

Possible Protective Effect of Kombucha Tea Ferment on Cadmium Chloride Induced Liver and Kidney Damage in Irradiated Rats

Nashwa Kamel Ibrahim

Abstract—Kombucha Tea Ferment (KT), was given to male albino rats, (1ml/Kg of body weight), via gavages, during 2 weeks before intraperitoneal administration of 3.5 mg/Kg body weight CdCl₂ and/or whole body γ -irradiation with 4Gy, and during 4 weeks after each treatment. Hepatic and nephritic pathological changes included significant increases of serum alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities, and creatinine and urea contents with significant decrease in serum total antioxidant capacity (TAC). Increase in oxidative stress markers in liver and kidney tissues expressed by significant increase in malondialdehyde (MDA) and nitric oxide (NO) contents associated to significant depletion in superoxide dismutase (SOD) and catalase (CAT) activities, and reduced glutathione (GSH) content were recorded. KT administration results in recovery of all the pathological changes. It could be concluded that KT might protect liver and kidney from oxidative damage induced by exposure to cadmium and/ or γ -irradiation.

Keywords—Cadmium, Kombucha, radiation, rats

I. INTRODUCTION

IONIZING radiation has always been a part of the human environment. Along with natural radioactive sources present in the earth crust and cosmic radiation, exposure to large doses resulting from accidental exposures or from special medical procedures (radiation therapy) also contribute to our continuous exposure to ionizing radiation. Radiation damage, is to a large extent, caused by the overproduction of reactive oxygen species (ROS), including superoxide anion (O₂⁻), hydroxyl radical ([•]OH), and hydrogen peroxide (H₂O₂), that overwhelm the levels of antioxidants, resulting in oxidative stress and cellular damage. ROS cause damage by reacting with cellular macromolecules such as nucleotides in nucleic acids, polyunsaturated fatty acids found in cellular membranes, and sulfhydryl bonds in proteins. If this damage is irreparable, then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur [1]. Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. Among the enzymes involved in antioxidative defense, particularly well documented are the antioxidant properties of the superoxide dismutases (SOD), glutathione

peroxidases (GSH-Px), and catalase (CAT). SOD catalyzes the reduction of O₂⁻ to H₂O₂. The majority of H₂O₂ is broken down to oxygen (O₂) and water (H₂O) by CAT. In addition to CAT, GSH-Px can also break down H₂O₂ and also any peroxides that form on lipids within the body [2]. The activity of GSH-Px depends on the presence of adequate amounts of reduced glutathione (GSH).

Humans are subject to exposure to cadmium pollution through contaminated air, food, water, manufactured goods and occupational hazards. Relatively large quantities are found in commercial phosphate fertilizer, thus the increases in soil and plant cadmium contents may lead to increases in dietary cadmium [3, 4]. Cigarettes made from tobacco grown in soil containing cadmium are another major source of cadmium intoxication [5]. Cadmium stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals [6]. Once absorbed, cadmium is rapidly cleared from the blood and concentrates in various tissues mainly in the liver and kidneys causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis [6, 7, 8].

Kombucha Tea Ferment (KT) is a sour beverage prepared from the fermentation of black tea and sugar with a symbiotic culture of acetic acid bacteria and yeasts such as *Bacterium xylinum*, *Bacterium xylinoides*, *Bacterium gluconicum*, *Saccharomyces ludwigii*, *Saccharomyces apiculatus varieties*, *Schizosaccharomyces pombe*, *Acetobacter ketogenum*, *Torula varieties*, *Pichia fermentans* reported to have potential health effects [9]. Fermentation and oxidation processes of Kombucha microorganisms produce a wide range of organic acids, vitamins and enzymes. Research indicated that KT improves resistance against cancer, prevents cardiovascular diseases, promotes digestion, stimulates immunity and reduces inflammation [10]. Researchers also indicate that KT possesses potent anti-stress, hepato-protective, antioxidant and immune- modulating properties [11]. The mechanisms by which KT ferment exerts its protective effect are unclear. Also little is known about the antioxidant activity [12] and the radio-protective properties of KT [13].

