direct protective effects.

Total protein, albumin and globulin levels are performed to evaluate the toxicological nature of various chemicals. Data from the present study, showed a significant decrease in the level of albumin, globulins and total protein in serum of aged rats (2^{nd} or senile group) as compared with adult rats (1^{st} or control group) as a result of oxidative stress associated with aging which reduced liver functions and decreased adaptive immunity **(Prabhakar, 2009).** Several lines of evidence strongly suggest that a reduced serum albumin concentration, although within the normal range, is associated with mortality risk **(Bourdon *et al*., 1999).**

Administrating aged rats with sumac extracts caused a significant increase in total protein and albumin levels compared with senile rats. (Table 3). Aging is indication of compromised liver excretory function and impairment of the liver synthetic function, which improved by treating with sumac extracts causing improvement in total protein, albumin and globulin levels.

According to the accumulated data, during aging thyroid function decreases progressively and it is well recognized that the symptoms of aging can easily be confused with those of hypothyroidism **(Silvestri *et al*., 2008).** This data matched with our results which found a decrease in the concentrations of T3 and T4 hormones in senile rats as compared with control group (Table 4). In addition, a reduction of testosterone level was observed in senile rats. This is consistent with **(Vermeulen and Kaufman 1995; Zirkin and Chen, 2000)** who also reported similar finding. This could be due to the changes in the antioxidant environment of aging cells that have been shown to be correlated with the reduced ability of these cells to produce testosterone **(Chen *et al*., 2007)**. It was also observed an increasing in the level of testosterone of senile rats after their treatment with sumac extract.

The improvement recorded in the studied parameters after oral administration of sumac extract could be attributed to the presence of a relatively high percentage of phenolic compounds with high antioxidant activity **(Nokatani, 2000)**. Epidemiological studies have strongly suggested that consumption of certain plant materials may reduce the risk of chronic diseases related to oxidative stress on account of their antioxidant activity and promote general health benefit **(Halliwell, 1997).**

5. Conclusion

In conclusion, the importance of this study is to give new information on the application of sumac extract in reducing physiological changes related to aging. Our data show a positive effect of sumac extract on antioxidant status. These findings suggest that sumac extract, reduce the risk of increasing anemia in aged rats and protect the liver from damage associated with aging. However, further studies are required to better understand the full potential of the Rhus genus as part of global green technology based on bioproducts and bioprocesses research program.

Corresponding author
M. M. Abbass
Biological Applications Dept., Isotopes Applications Division, Nuclear Research Center, Atomic Energy Authority
manalmounir71@yahoo.com

