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Phytochemical composition of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) and it's antioxidant activity with lead induced testicular toxicity in rats

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ABSTRACT

There is currently an upsurge of interest in phytochemicals as a new source of natural antioxidants to be used in foods and pharmaceutical preparations to replace synthetic antioxidants, which are being restricted due to their potential health risks and toxicity. The objective of the present study was to evaluate the phytochemical composition as well as the antioxidant activity of the ethanolic extracts of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) leaves & stem on testicular tissue that has been exposed to oxidative stress by lead acetate in albino rats. Phytochemical screening revealed the presence of various bioactive secondary metabolites as flavonoids, phenolics, coumarins, terpenes, tannins, and sterols compounds which might be responsible for their medicinal attributes. Anthraquinones and volatile oils were not detected in leaves and stem. The quantitative estimation of total flavonoids and total phenolics present in higher amount in stem than leaves. LD_{50} was calculated for leaves and stem extracts and was 3600 & 4600 mg/kg b.w., respectively. The oral administration of stem extract with a dose (460 mg kg^{-1}) reducing the oxidative stress in testicular tissue by increasing superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Keywords: *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl), phytochemical composition, antioxidant activity, testicular toxicity.

INTRODUCTION

Much attention has been focused on the use of antioxidants, especially natural antioxidants, to protect from free radicals. Sources of natural antioxidants are primarily, secondary metabolites that may occur in all parts of the plants such as fruits, vegetables, nuts, seeds, leaves, roots and bark. Many naturally occurring constituents in plant extracts show antimicrobial, antioxidant, antifungal, anticancer and anti-inflammatory activities to a greater or lesser extent ***(Patwardhan, 2005; Rad, et al., 2014)***. Crude extracts of fruits, herbs, vegetables and other plant materials rich in phenolics are increasingly of interest in the food industry, because they retard oxidative degeneration of lipids and there by improve the quality and nutritive value of food ***(Rice Evans, 1995; Ka¨hko¨nen, et al., 1999)***. Many studies have been extensively conducted in recent years to identify and quantify phenolic compounds and the relevant antioxidant activity of plants ***(Ham, et al., 2015).***

In the series of *Ficus* plants, *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) belongs to family Moraceae (mulberry family) has been used in folk medicine as a healer for skin allergy from external causes; sap of leaves used to treat scorpion stings; powder of wood used to treat sexual impotence, as a remedy for sterility. Moreover, decoction of aerial parts used in swelling and body pains, while in Oman leaves crushed and mixed with lemon to treat indigestion; leaves also used on bruised fingers and toes and for removing warts ***(Drummond and Coates Palgrave, 1973)***.

Consequently, the objective of this study was to identify the phytochemical composition and the antioxidant efficacy of the ethanolic extract of aerial parts (leaves & stem) of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl).

MATERIALS AND METHOD

Source and preparation of plant material

The fresh aerial parts (leaves & stem) of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) were collected from Elba mountain habitat in the remotest south eastern corner of Egypt. The separated specimens were washed with dis. Water and were shade dried at lab-temperature till constant weight in Medicinal Plants Department, at Desert Research Center, Cairo, Egypt. The dried parts were then grounded into fine powdery form, finally stored in dry glass jar for phytochemical analyses.

Extraction of plant material

The powdered plant material (100 grams) from leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) were extracted separately with minimum amount of 70% ethanol, and purified according to standard procedures reported by ***Mabry, et al. (1970)*** and ***Harborne, (1984)***. The slurry was allowed to stand for 24 h with occasional stirring, and then filtered off. The residue was repeatedly extracted with an excess volume of 70% ethanol. Combined filtrates were evaporated under reduced pressure using rotavapour apparatus until a minimum amount of solvent remained. The extracts were stored at 4^{o}C for phytochemical analyses.

Phytochemical screening

The crude ethanolic 70% extracts of leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) plant were subjected to preliminary qualitative phytochemical screening for the presence of bioactive constituents such as carbohydrates and/or glycosides, alkaloids, flavonoids, saponins, tannins, phenolic compounds, terpenoids, steroids, and coumarins using standard phytochemical techniques as described by ***Clarke, 1975; Harborne, 1998***.

Quantitative determination of phenolics and flavonoids contents

The amount of total phenolics and flavonoids and in the extracts were determined according to methods of ***Chun, et al. (2013)***; ***Malla, et al. (2013)***.