So it was of particular interest to investigate whether KT administration to rats would decrease the toxicity associated with oxidative stress and thereby reducing the damage induced by cadmium and/or γ -radiation exposure. Hepatic and nephritic toxicity was assessed by determining serum levels of alanine and aspartate transaminases (ALT, AST), alkaline phosphatase

(ALP) activities, creatinine, urea contents and serum total antioxidant capacity (TAC). Levels of liver and kidney antioxidants such as superoxide dismutase (SOD) and catalase (CAT) activities, and the content of reduced glutathione (GSH) besides oxidative markers malondialdehyde (MDA) and nitric oxide (NO) contents were measured.

II. MATERIAL AND METHODS

Preparation of Kombucha Tea ferment (KT)

For the preparation of the medium: One hundred grams (100 g) of sugar was added to one liter (1L) of distilled water, and the solution was boiled for 15 minutes in a sterile conical flask. Six tea bags of black tea powder (Lipton, Egypt) were added to the flask (12 g/L, 1.2%) and allowed to cool to room temperature for one hour. Kombucha culture was kept under aseptic conditions. Fermentation was carried out by incubating Kombucha culture in the prepared medium at $28 \pm 1^\circ\text{C}$ for 8-10 days. Then, the medium (brew) was centrifuged at 3000 rpm for 30 minutes aseptically and stored in polypropylene vials at -20°C for further use [12].

Cadmium treatment

Cadmium chloride (CdCl_2) was dissolved in saline solution. To induce cadmium toxicity, each animal received a single intraperitoneal dose of CdCl_2 (3.5mg/Kg of body weight) according to Tzirogiannis [14].

Radiation treatment

Irradiation was performed through the use of a Canadian Gamma Cell-40 (137Cs) at the National Center for Radiation Research and Technology, Cairo, Egypt. The dose rate of Gamma cell (0.5 Gy/minute) was calculated according to the Dosimetry Department in our Institute (NCRRT), where 0.5Gy is emitted from the Cesium source/ minute.

Experimental design

Male albino rats weighing (150-200g) purchased from the Egyptian Organization for Biological Product and Vaccines in Cairo, Egypt, were housed in cages under good ventilation and illumination condition and allowed balanced standard diet and water *ad-libitum*. Animals were divided into 8 groups of 8 rats each. 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).

TABLE I
ANIMAL GROUPS AND TREATMENTS

Groups	Treatments
Control	Rats didn't receive any treatment
KT	Rats supplemented with Kombucha Tea Ferment by gavages, at a dose of 1ml/Kg of body weight, daily, during 6 weeks.
CdCl_2	Rats injected with 3.5mg CdCl_2 /Kg of body weight via intraperitoneal administration (i.p.).
IRR	Rats whole body gamma irradiated with 4Gy administered as one shot dose.
KT + CdCl_2	Rats supplemented with Kombucha Tea Ferment during 2 weeks before CdCl_2 injection and 4 weeks

after injection.

KT + IRR Rats supplemented with Kombucha Tea Ferment during 2 weeks before irradiation and 4 weeks after irradiation.

CdCl_2 + IRR Rats injected with CdCl_2 and whole body gamma irradiated.

KT + CdCl_2 + IRR Rats supplemented with Kombucha Tea Ferment during 2 weeks before injection of CdCl_2 and whole body gamma irradiation and 4 weeks after irradiation.

Biochemical analysis

At the end of experimental period rats were sacrificed. Blood samples were collected in sterile heparinized tubes by heart puncture. Liver and kidneys were quickly removed.

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) USA. The kits used were purchased from Bio-Diagnostic

The quantitative determination of serum ALT and AST were done using the method of Reitman and Frankle [15]. ALP activity was estimated according to the method of Kind and King [16], TAC was determined using the total antioxidant capacity Kits [17]. Urea and creatinine concentrations were measured using the method of Halled and Cook [18], and Henery [19], respectively.