References

1. Aebi, H. (1984): Catalase *in vitro*. Methods enzymol., 105:121-126.
2. Beutler, E.; Duron, O. and Kelly, B.M. (1963): Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61:882-888.
3. Beretta, G.; Rossoni, G.; Santagati, N. A.; Facino, R. M. (2009): Anti-ischemic activity and endothelium-dependent vaso-relaxant effect of hydrolysable tannins from the leaves of *Rhus coriaria* (Sumac) in isolated rabbit heart and thoracic aorta. Planta Medica, 75: 1482–1488.
4. Bourdon, E.; Loreau, N.; Blache, D. (1999): Glucose and free radicals impair the antioxidant properties of serum albumin. FASEB Journal, 13: 233–244.
5. Candan, F. (2003): Effect of *Rhus coriaria* L. (Anacardiaceae) on superoxide radical scavenging and xanthine oxidase activity. Journal of Enzyme Inhibition and Medicinal Chemistry, 18: 59–62.
6. Chakraborty, A.; Ferk, F.; Simic´ , T.; Brantner, A.; Dusinska, M.; Kundi, M.; Hoelzl, C.; Nersesyan, A. and Knasmu¨ ller, S. (2009): DNA-protective effects of sumach (*Rhus coriaria* L.), a common spice: results of human and animal studies. Mutation Research, 661: 10–17.
7. Chen, H. Pechenino, A.S.; Liu, J. ; Beattie, M.C.; Brown, T.R. and Zirkin, B.R. (2007): Effect of glutathione depletion on leydig cell steriodogenesis in young and old brown Norway rats. Endocrinology, 149 (5): 2612-2619.
8. Correa de Costa, V.M.; Morreira, D.G. and Rosenthal, D. (2001): Thyroid function and aging: Gender- related differences. J. Endocrinol., 171: 193-198.
9. Doumas, B.T.; Baysa, D.D.; Carter, R.J.; Peters, T. and Schaffer, R. (1981): Determination of serum total protein. Clin. Chem., 27:1642
10. Draper, H. and Hadley, M. (1980): Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 186:421-31.
11. Drupt, F. (1974): Colorimeteric method for Determination of serum albumin. Pharm. Bio. Sciences, 9:777
12. Fadel, H.; Marx, F.; El-Sawy, A. and El-Ghorab,A. (1999): Effect of extraction techniques on the chemical composition and antioxidant activity of Eucalyptus *camaldulensis* var. *brevirostris* leaf oils. Z Lebensm Unters Fordch A. 208 : 212-216.
13. Fu, N. (1991): Protective effect of tannic acid against TPA- and cigarette smoke condensate-induced DNA strand breaks in human white cells and its antioxidant action. Zhongguo Yi Xue Ke Xue Yuan Xue Bao, 13: 347-352.
14. Halliwell, B. (1997): Antioxidants and human disease: a general introduction. Nutr. Reviews, 55: 544-552.
15. Halliwell, B. (2000): The antioxidant paradox. Lancet, 355: 1179-80.
16. Jurivich, D.A.; Qui, L. and Welk, J. F. (1997): Attenuated stress responses in young and old human lymphocytes. Mech. Aging Develop., 94: 233-249.
17. Kroliczewska, B.; Mis´ta, D.; Zawadzki, W.; Wypchlo, A. and Kro´ liczewski, J. (2011): Effects of a skullcap root supplement on haematology, serum parameters and antioxidant enzymes in rabbits on a high-cholesterol diet. Journal of Animal Physiology and Animal Nutrition, 95: 114–124.
18. Lind, D.A. and Masson, R.D. (1996): Statistical Techniques in Busines and Economics. 9th ed. Irwin McGraw- Hill, USA , P. 433.
19. Mavlyanov, M.; Islambekov, Sh.Yu.; Ismailov, A.I. and Kamaev, F.G. (1995): Phenolic compounds of some tannide-bearing plants and their pharmacological activity. Chemistry of Natural Compounds, 31: 268.
20. Mavlyanov, M.; Islambekov, Sh.Yu.; Ismailov, A.I.; Karimdzhanou, A.K. and Ismalov, A.I. (1997): Anthocyanins and organic acids of the fruits of some species of sumac. Chemistry of Natural Compounds, 33: 209.
21. Maxwell, S.R.J. (1995): Prospects for the use of antioxidant therapies. Drugs, 49: 345-61.
22. Mothana, R. A.; Gruenert, R.; Bednarski, P. J. and Lindequist, U. (2009): Evaluation of the *in vitro* anticancer, antimicrobial and antioxidant activities of some Yemeni plants used in folk medicine. Die Pharmazie, 64: 260–268.
23. Mothana, R. A.; Lindequist, U.; Gruenert, R. and Bednarski, P. J. (2009): Studies of the *in vitro* anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra. BMC Complementary and Alternative Medicine 9:7-17.
24. Mohammadi, S.; Montasser Kouchari,S. and Monavar Feshnai, A. (2010): Antidiabetic properties of the ethanolic extract of *Rhus coriaria* fruits in rats. DARU Journal of Pharmaceutical Sciences**,** 18 (4):270-275.
25. Muthuswamy, A.D.; Vedagiri, K.; Ganeson, M. and Chinnakannu, P. (2006): Oxidative stress – mediated macromolecular damage and dwindle in antioxidant status in aged rats brain regions : role of L- carnitine and DL- alpha- lipoic acid. Clin. Chem. Acta., 368: 84-92.
26. Nakatani, N. (2000): Phenolic antioxidants from herbs and spices. Biofactors, 13(1-4): 141-146.
27. Oh, P. S.; Lee, S. J.; Lim, K. T. (2006): Hypolipidemic and antioxidative effects of the

