Journal of

Pharmacology and Toxicology

Protective Effect of *Moringa oleifera* Lam. and *Lannea kerstingii* Extracts Against Cadmium and Ethanol-induced Lipid Peroxidation

[1]A. Diallo, [1]K. Eklu-Gadegkeku, [2]T. Mobio, [2]S. Moukha,
[1]A. Agbonon, [1]K. Aklikokou, [2]E.E. Creppy and [1]M. Gbeassor
[1]Department of Animal Physiology, Faculty of Sciences, University of Lome, Togo
[2]Department of Toxicology, Laboratory of Toxicology and Applied Hygiene,
University Victor Segalen Bordeaux 2, Bordeaux, France

Abstract: The present study had evaluated the protective effect of hydroalcoholic (50-50: v/v) and aqueous extracts of *L. kerstingii* and *M. oleifera* against lipid peroxidation induced *in vivo* and *in vitro* by either cadmium or ethanol. In a first series of experiments, lipid peroxidation induced *in vitro* by cadmium ($5 \ \mu g \ mL^{-1}$) is decreased by hydroalcoholic extracts of *M. oleifera* and *L. kerstingii* ($100 \ \mu g \ mL^{-1}$) by 94% and 50% (p<0.001) respectively whereas their aqueous extracts ($100 \ \mu g \ mL^{-1}$) reduced the cadmium induced lipid peroxidation by 94% (p<0.001) and 44% (p<0.001) respectively. *In vivo*, the pretreatment with hydroalcoholic extracts of *M. oleifera* and *L. kerstingii* at $1 \ g \ kg^{-1}$ b.wt. reduced significantly ethanol-induced lipid peroxidation, in liver, by 53 and 50% (p<0.001), respectively. Similar results were found in the kidney even though lipid peroxidation is slightly increased by ethanol in this organ.

Key words: Antioxidative effect, free radicals, *Moringa oleifera*, *Lannea kerstingii*, cytotoxicity

INTRODUCTION

Free radicals are responsible for lipid peroxidation and have received much attention recently in connection with a variety of pathologies such as cancer (Zhu *et al.*, 2002).

Cadmium a very toxic and ubiquitous metal and ethanol (95%) are known to induce lipid peroxidation (Traore *et al.*, 2000; Bashandy and Alhazza, 2008). It is also known that the most effective way to prevent oxidative damages remains the use of antioxidant substances or nutrients which can be found in fruits and vegetables (Keen *et al.*, 2005; Njayou *et al.*, 2008).

Moringa oleifera Lam. (Moringaceae) and *Lannea kerstingii* Engl. and K. Krause (Anacardiaceae) are medicinal plants respectively used to treat asthenia and anaemia. Pharmacological studies of extracts of *M. oleifera* have revealed several properties such as hypolipidaemic (Mehta *et al.*, 2003), hypotensive (Faizi *et al.*, 1998), anticancer (Costa-Lotufo *et al.*, 2005) and hepato-protective (Pari and Kumar, 2002). *Lannea kerstingii* has been studied for its trypanocidal effect (Atawodi *et al.*, 2003).

Based on the above-mentioned report, we have undertaken this work in order to study (1) the protective effect of *M. oleifera* leaves and *L. kerstingii* stem bark extracts against lipid peroxidation induced in mouse by ethanol and in Caco-2 cells by cadmium, (2) the cytotoxicity on Caco-2 cells and (3) the toxicity of extracts of *M. oleifera* leaves and *L. kerstingii* barks given orally to mice.

Corresponding Author: Aboudoulatif Diallo, Department of Animal Physiology, Faculty of Sciences,
University of Lome, Togo Tel: +2282351525 Fax: +2282218595

MATERIALS AND METHODS

Chemicals

Dulbecco's Modified Eagle Medium (DMEM), Foetal Calf Serum (FCS), Ethylenediamine Tetraacetic Acid (EDTA), Phosphate-Buffered Saline (PBS), alpha-tocopherol (vitamin E) and trypsine-EDTA mixture were purchased from Sigma-Aldrich (Lyon, France). All other chemicals used were of analytical grade.