The liver and kidney were homogenate in saline solution. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for biochemical analysis. The extent of lipid peroxidation was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) according to Yoshioka [20]. Superoxide dismutase (SOD) and catalase (CAT) activities were determined according to Minami and Yoshikawa [21], and Aebi [22], respectively. The content of reduced glutathione (GSH) was determined according to Beutler [23]. Nitric oxide concentration was measured by the method of Geng [24].

Statistical analysis

The SPSS 11.0 statistical software package programmed for Windows was used for statistical calculations. Data were analyzed using one way analysis of variance (ANOVA). Post-hoc Duncan test was used to determine significant differences between means. Values were expressed as mean \pm SE. (n=8). Differences between means were considered significant at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Exposure of mammals to ionizing radiations, leads to the development of a complex, dose-dependent series of changes, including injury to different organs which cause changes in the structure and function of cellular components, resulting in tissue damage and death. Oxidative stress with subsequent production of reactive oxygen species (ROS) has been postulated as one of the mechanisms of radiation toxicity [25].

Transaminases play an important role in protein and amino acid metabolism. They are found in the cells of almost all body tissues and when diseases or injuries affected these tissues; they are released into blood stream. Also ALP is considered as an enzyme of hepatocytes plasma membrane, thus an increase

in serum ALP activity has been related to damage of the liver cell membranes [26].

In the present study, a significant increase ($P \leq 0.05$) in ALP, AST and ALT activities (Table 1), and creatinine and urea levels (Table 2), was recorded in the serum of irradiated rats which might result from radiation-induced cell membrane damage followed by the release of intracellular molecules to the blood stream.

In agreement with this postulation, a significant increase ($P \leq 0.05$) in serum TAC was recorded (Table 2). Moreover, a significant increase ($P \leq 0.05$) in the level of TBARS (Fig 1) and NO (Fig 2) associated with a significant decrease ($P \leq 0.05$) in the activity of SOD (Fig 3) and CAT (Fig 4), and in the content of GSH (Fig 5) was recorded in the liver and kidney of irradiated rats.

The elevated level of TBARS might probably result from the interaction of the excess of $\cdot\text{OH}$, resulting from the radiolysis of water upon exposure to ionizing radiation, with polyunsaturated fatty acids in the phospholipids portion of cellular membranes [1].

The significant decrease ($P \leq 0.05$) in the activity of SOD and CAT might be, also, attributed to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation [27]. The depletion in GSH may be due to its reaction with free radicals resulting in the formation of thiyl radicals that associate to produce oxidized glutathione (GSSG). GSH can, also, react with peroxynitrite anion (ONOO^-) to form S-nitrosoglutathione [28].

Exposure to cadmium induces oxidative stress by increasing lipid peroxidation [8, 29] and altering the antioxidant status [7], which results in a loss of membrane functions [30]. Once absorbed, cadmium is rapidly cleared from the blood and concentrates mainly in kidneys [31] and liver [32]. The cellular processes underlying cadmium nephrotoxicity are poorly understood: cadmium reacts with thiol groups and may substitute for zinc in critical metabolic processes [33], but it also causes DNA strand breaks, lipid peroxidation, and generation of oxidatively modified proteins [34, 35].

Cadmium, not being a Fenton metal, does not appear to generate free radicals by itself [34] but it has been shown to produce hydroxyl radicals in the presence of metallothioneins, containing Fenton metals [36]. This suggests that Cd-mediated production of ROS takes place as a consequence of Cd-induced displacement of endogenous redox active metals (Fe, Cu) and subsequent damage to critical organelles (e.g., mitochondria) or is due to a decrease of endogenous radical scavengers, such as glutathione and/or protein sulfhydryls [34].

Cadmium hepatotoxicity is probably affected in two ways: on the one hand by occurrence of inflammatory state, on the other hand-by direct toxic action of cadmium on liver cells [4].

In the present study, a significant increase ($P \leq 0.05$) in ALP, AST and ALT activities (Table I), and creatinine and urea levels (Table II), were recorded in the serum of cadmium treated rats, probably due to the leakage of these molecules from tissues to the blood stream [37].