plant glycoprotein (36kDa) from Rhus verniciflua stokes fruit in Triton WR-1339- induced hyperlipidemic mice. Bioscience, Biotechnology and Biochemistry, 70: 447–456.
28. Ozcan, M. (2003): Antioxidant activities of rosemary, sage, and sumac extracts and their combinations on stability of natural peanut oil. Journal of Medicine Food, 6: 267–270.
29. Posadas, S.J. ; Caz, V.; Largo, C.; Dela Gandara, B.; Matallanas, B.; Reglero, G. and De Miguel, E. (2009): Protective effect of supercritical fluid rosemary extract, rosmarinus officials on antioxidants of major organs of aged rats. Experimental Gerontology, 44: 383-389.
30. Prabhakar, M.; Erishier, W.B. and Longo, D.L. (2009): Bone marrow, thrymus and blood: changes across the lifespan. Aging Health, 5 (3): 385-393.
31. Ram, A.; Lauria, P.; Gupta, R.; Kumar, P. and Sharma, V. N. (1997): Hypocholesterolaemic effects of *Terminalia arjuna* tree bark. Journal of Ethnopharmacology, 55: 165–169.
32. Rayne, S. and Mazza, G. (2007): Biological activities of extracts from sumac (Rhus spp.): a review. Plant Foods for Human Nutrition, 62: 165–175.
33. Reitman, S. and Frankel, S. (1957): A colorimetric method for determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. Am. J. Clin. Pathol., 25:56
34. Roche, M.; Rondeau, P.; Singh, N. R.; Tarnus, E.; Bourdon, E. (2008): The antioxidant properties of serum albumin. FEBS Letters, 582: 1783–1787.
35. Rozek, A.; Achaerandio, I.; Almajano, M. P.; Guell, C.; Lopez, F. and Ferrando, M. (2007): Solid foodstuff supplemented with phenolics from grape: antioxidant properties and correlation with phenolic profiles. Journal of Agricultural and Food Chemistry, 55: 5147–5155.
36. Sarker, S.; Yadav, P. and Bhatnagar, D. (1998): Effect of cadmium on glutathione metabolism and glucose 6-phosphate dehydrogenase in rat tissues: role of vitamin E and selenium. Trace Elem. Electro., 15(2): 101-105.
37. Silvestri, E.; Lombardi, A.; Lange, P.; Schiavo, L.; Lanni, A.: Goglia, F.; Visser, T.J. and Moreno, M. (2008): Age-related changes in renal and hepatic cellular mechanisms associated with variations in rats serum thyroid hormone levels. Am. J. Physiol. Endocrinol. Metab., 294:1160-1168.
38. Stadman, E. R. (2001): Protein oxidation in aging and age- related diseases. Ann. N.Y. Acad. Sci., 928: 22-38.
39. Sum, W.B.; Han, B.L.; Peng, Z.M.; Li, K.; Ji, Q.; Chen, J.; Wang, H.Z. and Ma, R.L. (1998): Effect of aging on cytoskeleton system of kupffer cell and its phagocytic capacity. World J. Gastroenterol., 4:77-79.
40. Velioglu, Y.S.; Mazza, G.; Gao, L. and Oomah, B.D. (1998): Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem., 46: 4113-17
41. Vermeulen, A. and Kaufman, J. M. (1995): Ageing of the hypothalamo- pituitary- testicular axis in men. Horm. Res., 43: 25-38.
42. Videan, E.N.; Fritz, J. and Murphy, J. (2008): Effect of aging on hematology and serum clinical chemistry in chimpanzees (pan trologodytes). Am. J. Primatal. 70 (4): 327-338.
43. Wolford, D.A.; Schroer, R.A. Gallo, P.P; Gohs, F.X.; Brodeck, M. ; Folk H.B. and Ruhren, R. (1987): Age – related changes in serum chemistry and hematology values in normal Sprague- dawley rats. Toxicol. Sci., 8 (1):80-88.
44. Zalacain, A.; Prodamov, M.; Carmona, M. and Alonso, G.L. (2003): Screening method for the detection of artificial colours in saffron using derivative UV-Vis spectrometry after precipitation of crocetin; Biosystem Eng., 84(2): 211.
45. Zargham, H. and Zargham, R. (2008): Tannin extracted from Sumac inhibits vascular smooth muscle cell migration. McGill Journal of Medicine, 11: 119–123.
46. Zirkin, B. R. and Chen, H. (2000): Regulation of leyding cell- steroidogenic function during aging .Biol. Reprod., 63:977-981.