The antioxidant study

Chemicals

Lead acetate and chemicals used were of high analytical grade purchased from El-Nasr pharmaceutical chemical Co. (ADWIC), Egypt and Aldrich-Sigma Co. (U.S.A).

Animals

A total of 210 male albino mice weighing 20-25 g and 60 adult male Sprague-Dawely albino rats (110-120g) were used throughout this study and purchased from Egyptian Organization for Biological Products and Vaccines (Helwan farm). Animals were housed in separate screen bottom cages (5/cage) under controlled environmental conditions (20-25 ºC, 55-60 % relative humidity and 12 hours light dark cycle), where food and water were offered *ad-libitum*. Animals were maintained on a commercial pellet diet having the following composition: protein (21% w/w), barley (37% w/w) and corn (15% w/w) ***(lewi and***

Marsboom, 1981). Animals were accommodated to the laboratory conditions for two weeks before starting the experiments.

Determination of acute toxicity & median lethal dose (LD_{50}) of leaves and stem

The acute toxicity of plant extracts (leaves & stem) was estimated in mice orally. Mice were dividing animals into ten groups (10 mice each). Each group received one dose of 500, 1000, or 4500 mg/kg for leaves extract while for stem extract the doses were 500, 1000 or 5500 mg/kg. Control animals received the vehicle and kept under the same conditions. Animals were observed for 24h for effects of toxicity and number of deaths. At the end of the study period, expired animals were counted for the calculation of LD_{50}. The arithmetic method of ***Karber, (1931)*** was used for the determination of LD_{50}.

$$LD_{50} = LD_{100} - \sum (a \times b) / n$$

n= total number of animal in a group.

a= the difference between two successive doses of administered extract/substance.

b= the average number of dead animals in two successive doses.

LD_{100}= Lethal dose causing the 100% death of all test animals.

Study design

The Sprague-Dawely albino rats were randomly divided into 6 groups (10 animals each):

Group A: Rats fed on the standard diet and served as a normal control group.

Group B: Rats received daily an oral dose of 30 mg/kg lead acetate.

Groups C and D: Rats treated daily with an oral dose of leaves extract at doses level of 360 and 180 mg/kg b.w., respectively, and lead acetate at a dose level of 30 mg/kg b. w.

Groups E and F: Rats treated daily with an oral dose of stem extract at doses level of 460 and 230 mg/kg b.w., respectively, and lead acetate at a dose level of 30 mg/kg b. w.

Rats were allocated in their groups for 60 constitutive days.

Specimen collection

At the end of the experimental period, rats were sacrificed by cervical dislocation; The testes tissue samples were dissected quickly, and separated then washed with normal saline for determination of oxidative stress parameters.

Preparation of testicular tissue homogenate

Prior to dissection, testicular tissue was perfused with a PBS (phosphate buffer saline) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells. Exactly 0.5 g of testicular tissue was weighed and homogenized in 10 ml cold buffer (50 mM potassium phosphate, pH 7.5, containing 1mM EDTA), to yield ultimately 5% (w/v) whole testis homogenate. The homogenates were centrifuge at 5000 rpm for 15 min at 4°C then the supernatant used for determination of MDA, SOD and GPx.

Estimation of antioxidant efficacy

Concentration of malondialdhehyde (MDA) and the activities of glutathione peroxidase (GPx) & superoxide dismutase (SOD) were estimated using commercial kits (Biodiagnostic, Egypt) according to the methods described by ***Ohkawa, et al. (1979)***; ***Nishikimi, et al. (1972)***; ***Paglia and Valentine, (1967)***, respectively.

Statistical analysis

Data were presented as mean±SE. One way analysis of variance (ANOVA) followed by post hoc – least significant difference analysis (LSD) was performed using the statistical package for social science

RESULTS

Phytochemical screening

The preliminary phytochemical screening of leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) plant proved the presence of glycosides and/or carbohydrates, saponins, alkaloids, flavonoids, tannins, sterols and/or terpenes, phenolics and amino acids were detected in plant leaves and stem. Cardiac glycosides were detected in plant leaves but not detected in plant stem. The volatile oils and anthraquinones were not detected in both leaves and stem as represented in table (1).

