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Protective role of rosemary against trichroacetate-induced toxicity in kidney of male rats.

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The present study was designed to evaluate the potential protective role of the aqueous extract of rosemary (*Rosmarinus officinalis*) (AER) against trichloro acetate (TCA) induced renal-toxicity in male albino rats. 40 male albino rats were divided into 4 groups of ten: group A served as control; group B was given AER (200 mg/kg/day) by gavage; group C received TCA at the dose 50 mg/kg/day and group D was treated with AER (200 mg/kg/day) and received TCA (50 mg/kg/day). The experiment was carried out for two months. The toxicity of TCA for rats was revealed by an increase in kidney biomarkers [urea, ceratenin and uric acid]. The TCA treatment caused a significant increase in MDA (malondialdehyde) level and the activities of CAT (catalase), SOD (superoxide dismutase) and GPx (glutathione peroxidase) in kidney tissues. These biochemical effects were accompanied by histological indicators of kidney damage. Treatment with AER recovered the kidney toxicity (induced by TCA treatment), as demonstrated by perfection of kidney biomarkers; as well as antioxidant parameters (CAT, SOD, GPx and MDA) and amelioration of histopathology changes in kidney tissues. It could be concluded that AER supplementation for 2 months in TCA induced toxicity in rats benefited renal antioxidant status and improved kidney injury and damage in male albino rats exposed to TCA.

Keywords: Rosemary – Trichloro acetate – kidney biomarker – Antioxidant.

INTRODUCTION

Now-a-days, heart disease and diabetes provide the first and second leading cause of death in the world, respectively and people are suffering from these diseases are likely to be more prone to kidney disease. Therefore, there is an urgent need to find alternative sources of current methods of treating kidney diseases, which cost a lot of money in addition to the exhaustive side effects of the patients (Roy et al., 2015). Nephrotoxicity occurs as a disturbance in renal function due to various drug interactions and chemicals (Watkins et al., 2006).

There is a serious organic matter has been detected in various water surfaces, such as drinking water and swimming pools, which is a trichloroacetate (TCA), which consists mainly during water chlorination (Coleman et al., 1980). TCA is also found in fruits, vegetables and grains (Reimann et al., 1996), which may have been transported to them mostly from washing or cooking water, which increases the exposure of human beings from different sources (U.S. EPA, 2005). During the liver detoxification of foreign substances such as TCA, the cytochrome P-450 enzymes remove the chlorine from it, leaving many radicals and reactive oxygen species (ROS) [Larson and Bull, 1992]. It is also known that ROS work to oxidize the lipids in the cellular membranes, which eventually lead to damage of protein, lipid and DNA, causing at the end toxicity for different body organs (Austin et al., 1996). The

kidney is a body member that is strongly affected by chlorine-containing compounds such as TCA, where it causes kidney toxicity and damage to kidney cells (Ali et al., 2016).

Currently, researchers in different countries of the world have noticed the important roles that our normal foods, which contain many natural antioxidants (vitamins and phytochemicals), can serve as a defensive line against ROS and oxidative damage. Rosemary (*Rosmarinus officinalis L.*) is an important plant that grows in Egypt and in many countries of the world. It has also been used in ancient medicine to treat common diseases such as cold, flu and anemia (AL-Sereiti et al., 1999; Ugulu et al., 2009). Rosemary contains important phenolic compounds that are known to play an important role as antioxidants and as anti – inflammatory agents such as caffeic acid, carnasol and rosmarinic acid (Afonso MS, Sant'Ana, 2008; Pérez-Fons et al., 2010).

The present study is aimed to evaluate the kidney-protective and antioxidant activity of aqueous extract of rosemary against TCA-induced kidney toxicity in rats. Also, the chemical constituents and antioxidant activity of rosemary (aqueous extract) was determined.

MATERIALS AND METHODS

Plant material

Fresh leaves of rosemary were purchased from local market in Jeddah, Saudi Arabia and it was identified and authenticated by the Department of Botany and Microbiology, University of King Abdulaziz, Jeddah, KSA.