TABLE I
EFFECT OF KOMBUCHA TEA FERMENT SUPPLEMENTATION ON SERUM ENZYMES ACTIVITIES IN RATS INJECTED CdCl_2 AND/OR γ -IRRADIATED

Animal Groups and Treatments	Aspartate amino transferase (U/L)	Alanine amino transferase (U/L)	Alkaline phosphatase (IU/L)
Control: didn't receive any treatment	30.65 ± 1.66	65.38 ± 2.25	115.51 ± 3.93
KT: Supplemented Kombucha Tea Ferment	31.60 ± 1.33	64.91 ± 2.52	113.16 ± 5.21
CdCl_2: Injected CdCl_2	59.60 $\pm 4.33^{ab}$	80.39 $\pm 2.62^{ab}$	136.27 $\pm 5.20^{ab}$
IRR: irradiated	45.27 ± 2.52	74.02 $\pm 1.80^{ab}$	127.35 ± 5.28
KT+CdCl_2: Supplemented Kombucha Tea Ferment and injected CdCl_2	43.00 $\pm 1.50^c$	67.40 $\pm 2.29^c$	121.20 ± 1.69
KT+IRR: Supplemented Kombucha Tea Ferment and irradiated	40.65 $\pm 2.76^{ab}$	72.33 $\pm 1.21^{ab}$	118.10 ± 1.73
CdCl_2+IRR: Injected CdCl_2 and irradiated	65.43 ± 1.22	88.17 $\pm 4.85^{abc}$	140.21 $\pm 3.34^{ab}$
KT+CdCl_2+IRR: Supplemented Kombucha Tea Ferment and injected CdCl_2 and irradiated	37.66 $\pm 3.45^{abcd}$	78.33 $\pm 4.42^{ab}$	122.17 $\pm 0.73^c$

Data are presented as mean value \pm Standard Error (n=8).

^a Significantly different from control. ^b Significantly different from KT

^c Significantly different from CdCl_2 ^d Significantly different from IRR

Antioxidant enzymes CAT, SOD and GPX form the first line of defense against ROS and the decrease in their activities contribute to the oxidative insult on the tissue. SOD detoxifies superoxide radicals and thus provides cytoprotection against free-radical-induced damage. Reports about SOD activity in Cd-treated rats are contradictory, some studies report an increase [38] and some others report a decrease [29] in activity. Researchers demonstrated, also, that SOD activity is strongly inhibited by cadmium, probably by interactivity with metal moieties of SOD (Cu, Zn or Mn) and thus reducing its activity [8]. Furthermore, cadmium interacts with enzyme, altering its functional activity [39].

In the present study, cadmium exposure induced a significant decrease ($P \leq 0.05$) in the activity of SOD (Fig 3) and CAT (Fig 4), and in the content of GSH (Fig 5) associated to with a significant increase ($P \leq 0.05$) in the level of TBARS (Fig 1) and NO (Fig 2) in the liver and kidney of rats. In the same line a significant decrease was recorded for serum total antioxidant capacity (TAC) (Table II).

TABLE II
EFFECT OF KOMBUCHA TEA FERMENT SUPPLEMENTATION ON SERUM CREATININE, UREA AND TOTAL ANTIOXIDANT CAPACITY IN RATS INJECTED CdCl_2 AND/OR γ -IRRADIATED

Animal Groups and Treatments	Creatinine (mg/dl)	Urea (mg/dl)	Total Antioxidant capacity (mmol/l)
Control: didn't receive any treatment	0.52 ± 0.06	30.00 ± 2.51	1.11 ± 0.05
KT: Supplemented Kombucha Tea Ferment	0.57 ± 0.06	27.87 ± 1.54	1.66 ± 0.02
CdCl_2: Injected CdCl_2	1.10	64.60	0.86