Plant Materials

Moringa oleifera leaves and *Lannea kerstingii* barks were collected locally from rural areas close to Lome (Togo) in September 2005. They were identified by the Department of Botany, University of Lome. A specimen of each plant was also deposited in the herbarium of the Department of Botany. The extraction was conducted in Animal Physiology Department, Faculty of Sciences, University of Lome, Togo.

Leaves and barks were shade dried and pulverised. The powder was extracted with water or ethanol-water (50-50: v/v). Dried powder was soaked in water and heated in bath water for 3 h (aqueous extract) or soaked in ethanol-water for 48 h (hydroalcoholic extract). Each extracted solution was filtered and evaporated by using a rotary evaporator. *Moringa oleifera* yielded respectively 18.67 and 21.47% for aqueous and hydroalcoholic extraction. *Lannea kerstingii* yielded 10.41% for the aqueous extract and 15.90% for the hydroalcoholic extract. Extracts were dissolved in double distilled water for final use.

Animals

Male and female mice (n = 38) weighing between 18 and 20 g were used for the *in vivo* experiments. The animals were purchased from the animal centre R. Janvier (France). They were housed in large cages in an environmentally controlled condition and they were fed on Standard Laboratory chow with water *ad libitum* and were acclimated for a week before experiments. This study was conducted in Toxicology Department, Laboratory of Toxicology and Applied Hygiene, University Victor Segalen Bordeaux 2, France.

Cell Culture

The Caco-2 cells, a human colon cancer line, were obtained from Dr. Jing Yu, (Tuffs School of medicine; Medford, Mass., USA). Cells were routinely cultured in a humidified 5% CO_2 -95% air mixture at 37°C and were grown in DMEM medium, (Sigma, France), supplemented with 10% foetal bovine serum, 8 mM L-glutamine, penicillin (100 μl mL^{-1}) and streptomycin (100 μg mL^{-1}).

Induction of Lipid Peroxidation by Cadmium in Caco-2 Cells

Lipid peroxidation in Caco-2 cells was induced according to the method described by Matias *et al*. (1999). Briefly, Caco-2 cells were cultured (1×10^5 cells mL^{-1}) in 24 wells non coated microplates, for 19 h at 37°C. Plant extracts (100, 200 and 300 μg mL^{-1}) and vitamin E (50 μg mL^{-1}) were added. After 24 h of contact, the supernatant was removed. Cadmium was added for 24 h. Then cells were trypsinised, centrifuged and resuspended in SET buffer (0.1 M NaCl, 20 mM EDTA, 50 mM Tris-HCl, pH 8.0) for the determination of MDA level.

Induction of Lipid Peroxidation by Ethanol in Mouse Liver and Kidney after the Administration of Plant Extracts

The protective effect of hydroalcoholic extracts of *M. oleifera* and *L. kerstingii* on the liver and kidney lipid peroxidation was determined by the MDA-TBA adduct according to the modified method

described by Shieh *et al.* (2001). Briefly, the mice were divided into eight groups (n = 4): I (control); II (ethanol,); III or IV, V or VI and VII or VIII (respectively, ethanol 95% + *M. oleifera* or *L. kerstingii* extract 1, 2 and 3 g kg^{-1} b.wt.). Groups III, IV, V, VI, VII and VIII, received orally 0.1 mL of ethanol 30 min after plants extract administration. Group I received only tap water and group II only ethanol. One hour after the treatment with ethanol, all mice were sacrificed under ether anaesthesia. The liver and kidney of each animal were excised, rinsed in ice cold saline to clear them of blood, weighed and homogenised in SET buffer (approximately 10% w/v) by using a potter Elvehjem homogenizer with a Treflon pestle. Homogenates were then centrifuged at 600 g for 5 min at 4°C. One milliliter of each supernatant was centrifuged at 10,000 g for 15 min at 4°C and final supernatants were used to determine lipid peroxidation.