Aqueous extract preparation

The rosemary leaves were dried under environmental temperature and powdered using a grinder. About 50 g of dried, ground plant materials were soaked in distilled water (5000 ml) for 48 hours. The final extract was filtrated, solvent removed and stored at 4^{o}C for further use (Abozid and Farid, 2018).

Antioxidant activity *In vitro*

The amount of total phenolic compounds in aqueous extract of rosemary (AER) was determined with Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (1990) by using gallic acid (GA) as standard, and aluminum chloride colorimetric method was used for total flavonoids determination (Aiyegoro and Okoh, 2010) by using quercetin (QE) as standard, while the reducing power of aqueous extract of rosemary (AER) was determined according to the method of Ebrahim zadeh et al., (2008).

Identification and determination of phenolic compounds by HPLC

The phenolic compounds were identified and analyzed using high performance liquid chromatography (HPLC) by comparing the retention time of our samples with standard samples by using Shimadzu LC 20 AT HPLC fitted with a SIL 20A auto sampler and a SPD-20 UV Visible detector with a class LC 10 chromatography workstation (Zuo et al., 2002).

Experimental animals and experimental design

Forty male albino wistar rats weighting between 180 - 200 g each were purchased from the Central Animal House in Jeddah, Saudi Arabia. The experiments and the protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Saudi Arabia, Jeddah. All experimental procedures were completed according to the ethical guidelines of International Association for the Study of Pain (Zimmermann, 1983).

Rats were housed in stainless steel cages placed in a well-ventilated rat house, preserved for 15 days to adapt them to laboratory conditions on free supply of water and food provided *ad libitum*. After that, rats were divided into 4 groups of ten. The animal experiments were conducted for 2 months. Group I was received daily 0.5 mL of 0.9% NaCl solution orally for 2 months and was used as control (CG); group II was administrated orally with aqueous extract of rosemary (AER) (200 mg/kg body weight) by gavage (RG); group III was received TCA at dose 50 mg/kg/day (TG); group V was received the rosemary (200 mg/kg/day) and receive TCA at dose 50 mg/kg/day (RTG).

Serum collection and tissue preparation.

The rats were anesthetized and scarified after the examination period (2 months), the blood samples were collected from a cardiac puncture and put immediately into glass tubes and centrifuged at 3000 rpm for 20 min at 4°C to separate serum samples which stored in aliquots at -20 ^{0}C till used. The kidneys were immediately removed, washed with physiologic saline solution and weighed. Small pieces were fixed in 10 % neutral buffered formalin for histopathology and the remaining part was homogenized in ice-cold Tris-buffered saline (TBS), pH 7.4, and

centrifuged at 3000 rpm for 10 min at 4°C. The homogenate was collected in aliquots, stored at −20 °C until use.

Measurement of biochemical parameters

Uric acid was determined in serum according to Rock et al., (1987) while serum urea and creatinine were measured according to Wootton and Freeman, (1982).

All antioxidant parameters were determined in kidney tissues. The lipid peroxidation end product, MDA was measured according to Ohkawa et al.,(1979) Also, Catalase (CAT) activity was determined as described by Aebi (1984), Superoxide dismutase (SOD) activity was measured using the method of Nishikimi et al., (1972) and Kinetic determination of glutathione peroxidase (GPx) activity was done according to the method of Paglia and Valentine (1967).

Histopathology of the rat kidneys

The histopathological examination of the cells of the kidney and the preparation of the slides were examined under the microscope and photographed to observe the changes that occurred to the cells as a result of the treatments used in the experiment was done according to (Carleton, 1979).

Statistical analysis

The numbers in the tables are mean for each group + standard deviation (SD).

The data were analyzed using the ANOVA test (one-way analyses of variance) and student's t-tests by using a computer program (P-value ≤ 0.05 considered significant).