	$\pm 0.07^{ab}$	$\pm 0.07^{ab}$	$\pm 0.04^{ab}$
IRR: Irradiated	0.79	37.40	0.96
	$\pm 0.02^a$	$\pm 0.97^{abc}$	$\pm 0.23^{ab}$
KT+CdCl₂: Supplemented Kombucha Tea Ferment and injected CdCl ₂	0.90	56.23	1.04
	± 0.04	$\pm 1.46^{bc}$	$\pm 0.05^{bc}$
KT+IRR: Supplemented Kombucha Tea Ferment and irradiated	0.63	32.09	1.09
	$\pm 0.22^{ad}$	$\pm 0.41^{bd}$	$\pm 0.06^b$
CdCl₂+IRR: Injected CdCl ₂ and irradiated	0.94	59.96	0.55
	$\pm 0.35^{cd}$	$\pm 1.64^{abd}$	$\pm 0.03^{abcd}$
KT+CdCl₂+IRR: Supplemented Kombucha Tea Ferment and injected CdCl ₂ and irradiated	0.72	50.42	1.05
	$\pm 0.40^{abcd}$	$\pm 1.52^{abcd}$	$\pm 0.05^{abcd}$

Data are presented as mean value \pm Standard Error (n=8).

^a Significantly different from control. ^b Significantly different from KT

^c Significantly different from CdCl₂ ^d Significantly different from IRR

It is well documented that free radical scavengers and antioxidants are useful in protecting against oxidative stress toxicity [40]. In the present study, KT ferment administration has ameliorated the increase of ALT, AST and ALP activities as well as the concentration of creatinine and urea in the serum of cadmium treated rats, irradiated rats, as well as, rats subjected to both treatment. Furthermore, oral administration of KT ferment was able to improve the levels of endogenous antioxidants SOD, CAT and GSH in the liver and kidney and to decrease the level of TBARS and NO in liver and kidney tissues of Cd-treated, irradiated and Cd-treated irradiated rats.

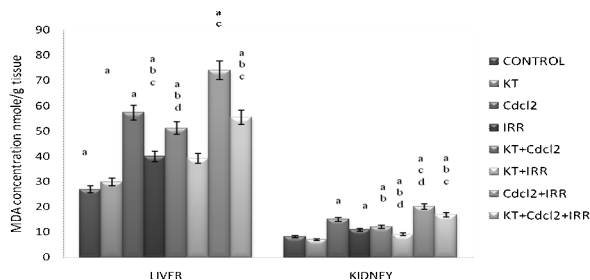


Fig. 1. Effect of KT supplementation on liver and kidney malondialdehyde (MDA) concentration in rats injected CdCl₂ and/or whole body γ -irradiated.

Control: no treatment, KT: Supplemented KT, CdCl₂: Injected with CdCl₂, IRR: irradiated, KT + CdCl₂: Supplemented KT and injected CdCl₂, KT + IRR: Supplemented KT and irradiated, CdCl₂ + IRR: Injected CdCl₂ and irradiated, KT + CdCl₂ + IRR: Supplemented KT and injected CdCl₂ and irradiated

^a Significantly different from control. ^b Significantly different from KT

^c Significantly different from CdCl₂ ^d Significantly different from IRR

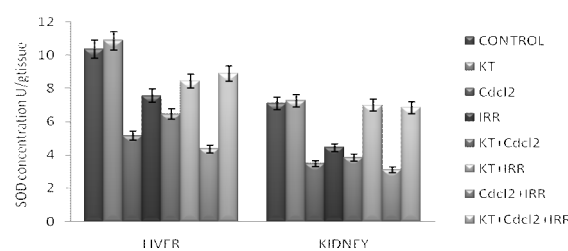


Fig. 2. Effect of KT supplementation on liver and kidney Superoxide dismutase (SOD) activity in rats injected CdCl₂ and/or whole body γ -irradiated. Legends as in Fig. 1.

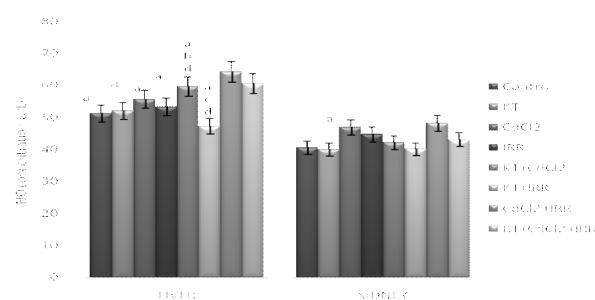


Fig. 3. Effect of KT treatment on liver and kidney nitric oxide (NO) concentration in rats injected with CdCl₂ and/or whole body γ -irradiated. Legends as in Fig. 1.