Determination of MDA-TBA Adduct

Lipid peroxidation was measured by quantification of MDA-TBA adducts formed during incubation, as previously described by Traore *et al.* (2000) and related to the protein content of tissue homogenates. The protein content was determined through the use of Bradford (1976) method. The same method was applied to Caco-2 cells homogenates.

Neutral Red Uptake Assay

Caco-2 cells were seeded in 96-wells microplates 10000 cells 200 µL^{-1} well^{-1} and routinely cultured in a humidified incubator for 24 h. Cells were maintained in culture and exposed to plant extracts over a range of concentrations (50-500 µg mL^{-1}). After 72 h exposure to extracts, Neutral Red (NR) uptake test was performed according to the procedure described by Yusup *et al.* (2005). Briefly, at the end of the treatment (72 h), the medium with or without extracts was discarded and 200 µL of freshly prepared neutral red solution (50 µg mL^{-1}) were added to each well. Cells were then re-incubated for an additional 4 h at 37°C. Thereafter, the cells were carefully washed twice with 200 µL of PBS to eliminate extracellular NR. The incorporated dye was eluted from the cells by adding 200 µL elution medium (50% ethanol supplemented with 1% acetic acid, v/v) to each well followed by gentle shaking of the microplate for 15 min. The plates were then read at 540 nm using a microplate reader (Dynatech MR 4000, Dynatech, Boston, MA, USA).

Survived cells in treated wells were expressed as percentage of control wells. The IC$_{50}$ (50% viability inhibitory effect) was determined and expressed in µg mL^{-1}.

MTT Assay

This test was carried out according to the method described by Kouadio *et al.* (2005). Caco-2 cells were seeded in 96-wells microplates (10000 cells 200/µL/well) and routinely cultured in a humidified incubator for 24 h. Cell culture media were removed and extracts were added in concentration ranging from 50 to 500 µg mL^{-1}. Cells were then incubated for 72 h. In this test, a control group (DMEM without extract) and a blank group (without cells or medium) were also included. The medium with or without extract was then discarded and 100 µL of tetrazolium salt MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) solution (0.5 mg mL^{-1} in DMEM) were added to each well. Cells were re-incubated for an additional 2 h; 100 µL of 10% SDS in 0.01 M HCl were added to each well to dissolve the formazan crystals. The plates were then read on a microplate reader (DYNATECH MR 4000, Dynatech, Boston, MA, USA) at 560 nm. Four wells were used for each concentration of extract.

Acute Toxicity Test

The limit test dose of 5,000 mg kg^{-1} was used as described by the Organization for Economic Cooperation Development (OECD, 2002) guideline. Three female mice each sequentially dosed at

interval of 48 h (short term observation period) were used for the test. Animals were observed individually for any sign of acute toxicity and behavioural changes 1 hour post dosing and at least once daily for 14 days.

Statistical Analysis

The results are expressed as Mean±SEM. Statistical analysis was performed by Analyse of Variance (ANOVA) followed by Fischer LSD test using Systat 5.0. Results were considered significant if the probability $p < 0.05$.

RESULTS

Cadmium increased significantly ($p < 0.001$) Caco-2 cells MDA level by 925% (Table 1) and ethanol increased the liver and kidney MDA level (Table 2), respectively by 48% and 16% ($p < 0.05$) compared to control.

Pretreatment of Caco-2 cells with vitamin E (positive control), aqueous (100, 200 and 300 µg mL^{-1}) and hydroalcoholic extract (100, 200 and 300 µg mL^{-1}) of *M. oleifera* decreased MDA level in cadmium-treated cells. This effect did not appear concentration dependant. The treatment of Caco-2 cells with aqueous (100 µg mL^{-1}) and hydroalcoholic extract (100 µg mL^{-1}) of *L. kerstingii* also decreased significantly ($p < 0.001$) cadmium-induced MDA production by 44% and 50%, respectively. At 200 µg mL^{-1} and 300 µg mL^{-1}, *L. kerstingii* hydroalcoholic and aqueous extract was more effective (Table 1). Similarly to *M. oleifera* extracts this effect did not appear concentration dependant. *M. oleifera* and *L. kerstingii* administered orally showed a significant decrease ($p < 0.001$) of MDA level