RESULTS

Phytochemicals of aqueous extract of rosemary (AER)

Extract analysis using HPLC and spectrophotometric assays provided qualitative insights the bioactive phytochemicals of AER. Table (1) shows AER containing 18 mg GAE/ 100g DM of total phenolics content (TPC) and 8.6 mg QE/100 g DM of total flavonoids content (TFC).

A total of six phenolic compounds (Caffeic acid, ferulic acid, apigenin, luteolin, gallic acid and gallic acid methyl ester) were characterized by HPLC. Luteolin and caffeic acid were present in high levels (3.96 and 3.24 mg/100g DM, respectively). Traces of ferulic acid, apigenin, gallic acid and gallic acid methyl ester were also detected.

Table 1: Phytochemical parameters of aqueous extract of rosemary (AER)

Phytochemica parameters	Amounts
Total phenolic content (TPC) mg GAE/100g DM	18 ± 0.53
Total flavonoids content (TFC) mg QE/100g DM	8.6 ± 0.22
Polyphenolic compounds (mg/100g DM)	
Luteolin	3.96 ± 0.01
Caffeic acid	3.24 ± 0.06
Apigenin	0.81 ± 0.03
Ferulic acid	0.684 ± 0.07
Gallic acid	0.378 ± 0.02
Gallic acid methyl ester	0.306 ± 0.04

Data are reported as the mean ± SD of three replicates.

Antioxidant activity of AER

Figure (1) showed the dose– response curves for the reducing powers of the AER compared with ascorbic acid; the reducing powers of AER increased by increasing the extract concentration. At the highest concentration (800 ug/ml) AER showed highest activity (0.856). Figure (1) also indicates that vitamin C has a higher reducing power than the AER at all concentrations.

Effect of TCA and AER on biochemical kidney markers in serum

Data tabulated in Table (2) revealed that TCA supplementation in TG induced a significant increase in blood urea, uric acid and creatinine as compared with normal rats (CG); while the treatment only with aqueous extract of rosemary (RG) didn't cause any significant changes compared with CG. Treatment with AER in rats treated with TCA in drinking water (RTG) allowed these parameters to significantly reduce and come near the control group values.

Enzymatic antioxidant status and MDA levels in kidney.

Rats in TG (treated with TCA) showed significantly increased in SOD, CAT and GPx activities as compared with CG (Table 3). TCA induced also a significant elevation in the MDA content in kidney tissues as compared with CG. Oral supplementation with 200 mg/kg/day of AER against TCA treatment (RTG) caused a significant decreased in all antioxidant enzymes (SOD, CAT and GPx) activities compared with rats treated with TCA only (TG).

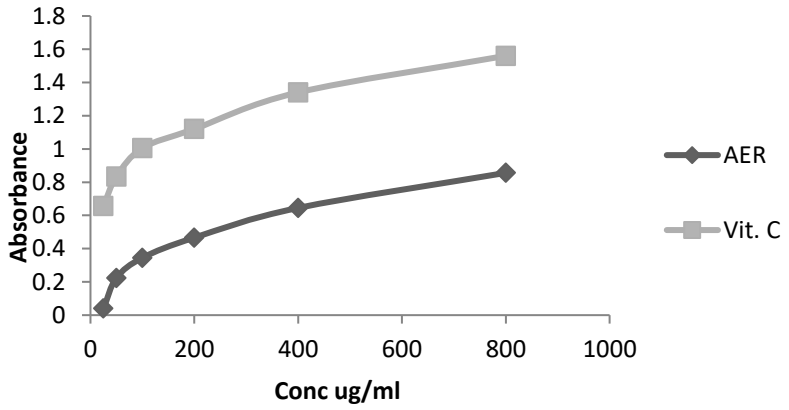

Figure 1: Reducing ability of aqueous extract of rosemary (AER) compared with ascorbic acid at various concentrations.