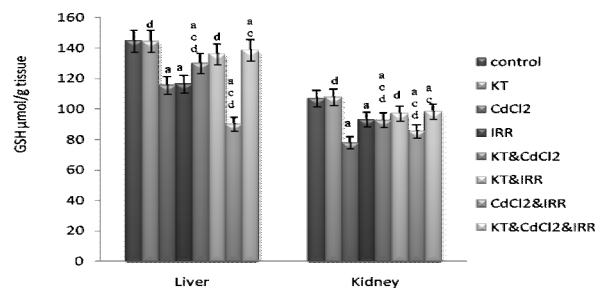


Fig. 4. Effect of KT treatment on liver and kidney glutathione (GSH) content in rats injected with CdCl₂ and/or whole body γ -irradiated. Legends as in Fig. 1.

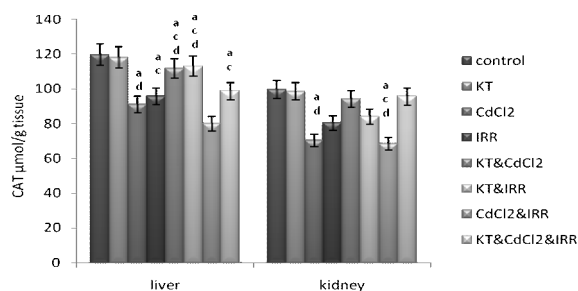


Fig. 5. Effect of KT treatment on liver and kidney catalase (CAT) activity in rats injected with CdCl₂ and/or whole body γ -irradiated. Legends as in Fig.1.

The beneficial effect of KT ferment may be attributed to the presence of vitamin C [41] a potent antioxidant and β -glucan, a bi-product of the kombucha fermented tea, considered a potent free radical scavenger and non-specific stimulator of immune response [13]. Furthermore, the protective mechanism of KT ferment could be related to the presence of hyaluronic acids, which reduce free radical- induced damage [42] and the glucuronic acid, (a powerful antioxidant) that provides a protection from oxidative stress. This acid also enables to bind up toxins (both environmental and metabolic) in liver and kidney, via UDP-glucuronyl transferase and bring them to the excretory system [42, 13].

IV. CONCLUSION

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

REFERENCES

- [1] D.R. Spitz, E.I.Azzam, J.J. Li, and D. Gius, "Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology". *Cancer Metast Rev* vol. 23, pp. 311-322, 2004.
- [2] J.Sun, Y. Chen, M. Li, and Z. Ge, "Role of antioxidant enzymes on ionizing radiation resistance". *Free Radic Biol Med* vol.24, pp. 586-93, 1998.
- [3] A.Klos, "Lead, cadmium and mercury content in metals planned for consumption in selected kindergartens in Warsaw". Proceeding of the 4th International Scientific-Technical Conference, (STC'01), Warsaw, 2001, pp. 4-5.
- [4] E.Kowalczyk, K. Anna, F. Pawel, K. Maria, N. Jan, B. Jan, K. Jozef, and T. Piotr, "Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium". *Acta Biochem. Polonica* vol.50, pp. 543-548, 2003.
- [5] A.B.Ericson, Gallen, P.Weterholm, "Cigarette smoking as an etiological factor in cleft lip and palate". *Am J Obstet Gynecol* vol.135, pp. 348-351, 1979.
- [6] M.Waisberg, P. Joseph, B. Hale, and D. Beyersmann, "Molecular and cellular mechanisms of cadmium carcinogenesis: a review". *Toxicology* vol.192, pp. 95-117, 2003.
- [7] Z.A.Shaikh, T.A. Vu, and K. Zaman, "Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants". *Toxicol Appl Pharmacol* vol.154, pp. 256-263, 1999.
- [8] E.Casalino, G. Calzaretto, C. Sblano, and C. Landriscina, "Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium". *Toxicology* vol.179, pp. 37-50, 2002.