Table (1): Phytochemical constituents in leaves and stem of *F. cordata* Thunb. subsp. *salicifolia* (Vahl)

Bioactive constituents	Leaves	Stem
Volatile oils	-ve	-ve
Anthraquinones	-ve	-ve
Glycosides and/or carbohydrates	+ve	+ve
Saponins	+ve	+ve
Alkaloids	+ve	+ve
Flavonoids	+ve	+ve
Tannins	+ve	+ve
Sterols and/or terpenes	+ve	+ve
Cardiac glycosides	+ve	-ve
Phenolics	+ve	+ve
Amino acids	+ve	+ve

+ve: presence –ve: absence

- ***Flavonoids &phenolics quantification***:

Table (2) declared that stem extract contains the highest total flavonoids and phenolics.

Table (2): Total flavonoids and phenolics concentration in leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl)

Item	Mean ± SE	
	Leaves	Stem
Total flavonoids (mg/gm rutin)	283.70 ± 2.028	316.00± 1.528
Total phenolics (mg/gm gallic acid)	280.70 ± 2.906	329.70± 1.202

Data are presented as mean ± SE for 3 replicates.

***Acute toxicity & median lethal dose (LD_{50}) of leaves and stem of F. cordata* Thunb. subsp. *salicifolia* (Vahl)**

Tables (3&4) illustrated that, the lethal dose (LD_{100}) was 4500 & 5500 mg/kg b.w. for leaves and stem extracts, respectively. So, LD_{50} calculated for leaves and stem extracts was 3600 & 4600 mg/kg b.w., respectively. Thus, the used doses were 1/10 and 1/20 of LD_{50} which were 460 & 230 mg/kg b.w. for stem extract and 360 & 180 mg/kg b.w. for leaves extract.

Table (3): Calculated LD_{50} of *Ficus cordata* Thunb. Subsp. *salicifolia* (Vahl) leaves extract

Groups	Dose (mg/ml)	No. of dead animals	
1	Vehicle	0	
2	500	0	
3	1000	0	
4	1500	0	
5	2000	0	
6	2500	0	
7	3000	1	
8	3500	5	
9	4000	7	
10	4500	10	
Groups	**Dose difference (a)**	**Mean mortality (b)**	**Probit (aXb)**
2	0	0	0
3	500	0	0
4	500	0	0
5	500	0	0
6	500	0	0
7	500	0.5	250
8	500	3	1500
9	500	6	3000
10	500	8.5	4250

$$LD_{50} = LD_{100} - \sum (aXb)/ n$$
$$LD_{50} = 4500 - 9000/10$$
$$LD_{50} = 3600 \text{ mg/kg b.w.}$$

Table (4): Calculated LD_{50} of *Ficus cordata* Thunb. Subsp. *salicifolia* (Vahl) stem extract

Groups	Dose (mg/ml)	No. of animals dead	
1	Vehicle	0	
2	500	0	
3	1000	0	
4	1500	0	
5	2000	0	
6	2500	0	
7	3000	0	
8	3500	0	
9	4000	2	
10	4500	5	
11	5000	6	
12	5500	10	
Groups	**Dose difference (a)**	**Mean mortality (b)**	**Probit (aXb)**
2	0	0	0
3	500	0	0
4	500	0	0
5	500	0	0
6	500	0	0
7	500	0	0
8	500	0	0
9	500	1	500
10	500	3.5	1750
11	500	5.5	2750
12	500	80	4000

$$LD_{50} = LD_{100} - \sum (aXb)/ n$$
$$LD_{50} = 5500 - 9000/10$$
$$LD_{50} = 4600 \text{ mg/kg b.w.}$$

Antioxidative efficacy

Data present in table (5) illustrated that lead acetate administration induced significant elevation in the concentration of testicular MDA ($p<0.001$) and reduced the activity of SOD & GPx ($p<0.001$) compared to normal control group.

Unfortunately, oral administration of leaves extract at doses 360 and 180 mg/kg b.wt and stem extract at dose 230 mg/kg b.wt showed non signicant changes in testicular SOD & GPx as well as MDA compared with Pb-intoxicated group.

Oral administration of stem extract at dose 460 mg/kg b.wt showed significantly increased ($p<0.001$) in the testicular GPx & SOD, while MDA was significantly decreased ($p<0.01$) as compared to Pb-intoxicated group.

Table (5): Testicular concentration of malondialdehyde (MDA) and testicular activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the different experimental groups expressed as Mean ±SE.