Table 2: Effect of aqueous extract of rosemary (AER) on serum kidney functions in different groups

Groups / Parameters	CG	RG	TG	RTG
Urea (mg/dl)	30.92 ± 4.43 a	33.17 ± 3.36 a	48.25 ± 4.5 c	40.33 ± 3.4 b
Creatinine (mg/dl)	0.72 ± 0.11 a	0.69 ± 0.13 a	0.95 ± 0.11 c	0.83 ± 0.09 b
Uric acid (mg/dl)	1. 8 ± 0.25 a	1.9 ± 0.28 a	2.6 ± 0.21 c	2.1 ± 0.19 b

Data are expressed as means ± SD (n=10 rats per group). Comparison between groups was made using student's t-tests. Values in the same columns not sharing a common letter (a–c) differ significantly at $p<0.05$.

Table 3: Effects of aqueous extract of rosemary (AER) on the activity of SOD, CAT, GPx and MDA content in rat kidney in different groups.

Groups / Parameters	CG	RG	TG	RTG
SOD(U/mg protein)	22.32 ± 2.01 a	21.19 ± 2.92 a	44.75 ± 2.8 c	35.78 ± 2.3 b
CAT (U/mg protein)	89.63 ± 1.8 a	88.32 ± 2.85 a	121.6 ± 3.2 c	108.7 ± 2.5 b
GPx (U/mg protein)	13.77 ± 1.88 a	14.11 ± 2.11 a	33.66 ± 3.88 c	24.15 ± 2.9 b
MDA (nmol/g tissue)	35.08 ± 1.46 a	34.55 ± 2.13 a	66.45 ± 2.22 c	51.3 ± 2.85 b

Data are expressed as means ± SD (n=10 rats per group). Comparison between groups was made using student's t-tests. Values in the same columns not sharing a common letter (a–c) differ significantly at $p<0.05$.

Moreover, AER regulation alleviated lipid peroxidation prompted by TCA treatment and significantly improved MDA levels in kidney (Table 3).

Histopathology of rat kidney tissue

Microscopic screening of the kidney in CG group showed normal kidney with central glomeruli surrounded by Bowman's space, normal tubules lined by cuboidal epithelium (Fig 2A)and the RG group also showed no negative changes in the cells of the kidney, indicating that treatment with water extract of rosemary plant did not adversely affect the kidney cells (Fig 2 B). In contrast, supplementation of TCA for two months prompted damage to the kidney cells with disarrangement of kidney strands. The kidney sections in TG group showed tubular necrosis, degeneration, vacculation and interstatial vascular congestion (Fig 2C). However, treatment with AER (RTG) has greatly improved the deterioration of kidney cells (Fig 2D). Based on the kidney histopathological changes, we can confirm that the treatment of TCA caused kidney damage, while the water extract of rosemary showed a protective effect against this toxicity, resulting in kidney cell protection.