- [9] G. B.Morales, H.H. and Sanchez, "Manufacture of a beverage from cheese whey using "tea fungus" fermentation". *Rev Latinoam Microbiol* vol. 45, pp. 5, 2003.
- [10] C.Dufresne, and E. Farnworth, "Tea, kombucha health: a review". *Food Res Int* vol. 336, pp. 409-421, 2000.
- [11] T. Pauline, P. Dipti, B. Anju, S. Kavimani, S.K. Sharma, A.K. Kian, S.K. Sarada, M. Sairam, G. Ilavazhagan, K. Devendra, and W. Selvamurthy, "Studies on toxicity, anti-stress and hepato-protective properties of Kombucha tea". *Biomed Environ Sci* vol.14, no.3, pp. 207, 2001.
- [12] M. Sai Ram, B. Anju, T. Pauline, P. Dipti, A. K Kian, S.S. Mongia, S.K. Sharma, B. Singh, R. Singh, G. Ilavazhagan, D. Kumar, and W. Selvamurthy, "Effect of kombucha tea on chromate (VI)-induced oxidative stress in albino rats". *J Ethnopharmacol* vol.71no.1-2, pp.235, 2000.
- [13] O. A. Gharib, "Does kombucha tea reduce the damage induced by radiation exposure?" *Egypt J Rad Sci Applic* vol.20no.1, pp. 141, 2007.
- [14] K .N. Tzirogiannis, G. I. Panoutsopoulos, M. D. Demonakou, R. I .Hereti, K. N .Alexandropoulou, A. C. Basayannis, and M. G. Mykoniatis, "Time-course of cadmium-induced acute hepatotoxicity in the rat liver: the role of apoptosis". *Arch Toxicol* vol.77, pp. 694-701, 2003.
- [15] S. Reitman, S. Frankel, "A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases". *Am J Clin Pathol* vol.28no.1, pp. 56, 1957.
- [16] D. P. Kind, and E. G. King, 1954. *Practical Clinical Biochemistry 1*: H. Varley, AH Gownlock and M Bell, eds, 5th edition, William Heinmann Medical Books Ltd, London 1954, P. 892.
- [17] D. Koracevic, G. Koracevic, V. Djordjevic, S. Andrejevic, and V. Cosic, "Method for the measurement of antioxidant activity in human fluid". *J Clin Pathol* vol.54, no.5, pp.356, 2001.
- [18] C. J. Halled, and J. G. Cook, "Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation". *Clin Chim Acta* vol. 35, pp.33-40, 1971.
- [19] R. J. Henery, From *Principle and Techniques: Clinical chemistry* 2nd edition. New York: Harper & Row, 1974, pp.525.
- [20] T. Yoshioka, K. Kawada, T. Shimada, and M. Mori, "Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood". *Am J Obstet Gynecol* vol.135,pp. 372, 1979.
- [21] M. Minami, H. Yoshikawa, "A simplified assay method of superoxide dismutase". *Clinica Acta* vol.92, pp. 337, 1979.
- [22] H. Aebi, "Catalase in vitro". *Methods Enzymol* vol.105, pp.121-6, 1984.
- [23] E. Beutler, O. Duron, and B.M. Kelly, "Improved method for determination of blood glutathione". *Lab Clin Med* vol.61,no.5,pp.882, 1963.
- [24] Y. Geng, M. Almqvist, and G.K. Hansson, "DNA cloning and expression of inducible nitric oxide synthase from rat vascular smooth muscle cells". *Biochim Biophys Acta* vol.1218, pp.421, 1994.
- [25] T. Finkel, and N.J. Holbrook, "Oxidants, oxidative stress and biology of aging". *Nature* vol. 408, pp.239- 47, 2000.
- [26] M.M. Kaplan. "Serum alkaline phosphatase - another piece is added to the puzzle". *Hepato* vol. 6, no.3, pp.526, 1986.
- [27] K.C. Kregel, and H.J. Zhang, "An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations". *Am. J Physiol Regul Integr Comp Physiol* vol. 292,pp. 18-36, 2007.
- [28] D. Roy, K. Maya, M. Shawn, L. Larry, D. Christine, and J. Basil, "Dynamic responses of the glutathione system to acute oxidative stress in dystrophic mouse (mdx) muscles". *Am J Physiol Regul Integr Comp Physiol* vol.291, pp. 704-710, 2006.
- [29] S. Yalin, U. Comlekoglu, S. Bagis, N.O. Sahin, O.O. Ogenler, and R. Hatungil, "Acute effect of single- dose cadmium treatment on lipid peroxidation and antioxidant enzymes in orvriectomized rats". *Ecotoxicol Environ Saf* vol.65, pp.140-144, 2006.
- [30] S. Sarkar, P.Yadav, R. Trivedi, A.K. Bansal, and D. Bhatnager, "Cadmium-induced lipid peroxidation and the status antioxidant system in rat tissues". *J Trace Elem Med Biol* vol.9, pp. 144-149, 1995.
- [31] K. Mitsumori, M. Shibutani, S. Sato, H. Onodera, J. Nakagawa, Y. Hayashi, and M. Ando, "Relationship between the development of hepato-renal toxicity and cadmium accumulation in rats given minimum