Table 1: Effect of hydroalcoholic and aqueous extracts of *L. kerstingii* and *M. oleifera* on MDA level following lipoperoxidation induced with cadmium (5 µg mL^{-1}) in Caco-2 cells

Groups	Concentration (µg mL^{-1})	MDA (pmoles mg^{-1} of protein)
Control	-	364±32
Cd	5	3368±242[###]
Cd+Vitamin E	50	256±39**
Cd+hydroalcoholic extract of *L. kerstingii*	100	1897±240**
-	200	580±172**
-	300	495±253**
Cd+aqueous extract of *L. kerstingii*	100	1694±240**
-	200	422±143**
-	300	319±179**
Cd+hydroalcoholic extract of *M. oleifera*	100	203±39**
-	200	268±3**
-	300	223±34**
Cd+aqueous extract of *M. oleifera*	100	209±36**
-	200	252±5**
-	300	202±29**

Data are Means±SEM. **Significantly different as compared to cadmium with $p < 0.001$, [###]Significantly different as compared to control with $p < 0.001$

Table 2: Effect of hydroalcoholic extracts of *L. kerstingii* and *M. oleifera* on MDA level in liver and kidney, after lipoperoxidation induction with ethanol 95%

Groups	Dose (g kg^{-1} body wt.)	MDA (pmoles mg^{-1} of protein) in liver	MDA (pmoles mg^{-1} of protein) in kidney
Control	-	2266±98	1960±65
Ethanol 95%	-	3356±193[###]	2268±105[#]
Ethanol + extract of *L. kerstingii*	1	1562±47**	2090±167
-	2	1372±169**	1759±9*
-	3	1453±85**	1659±60**
Ethanol + extract of *M. oleifera*	1	1682±149**	2209± 113
-	2	1252±164**	1571±91**
-	3	1735±60**	2040±66*

Data are Means±SEM. **Significantly different as compared to ethanol with $p < 0.001$. *Significantly different as compared to ethanol with $p < 0.05$, [###]Significantly different as compared to control with $p < 0.001$, [#]Significantly different as compared to control with $p < 0.05$

Table 3: Effect of hydroalcoholic and aqueous extracts of *L. kerstingii* and *M. oleifera* on Caco-2 cells viability as measured by MTT assay and neutral red uptake

Extracts	IC_{50} ($\mu g\ mL^{-1}$)	
	MTT assay	Neutral red uptake assay
Hydroalcoholic extract of *L. kerstingii*	186	328
Aqueous extract of *L. kerstingii*	228	386
Hydroalcoholic extract of *M. oleifera*	284	274
Aqueous extract of *M. oleifera*	>500	>500

in liver by 53% and 50% compared to ethanol-treated group. Similar results were obtained, in liver, when extracts were administered at either 2 or 3 g kg⁻¹ b.wt. But in kidney, pretreatment with hydroalcoholic extract of *M. oleifera* and *L. kerstingii* at 1 g kg⁻¹ body wt did not decrease the level of MDA (Table 2). Furthermore the administration of *L. kerstingii* hydroalcoholic extract at 2 g kg⁻¹ body wt and 3 g kg⁻¹ body wt was more effective and decreased kidney MDA level respectively by 22% ($p<0.05$) and 26% ($p<0.001$). *M. oleifera* hydroalcoholic extract at 2 g kg⁻¹ body wt reduced also the kidney MDA level by 31% ($p<0.001$). But, at 3 g kg⁻¹ body wt, *M. oleifera* showed higher MDA production as compared to 2 g kg⁻¹ body wt (Table 2).

The limit dose of 5 g kg⁻¹ did not cause any mortality or any signs of acute toxicity in any of the three rats tested in the short term (i.e., 48 h) and long term (i.e., 14 days) observatory period. The LD_{50} of the extract according to OCDE guidelines is therefore greater than 5 g kg⁻¹.