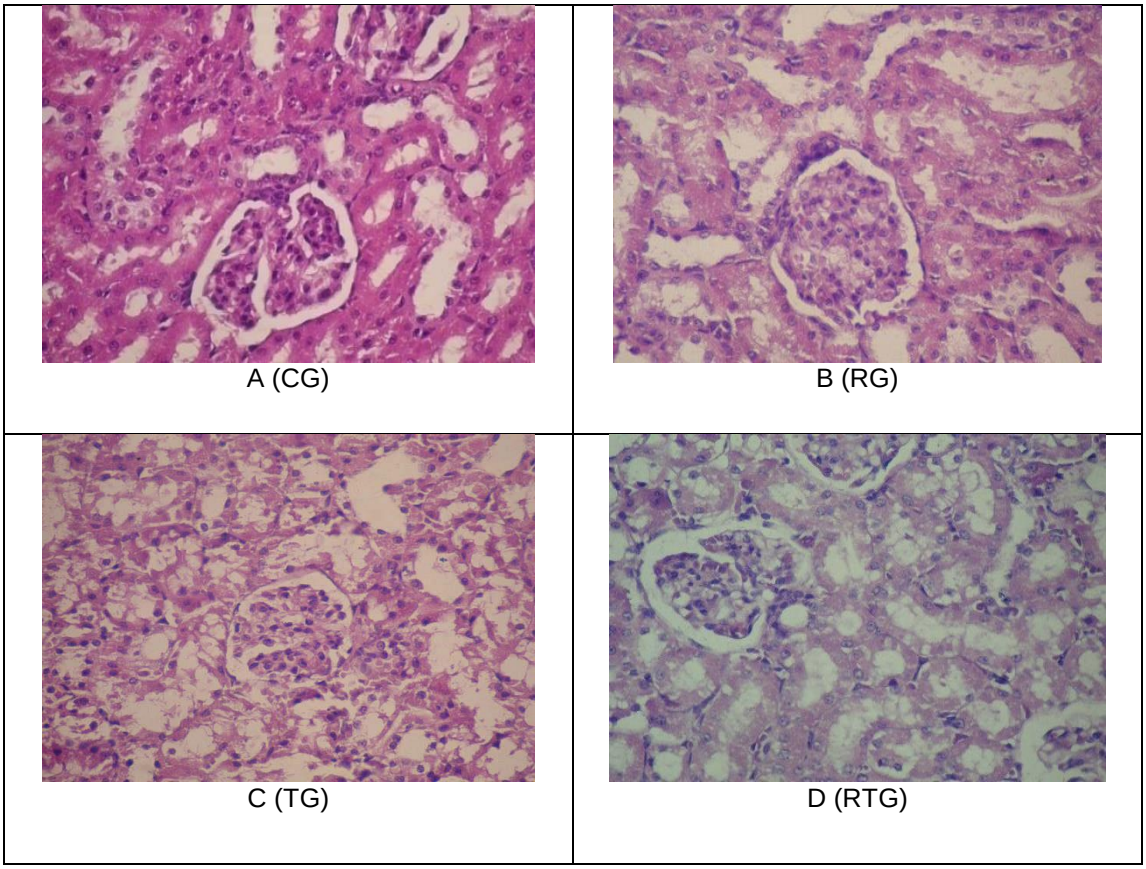

Figure 2: kidney histology for different groups. A (CG) showed normal kidney showing central glomeruli surrounded by Bowman's space, normal tubules lined by cuboidal epithelium. B (RG) showed kidney cells with mild tubular degeneration. C (TG) showed kidney with marked tubular necrosis, degeneration, vacculation and interstatial vascular congestion. D (RTG) showed kidney with moderate tubular degeneration and vacculation (H&E X40).

DISCUSSION

Oxidative stress is an important factor that causes damage to the cells of the body in general, leading to poisoning of organs, especially important organs that play a key role in the body such as kidney (Ueda et al., 2007). ROS causes increased in final oxidation product MDA by upsetting the balance between ROS and antioxidant defense system in our bodies (Kuo and Tarng, 2010).

Our results showed that AER was rich in total phenolic compounds and total flavonoids (Table 1) that contributed to its antioxidant activity to scavenge free radicals. This result is in harmony with Al-Sereiti et al., (1999); Hossain et al., (2010) and Zhang et al., (2012) who found that rosemary is rich in different phenoilc compounds and flavonoids. There is a close relationship between the reducing power and the antioxidant activity of the various extracts, which is considered to be an expression of the efficacy of these extracts as antioxidants (Oktay et al., 2003). In our study main polypenolic compounds in AER were luteolin (3.96 ± 0.01 mg/100g DM) which showed high antioxidant activity when tested by reducing power assay compared with BHT (Alesheikha et al., 2016). Flavonoids with a hydroxyl group in their structure (such as luteolin) have antioxidant properties as they donate hydrogen to a free radical (Miliauskas et al., 2004). On the other hand the second important phenolic compounds in AER is caffeic acid (3.24 ± 0.06 mg/100g DM) which also showed high antioxidant activity because of caffeic acid has two hydroxyl groups and therefore has good antioxidant properties (Masek et al., 2016). The antioxidant effect of AER may be related to its various phenolic compounds content, which is characterized by redox properties, making them act as hydrogen donor and put out singlet oxygen radicals (Negri et al., 2011).