Parameters / Groups and treatments	MDA (nmol/g. tissue)	SOD (U/g. tissue)	GPx (U/g. tissue)
Group A	14.52 ± 0.714	208.3 ± 3.017	24.96 ± 0.870
Group B	27.42 ± 2.097***	105.5 ± 6.754***	10.24 ± 0.996***
Group C	26.52 ± 2.625	105.1 ± 5.039	11.0 ± 1.136
Group D	22.98 ± 1.979	110.0 ± 5.662	11.86 ± 1.500
Group E	19.54 ± 1.021**	199.6 ± 5.442***	17.56 ± 0.568***
Group F	21.74 ± 1.154	113.7 ± 3.089	13.10 ± 1.223

: Significant at P < 0.01 *: Significant at P < 0.001.
Each group contains 10 rats.

DISCUSSION

Lead (Pb) is a widely distributed industrial metal and it also is naturally present in the environment. It is an environmentally persistent element and a major

global environmental hazard ***(Gillis, et al., 2012)***. Lead may cause cell damage, impairment of enzymes, functions or alter genetic material (DNA) ***(David, 2001)***. As environmental exposure to lead has increased, the toxic effects of it on various organ systems in the body have been recognized ***(Lyn, 2006)***.

It is known that, lead causes biochemical and physiological dysfunctions in humans and laboratory animals ***(Zhang, et al., 2010; Kalender, et al., 2013)***.

This study revealed that, lead intoxicated group for 60 consecutive days significantly reduced GPx and SOD activities in the testicular tissue homogenate compared to control animal. These results agree well with those of ***Roy, (2009) and Mohsen Vigeh, et al. (2012).*** Significantly elevation in MDA concentration was observed compared to control group which is in accordance with those of other studies ***Patra, et al. (2001) and Lyn, (2006)***.

Lead toxicity leads to the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide, that lead to direct depletion of antioxidant reserves as lead has also been shown to suppress blood levels of the antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) ***(Sugawara, et al., 1991; Han, et al., 2005)***. In any biological system where ROS production increases, antioxidant reserves are depleted and this resulted in oxidative stress and this was well extrapolated from the increase in lipid peroxidation products (LPP). An increase in LPP damages various cellular components of tissues as which occurred in testicular tissue ***(Batra, et al., 1998)***.

Malondialdehyde (MDA) is a good marker of membrane lipid peroxidation, resulting from the interaction between ROS and cellular membrane ***(Durak, et al.,***

2010). Lead has been documented to increase the MDA levels in various rat tissues such as liver, brain, kidney and testis ***(Flora, et al., 2004; El-Sokkary, et al., 2005; Sainath, et al., 2011)***. In the present study, MDA increased in the lead intoxicated group. This elevation in MDA concentration could be due to an increase in free radicals, resulting from the induction of oxidative stress.

Mammalian cells possess non-enzymatic and enzymatic antioxidant defense systems to prevent the oxidative stress that interact with and inactivate ROS ***(Farmand, et al., 2005; Demir, et al., 2011)***. SOD plays an important role in protecting the toxic effects of superoxide radical ***(Uzunhisarcikli and Kalender, 2011)*** and catalyzes the conversion of superoxide radicals to hydrogen peroxide ***(Uzun, et al., 2010)***. Therefore, increase in the SOD activity has been reported to be due to the first line of defense against free radicals and oxidative stress ***(Kalender, et al., 2013)***. GPx, a selenoenzyme, plays a major role in the reduction of hydrogen peroxide and hydroperoxide to non-toxic products ***(Renugadevi and Prabu, 2010)***.

In the current study, the administration of stem extract (460 mg/kg b.wt) per day for 60 consecutive days to toxicated rats (group E) improved the activity of GPx and SOD and reduced MDA concentration compared to lead acetate treated group. The potential effect of stem extract of *Ficus cordata* Thunb. Subsp. *salicifolia* (Vahl) was due to the presence of flavonoids and phenolics that possess antioxidant and radical scavenging activities ***(Sumczynski, et al., 2016)***.

Flavonoids inhibit oxidases ***(Procházkováa, et al., 2011***) and able to scavenge free radicals directly by hydrogen atom donation ***(Heim, et al., 2002)***. Other possible mechanism by which flavonoids act is through interaction with various antioxidant enzymes. Furthermore, some effects may be a reset of a

combination of radical scavenging and the interaction with enzyme functions ***(Nijveldt, et al., 2001)***.