- to large amounts of cadmium chloride in the long-term: preliminary study". *Arch Toxicol* vol.72, pp.545–552, 1998.
- [32] J.P. Groten, E.J. Sinkeldam, J.B. Luten, P.J. Bladeren, "Comparison of the toxicity of inorganic and liver-incorporated cadmium: A four week feeding study in rats". *Food Chem. Toxicol* vol.28, pp. 435-441, 1990.
- [33] M. Tanaka, M. Yanagi, K. Shirota, Y. Une , Y. Nomura , T. Masaoka ,and F. Akahori , " Effect of cadmium in the zinc deficient rat". *Vet Hum Toxicol* vol.37, pp. 203–208, 1995.
- [34] S.J. Stohs, and D. Bagchi , " Oxidative mechanisms in the toxicity of metal ions". *Free Rad Biol Med* vol.18,pp. 321–336, 1995.
- [35] H. Dally, and A. Hartwig, "Induction and repair inhibition of oxidative DNA damage by nickel (II) and cadmium (II) in mammalian cells". *Carcinogenesis* vol.18, pp. 1021–1026, 1997.
- [36] P. O'Brien, and H.J. Salacinski, "Evidence that the reactions of cadmium in the presence of metallothionein can produce hydroxyl radicals". *Arch Toxicol* vol.72, pp. 690–700, 1998.
- [37] J. Navarro, E. Obrador, J. Carretero, I. Petchen, J. Avino, P. Perez, and J.M. Estrela, "Changes in glutathione status and the antioxidant system in blood and in cancer cells associate with tumor growth *in vivo*". *Free Radic Biol Med* vol.26, no.3-4, pp.410, 1999.
- [38] B.I. Ognjanović, S.Z. Pavlović, R.V.A. Ikić Tajn, S.D. Maletić, Z.S. Saičić, and V.M. Petrović, "Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium". *Physiol Res* vol.52, pp. 563-570, 2003.
- [39] M. Nagoraj, S. Sunitha, and P. Varalaskhmi, "Effect of lupeol, a pentacyclic triterpene, on lipid peroxidation and antioxidant status in rat kidney after chronic cadmium exposure". *J Applied Toxicol* vol.20, pp.413-417, 2000.
- [40] M.W. Fariss, "Cadmium toxicity: Unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes". *Toxicology* vol.69, pp. 63-77, 1991.
- [41] S.M. Zaidi, T.M. Al-Qirim, and N. Banu, "Effects of antioxidants vitamins and glutathione depletion and lipid peroxidation induced by restraint stress in the rat liver". *Drugs RD* vol.6, no.3, pp.157, 2005.
- [42] P.J. Blanc, "Characterization of the tea fungus metabolites", *Biotechnol Lett* vol.18, no.2, pp.139-143, 1996.