In the present study, oral treatments with 200 mg/kg/day of AER for 60 days significantly reversed the altered serum kidney function parameters in the TCA-treated rats. The kidney protective effects of AER can be linked to the phenolic compounds and flavonoids, which are characterized by an antioxidant activity; that can fight the oxidative stress caused by TCA (Lee et al., 2008; Panuganti et al., 2006).

The biomarkers of kidney functions in serum supported the histopathological damages in kidney cells in the current work. These observations confirmed significant histological changes in the kidney cells as a result of TCA treatment. It might be due to its toxic impact by generation of free radicals causing destroy to the various membrane components of the kidney cells.

Determination of enzyme markers [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx)] and malondialdehyde (MDA), are widely accepted and used in antioxidant evaluation studies (Celik, 2007).

In order to evaluate the effect of AER on TCA-induced oxidative stress, and activities of antioxidant enzymes CAT, SOD, GPx and MDA level were measured.

The present study showed that TCA induced a significant increase in the activity of SOD, CAT and GPx compared with normal control CG.

These results are agreement with El Arem et al., (2014); Hassoun and Cearfoss, (2011), who confirmed the increase in these enzymes activity in the serum when animals treated with TCA. TCA treatment resulted in rise in SOD and CAT activities suggesting a partial role of SOD-CAT in protection against cellular toxicity and the contribution of superoxide anion (SA) and H_2O_2 to the previously observed oxidative cellular damage associated with TCA treatment (Hassoun and Cearfoss, 2011).

Levels of MDA in different tissues are a primary indicator of the degree of lipid peroxidation as it is the final product of polyunsaturated fatty acid peroxidation. Our results showed that the kidney content of MDA was significantly elevated in TCA-treated rats. This elevation might have resulted from an elevation of ROS as a result of the stressful conditions in the rats exposed to TCA. The significant decreased of MDA levels in rats treated with AER (RTG) may be due to inhibitory action of this extract on lipid peroxidation that may be related by the antioxidant activity which help in the protection of membrane integrity.

The ability of the AER to recover SOD, CAT and GPx activities and MDA levels to normal levels of animals treated with TCA might be attributed to antioxidant properties and the free radical scavenging of rosemary aqueous extract, since antioxidants appear to confront disease by increasing the activities of antioxidant enzymes and reduction lipid peroxidation (Bansal et al., 2005)

Thus, this results strongly further suggests anti lipoperoxidation activity of AER. The antioxidant activity and kidney protective effect of AER could be attributed to its high content from phenolic compounds and flavonoids as these

phyto components have been widely reported to possess antioxidant and anti-lipoperoxidative activities (Khalil et al., 2012).

CONCLUSION

The present study suggests that aqueous extract of rosemary (AER) has a potent antioxidant and kidney protective role in TCA treated rats.

It is possible that the antioxidant effect and protection of kidney cells of AER is due to the scavenging free radicals generated by TCA. Therefore, we need further studies on the various extracts of rosemary and its role in the protection of kidney cells.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors have read and agreed to the content and the publication of this paper.

REFERENCES

Abozid, M. M. and Farid. H. E. A. 2018. Protective role and antioxidant activity of aqueous extract of *Rosmarinusofficinalis* against trichloroacetate-induced toxicity in liver of male rats. Asian J Pharm Clin Res.,11 (6), 420-424.

Aebi, H. 1984. Methods in Enzymatic Analysis, vol. 3, Academic Press, New York.

Afonso, M. S., Sant'Ana, L. S. 2008. Effects of pretreatment with rosemary (*Rosmarinusofficinalis L.*) in the prevention of lipid oxidation in salted tilapia fillets. J Food Quality, 31, 586–595.