Phenolic compounds have been exploited as scavengers and inhibitors due to their antioxidant, antibacterial, anti-allergic, anti-inflammatory, anti-aging, and anti-tumor properties ***(Ayoub, et al., 2016)***. Therefore, phenolic compounds are increasingly applied in food, pharmaceutical and cosmetic productions ***(Vázquez, et al., 2008)***. Thus, the antioxidant activity of the extract may be attributed to its phenolics and flavonoids content.

CONCLUSION

These data suggested that, stem extract of *Ficus cordata* Thunb. Subsp. *salicifolia* (Vahl) possess antioxidant effects due to its high content of phenolics and flavonoids which reduced the oxidative stress in testes of rats intoxicated by lead.

REFERENCES

Ayoub, M.; de Camargo, A.C. and Fereidoon, S. (2016). Antioxidants and bioactivities of free, esterified and insoluble-bound phenolics from berry seed meals. Food Chem.; 197: 221–232.

Batra, N.; Nehru, B. and Bansal, M.P. (1998): The effect of zinc supplementation on the effects of lead on the rat testis. Reprod Toxicol; 21: 507-512.

Chun, K.; Kim, D. and Lee, C.Y. (2013). Composition of polyphenols and antioxidant activity of rabbit eye blueberry (*Vacciniu mashei*) in Nanjing. Journal of Agriculture and Food chemistry; 51: 8067-8072.

Clarke, E.G.C. (1975). Isolation and identification of drugs. London, UK: Pharmaceutical Press; 2: 905.

David, J.F. (2001). Symptoms toxicity of heavy metals. J. American Med. Asso.; 260: 1523 – 1533.

Demir, F.; Uzun, F.G.; Durak, D. and Kalender, Y. (2011). Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. Pestic Biochem Phys.; 99: 77-81.

Drummond, R.B. and Coates Palgrave, K. (1973). Common trees of the Highveld. Longman Rhodesia; 434: 81 - 83.

Durak, D. Kalender, S.; Uzun, F.G.; Demir, F. and Kalender, Y. (2010). Mercury chloride-induced oxidative stress and the protective effect of vitamins C and E in human erythrocytes *in vitro*. Afr J Biotechnol.; 9 (4): 488- 495.

El-Sokkary, G.H.; Abdel-Rahman, G.H. and Kamel E.S. (2005): Melatonin protects against lead-induced hepatic and renal toxicity in male rats. Toxicology; 213: 25-33.

Farmand, F.; Ehdaie, A.; Roberts, C.K. and Sindhu, R.K. (2005): Lead induced dysregulation of superoxide dismutases, catalase, glutathione peroxidase, and guanylate cyclase. Environ Res.; 98: 33-39.

Flora, S.J.S.; Pande, M.; Bhadauria, S. and Kannan, G.M. (2004). Combined administration of taurine and meso 2,3 dimercaptosuccinic acid in the treatment of chronic lead intoxication in rats. Hum Exp Toxicol.; 23: 157-166.

Gillis, B.S; Arbieva, Z. and Gavin, I.M. (2012): Analysis of lead toxicity in human cells. BMC Genomics, 313:344.

Ham, J.S.; Kim, H.Y. and Lim, S.T. (2015). Antioxidant and deodorizing activities of phenolic components in chestnut inner shell extracts. Ind. Crops Prod.; 73: 99–105.

Han, S.G.; kim, Y.; kashon, M. L. (2005). Correlates of oxidative stress and free radical activity in serum from asymptomatic shipyard welders. Am. J. Respirr Cit Care Med.; 172:1541-1548.

Harborne, J.B. (1984). In "Phytochemical Methods", Chapman and Hall. London, 287 pp.

Harborne, J.B. (1998). Methods of Extraction and Isolation. In: Phytochemical Methods, 3rd ed. Chapman & Hall, London. 60-66.

Heim, K.E.; Tagliaferro, A.R. and Bobilya, D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. J. Nutr. Biochem; 13: 572–84.

Ka¨hko¨nen, M.P.; Hopia, A.I.; Vuorela, H.J.; Rauha, J.P., Pihlaja, K.; Kujala, T.S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, 47; 3954–3962.

Kalender, S.; Uzun, F.G.; Demir, F.; Uzunhisarcikli, M. and Aslanturk, A. (2013). Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food Chem Toxicol.; 55: 456-462.

Karber, G. (1931). Beitrag zur kollecktiven Behandlung pharmakologischer Reihenversuche. Arch. Exptl. Pathol. Pharmakol.; 162: 480-483.