The hydroalcoholic extracts of *L. kerstingii* and *M. oleifera* were more efficient in reducing cell viability than their aqueous extracts (Tables). According to MTT assay *L. kerstingii* is the most cytotoxic exhibiting a lower IC_{50} value, but neutral red uptake assay showed that hydroalcoholic extract of *M. oleifera* has the lower IC_{50}.

DISCUSSION

This study aimed to evaluate the protective effect of *M. oleifera* leaves and *L. kerstingii* stem bark extracts against lipid peroxidation induced in mouse by ethanol and in Caco-2 cells by cadmium. It has been demonstrated previously that cadmium and ethanol increase free radicals production (Shieh *et al.*, 2001; Ognjanovic *et al.*, 2003; Bashandy and Alhazza, 2008).

Several studies had demonstrated the protective effect of plant extracts against lipid peroxidation (Rahmat *et al.*, 2004; Costa-lotufo *et al.*, 2005). This effect is due to the presence of some potent free oxygen radicals scavengers such as: polyphenol, terpenoid, sulphide, carotenoid, coumarin, saponin, curcumin, sterol and vitamins in these extracts (Winston, 1999).

Moringa oleifera is known to be a source of antioxidants, because of its total phenolic (Bajpai *et al.*, 2005), vitamin A (Nambiar and Seshadri, 2001) and vitamin E (Ching and Mohamed, 2001) contents. In our experimental conditions, *M. oleifera* showed an *in vitro* antioxidant activity, comparable to vitamin E (50 µg mL⁻¹) which is known to reduce lipid peroxidation (Ognjanovic *et al.*, 2003).

In comparison to *M. oleifera*, very little is known about *L. kerstingii*. In our study, *L. kerstingii* showed a remarkable protective effect on lipid peroxidation. These results are in line with previous data from Diallo *et al.* (2001) who reported that *Lannea velutina*, a species very close to *L. kerstingii*, has antioxidant and radical scavenging activities.

In kidney, the higher dose of *M. oleifera* tested (3 g kg⁻¹ b.wt.) showed lower antioxidant activity as compared to 2 or 1 g kg⁻¹ b.wt. This could be explained by the possible shift from antioxidant activity to pro oxidant activity when doses or concentrations are increased. Similar results are reported in the literature else where (Tafazoli *et al.*, 2005).

The result of the acute toxicity study indicated that the LD_{50} of the aqueous and hydroalcoholic extracts of *M. oleifera* leaves and *L. kerstingii* stem bark are more than 5,000 mg kg⁻¹. The limit test is primarily use in situations where the investigator has information indicating that the test material is likely to be non-toxic or of low toxicity (OCDE , 2002). The result of the acute oral toxicity study

therefore suggest that the extracts of *M. oleifera* leaves and *L. kerstingii* stem bark at the limit dose tested are essentially non-toxic and safe in oral formulation. The Caco-2 cells viability test indicated that hydroalcoholic extracts were more cytotoxic than aqueous extracts and *L. kerstingii* appears more cytotoxic than *M. oleifera*.

These results were however obtained with quite large amount of plant extracts (50 µg mL^{-1} and up to 500 µg mL^{-1}) due to overall low toxicity of these plants used in traditional medicine in Africa. Many studies had showed a cytotoxic activity of *M. oleifera* against tumour cell lines (Mehta *et al.*, 2003; Costa-lotufo *et al.*, 2005). The IC50 values obtained using MTT test were in HL-60, CEM, HCT-8 and B16 cells, 60, 12.7, 113.8 and 28.8 µg mL^{-1} respectively. In our investigation, the IC50 values in MTT test were 284 µg mL^{-1} for hydroalcoholic extract and over 500 µg mL^{-1} for aqueous extract but Caco-2 cells are known to be very resistant to cytotoxic drugs (Kralj *et al.*, 2003).

The values of IC$_{50}$ were lower in MTT test than in NR test except the case of *M. oleifera*. These two viability tests do not measure the same parameters. The MTT test measures mitochondrial activity, while NR test measures the integrity of endosomes, lysosomes and membranes (Zhang *et al.*, 1990). Active substances of *L. kerstingii* may target mitochondria, while *M. oleifera* extract seemed to target membranes, endosome and lysosome and reticulum endoplasmic.