Aiyegoro, O. A., Okoh, A. I. 2010. Preliminary phytochemical screening and In vitro antioxidant activities of the aqueous extract of *Helichrysumlongifolium*. BMC ComplemAltern Med, 10, 21 – 28.

Alesheikha, P., Feyzia, P., Kamalib, M., Sania, T. A. 2016. Evaluation of antioxidant activity of luteolin, isolated from *Dracocephalumkotschyi*. J Med Plant Nat Prod, 1, 41-47.

AL-Sereiti, M. R., Abu-Amerb, K. M., Sena, P. 1999. Pharmacology of rosemary (*Rosmarinusoificinalis L.*) and its therapeutic potentials. Indian J ExperimBiol, 37, 124-130.

Aly, H. M., El-Saeed, G. S. M., Ali, R. K. 2010.Impact of toxicity with commercial sodium chloride salt on kidney and DNA of male mice-Part 1. Global J Pharmaco, 10 (2), 35-40.

Austin, E. W., Parrish, J. M., Kinder, D. H., Bull, R. J. 1996. Lipid peroxidation and formation of 8-hydroxydeoxyguanosine from acute doses of halogenated acetic acids.FundamApplToxicol, 31, 77–82.

Bansal, A. K., Bansal, M., Soni, G. Bhatnagar, D. 2005. N-Nitrosodiethylamine induced oxidative stress in rat liver. ChemBiol Interact, 156, 101–111

Carleton, H. 1979. Histological Techniques.4th ed. Oxford University Press, New York, USA.

Celik, I. 2007. Determination of toxicity of trichloroacetic acid in rats: 50 days drinking water study. PesticBiochemPhys, 89, 39–45.

Coleman, W., Melton, R., Kopfler, F., Barone, K., Aurand, T., Jellison, M. 1980.Identification of organic compounds in a mutagenic extract of a surface drinking water by a computerized gas chromatography/mass spectrometry system (GC/MS/COM). Environ Sci Technol,14, 576-588.

Ebrahimzadeh, M. A., Pourmorad, F., Hafezi, S. 2008. Antioxidant activities of Iranian corn silk. Turk J Biol, 32, 43-49.

El Arem, A., Saafi, E. B., Ghrairi, F., Thouri, A., Zekri, M., Ayed, A. 2014. Aqueous date fruit extract protects against lipid peroxidation and improves antioxidant status in the liver of rats subchronically exposed to trichloroacetic acid. J PhysiolBiochem, 70, 451–464.

Hossain, M. B., Rai, D. K., Brunton, N. P., Martin-

Diana, A. B., Barry-Ryan, C. 2010. Characterization of phenolic composition in *Lamiaceae* spices by LC-ESI-MS/MS. J Agric Food Chem, 58, 10576–10581.

Hassoun, E. A., Cearfoss, J. 2011. Dichloroacetate- and trichloroacetate-induced modulation of superoxide dismutase, catalase, and glutathione peroxidase activities and glutathione level in the livers of mice after subacute and subchronic exposure.Toxicol Environ Chem, 93(2), 332–344.

Khalil, O. A., Ramadan, K. S., Danial, E. N., Alnahdi, H. S., Ayaz, N. O. 2012. Antidiabetic activity of *Rosmarinusofficinalis* and its relationship with the antioxidant property.Afric J Pharm Pharmaco, 6(14), 1031 – 1036.

Kuo, K., Tarng, D. 2010. Oxidative stress in chronic kidney disease. Adaptive Medicine, 2(2), 87-94.

Larson, J. L., Bull, R. J. 1992. Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice.ToxicolApplPharmacol, 115, 268–277.

Lee, H. J., Cho, H. S., Seung, E. P., Lee, S. Y., Kim, C. S., Kim, D. K. 2008. Rosmarinic acid protects human dopaminergic neuronal cells against hydrogen peroxide-induced apoptosis. Toxicology, 250, 109–115.