Lewi, P.J. and Marsboom, R.P. (1981). Page 197 in: Toxicology reference data-wistar rat. Elsevier/ North Holland Biomedical Press: New York.

Lyn, P. (2006): Lead toxicity, a review of the literature. Part 1: Exposure, evaluation and treatment. Altern. Med. Rev.; 11: 2-22.

Mabry, T.J.; Markham, K.R. and Thomas, M.B. (1970). In "The Systematic Identification of Flavonoids." Springer-Verlag, New York, Heidlberg, Berlin, 354 pp.

Malla, M.Y.; Sharma, M.; Saxena, R.C.; Mir, M.I.; Mir, A.H. and Bhat, S.H. (2013). Phytochemical screening and spectroscopic determination of total phenolic and flavonoid contents of *Eclipta alba* Linn, J. Nat. Prod. Plant Resour.; 3 (2):86-91.

Mohsen Vigeh, M.D.; Derek, R.S. and Ping-Chi, H. (2012). How does lead induce male infertility? Enviro. Research, 112: 225–229.

Nijveldt, R.J.; Nood E.; Hoorn D.E.C.; Boelens, P.G., Norren, K. and Leeuwen, P.A.M. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr.; 74: 418–25.

Nishikimi, M.; Roa, N.A. and Yogi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Bioph. Res. Common.; 46 (2): 849 - 854.

Ohkawa, H.; Ohishi, W. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem.; 95 (2): 351-358.

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

Patra, R.C.; Swarup, D. and Dwived, S.k. (2001). Antioxidant effects of α tocopherol, ascorbic acid and -methionine on lead induced oxidative stress to

the liver, kidney and brain in rats". Ind. Vet. Research Instit. Izatnagar, India, 32: 35–38.

Patwardhan, B. (2005). Ethnopharmacology and drug discovery. J. Ethnopharmacol.; 100(1–2): 50–52. doi:10.1016/j.jep.2005.06.006.

Procházkováa, D.; Boušováb, I. and Wilhelmováa, N. (2011). Antioxidant and prooxidant properties of flavonoids. Fitoterapia; 82: 513–523.

Rad, J.S.; Alfatemi, S.M.H. and Rad, M.S. (2014). *In vitro* assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts against multidrug resistant gram-positive and gram-negative bacteria. Int. J. Bio. Sci.; 4 (6): 217–222.

Renugadevi, J. and Prabu, S.M. (2010). Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp. Toxicol. Pathol.; 62: 171-181.

Rice Evans, C.; Miller, N.J.; Bolwell, G.P.; Bramley, P.M. and Pridham, J.B. (1995). The relative antioxidant activities of plant derived polyphenolic flavonoids. Free Radical Research, 22: 375–383.

Roy, C.A. (2009). Recent advances in heavy metals induced effect on male reproductive function. J. Med. Sci.; 2 (2): 37 - 42.

Sainath, S.B.; Meena, R.; Supriya, C.h.; Reddy, K.P. and Reddy, P.S. (2011). Protective role of *Centella asiatica* on lead-induced oxidative stress and suppressed reproductive health in male rats. Environ Toxicol Pharmacol.; 32: 146-154.

Sugawara, E.; Nakamura, k. and Miyake, T. (1991). Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. Br. J. Ind. Med.; 48:239-242.

Sumczynski, D.; Kotásková, E.; Družbíková, H. and Mlcˇek, J. (2016). Determination of contents and antioxidant activity of free and bound

phenolics compounds and *in vitro* digestibility of commercial black and red rice (*Oryza sativa* L.) varieties. Food Chem.; 211: 339–346.

Uzun, F.G.; Demir, F.; Kalender, S.; Bas, H. and Kalender, Y. (2010). Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food Chem Toxicol.; 48: 1714-1720.

Uzunhisarcikli, M. and Kalender, Y. (2011). Protective effects of vitamins C and E against hepatotoxicity induced by methyl parathion in rats. Ecotox Environ Safe.; 74: 2112-2118.

Vázquez, G.; Fontenla, E.; Santos, J.; Freire, M.S.; González-Álvarez, J. and Antorrena, G. (2008). Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. Ind. Crops Prod.; 28: 279–285.

Zhang., R; Niu, Y.; Li, Y.; Zhao, C.; Song, B.; Li, Y and Zhao, Y. (2010). Acute toxicity study of the interaction between titanium dioxide nanoparticles and lead acetate in mice. Environ Toxicol Pharmacol.; 30: 52-60.