Masek, A., Chrzescijanska, E., Latos, M. 2016. Determination of antioxidant activity of caffeic acid and p-coumaric acid by using electrochemical and spectrophotometric assays.Int J ElectrochemSci, 11, 10644 – 10658.

Miliauskas, G., Venskutonis, P., Van Beek, T. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem, 85, 231-237.

Negri, G., Teixeira, E. W., Alves, M. L., Moreti, A. C., Otsuk, I. P., Borguini, R. G. 2011. Hydroxycinnamic acid amide derivatives, phenolic compounds and antioxidant activities of extracts of pollen samples from Southeast Brazil. J Agric Food Chem, 59, 5516–5522.

Nepis.epa.gov. 2018.Retrieved on January 31, 2018 from: https://nepis.epa.gov/EPA/html/Pubs/pubtitleOW.html.

Nishikimi, M., Roa N. A., Yogi, K. 1972.Measurement of superoxide dismutase.BiochemBiophys Res Common, 46, 849-854.

Ohkawa, H., Ohishi, N., Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95, 351-358.

Oktay, M., Gulcin, I., Kufrevioglu, O. I. 2003. Determination of in-vitro antioxidant activity of fennel (*Foeniculumvulgare*) seed extracts. LebensumWiss U Technol, 36, 263-271.

Paglia, D. E., Valentine, W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med, 70, 158–169.

Panuganti, S. D., Khan, F. D., Svensson, C. K. 2006. Enhanced xenobiotic-induced hepatotoxicity and kupffer cell activation by restraint-induced stress. J PharmacolExpTher, 318, 26–34.

Pérez-Fons, L., Garzón, M. T., Micol, V. 2010.Relationship between the antioxidant capacity and effect of rosemary (*Rosmarinusofficinalis L.*) polyphenols on membrane phospholipid order. J Agric Food Chem, 58, 161–171.

Reimann, S., Grob, K., Frank, H. 1996. Chloroacetic acids in rainwater. Environ SciTechnol, 30, 2340-2344.

Rock, R. C., Walker, W. G., Jennings, C. D. 1987.Nitrogen metabolites and renal function. In: Tietz NW, editors. Fundamentals of clinical chemistry. 3rd ed. Philadellphia: WB Saunders.

Roy, S., Pradhan, S., Das, K., Mandal, A., Mandal, S., Patra, A. 2015. Acetaminophen Induced Kidney Failure in Rats: A Dose Response Study. J Biol Sci,15 (4), 187-193.

Spanos, G. A., Wrolstad, R. E. 1990. Influence of processing and storage on the phenolic composition of thompson seedless grape juice. J Agric Food Chem, 38, 1565-1571.

Ueda, S., Yamagishi, S., Kaida, Y., Okuda, S. 2007. Asymmetric dimethylarginine may be a missing link between cardiovascular disease and chronic kidney disease. Nephrol, 12, 582-90.

Ugulu, I., Baslar, S., Yorek, N., Dogan, Y. 2009. The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province. Turkey J Med Plants Res, 3, 345-367.

Watkins, P. B., Kaplowitz, N., Slattery, J. T., Colonese, C. R., Colucci, S. V., Stewart, P.W. 2006. Aminotransferase elevations in healthy adults receiving 4 grams of

acetaminophen daily: A randomized controlled trial. J Am Med Assoc, 296, 87-93.

Wootton, I. D., Freeman, H. 1982. Micro Analysis in Medical Biochemistry, 6th ed, Churchill Living Stone Publication, London.

Zhang, Y., Smuts, J. P., Dodbiba, E., Rangarajan, R., Lang, J. C., Armstrong, D. W. 2012. Degradation study of carnosic acid, carnosol, rosmarinic acid, and rosemary extract (*Rosmarinusofficinalis L.*) assessed using HPLC. J Agric Food Chem, 60, 9305–9314.

Zimmermann, M. 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16, 109–110.

Zuo, Y., Chen, H., Deng, Y. 2002. Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and pu-err teas using HPLC with a photodiode array detector. Talanta, 57 (2), 307-316.