In the present experiment, pretreatment with *M. oleifera* and *L. kerstingii* reduced cadmium and ethanol induced lipid peroxidation, this effect was not dose related and it seemed that the lower doses or concentrations already display high effect. From the results mentioned above, it can be concluded that, the use of *M. oleifera* and *L. kerstingii* has the capability to alleviate many of harmful effects of cadmium and ethanol. The findings of both acute oral and cytotoxicity could be an indication that *M. oleifera* and *L. kerstingii* have some large level of safety margin in oral formulation. These results support at least partially the traditional use of *M. oleifera* and *L. kerstingii* in the treatment of many diseases. However, further investigations are needed to know the nature of the actives substances and mechanism triggering these protective effects.

ACKNOWLEDGEMENTS

REFERENCES

Atawodi, S.E., T. Bulus, S. Ibrahim, D.A. Ameh, A.J. Nok, M. Mamman and M. Galadima, 2003. *In vitro* trypanocidal effect of methanolic extract of some Nigerian savannah plants. Afr. J. Biotechol., 2: 317-321.

Bajpai, M., A. Pande, S.K. Tewari and D. Prakash, 2005. Phenolic contents and antioxidant activity of some food and medicinal plants. Int. J. Food Sci. Nutr., 56: 287-291.

Bashandy, S.A. and I.M. Alhazza, 2008. The hepatoprotective effect of â-carotene against cadmium toxicity in rats. J. Pharmacol. Toxicol., 3: 457-463.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Ching, L.S. and S. Mohamed, 2001. Alpha-tocopherol content in 62 edible tropical plants. J. Agric. Food Chem., 49: 3101-3105.

Costa-Lotufo, L.V., M.T.H. Khan, A. Ather, D.V. Wilke, C.P. Jimenez, C. Pessoa, M.E. Amaral de Moraes and M. Odorico de Moraes, 2005. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. J. Ethnopharmacol., 99: 21-30.

Diallo, D., A. Marston, C. Terreaux, Y. Toure, B. Smestad and K. Hostettmann, 2001. Screening of Malian medicinal plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. Phytother. Res., 15: 401-406.

Faizi, S., B.S. Siddiqui, R. Saleem, K. Aftab, F. Shaheen and A.H. Gilani, 1998. Hypotensive constituents from the pods of *Moringa oleifera*. Planta Med., 64: 225-228.

Keen, C.L., R.R. Holt, P.I. Oteiza, G.C. Fraga and H.H. Schmitz, 2005. Cocoa antioxidants and cardiovascular health. Am. J. Clin. Nutr., 81: 298S-303S.

Kouadio, J.H., T.A. Mobio, I. Baudrimont, S. Moukha, S.D. Dano and E.E. Creppy, 2005. Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. Toxicology, 213: 56-65.

Kralj, M., K. Husnjak, T. Körbler and P. Jasminka, 2003. Endogenous p21WAF1/CIP1 status predicts the response of human tumor cells to wild-type p53 and p21WAF1/CIP1 overexpression. Cancer Gene Ther., 10: 457-467.

Matias, W.G., A. Traore, M. Bonini, A. Sanni and E.E. Creppy, 1999. Oxygen reactive radicals production in cell culture by okadaic acid and their implication in protein synthesis inhibition. Hum. Exp. Toxicol., 18: 634-639.

Mehta, L.K., R. Balaraman, A.H. Amin, P.A. Bafna and O.D. Gulati, 2003. Effect of fruits of *Moringa oleifera* on lipid profile of normal and hypercholesterolaemic rabbits. J. Ethnopharmacol., 86: 191-195.

Nambiar, V.S. and S. Seshadri, 2001. Bioavailability trials of beta-carotene from fresh and dehydrated drumstick leaves (*Moringa oleifera*) in rat model. Plant Foods Hum. Nutr., 56: 83-95.

Njayou, F.N., P.F. Moundipa, A.N. Tchana, B.T. Ngadjui and F.M. Tchouangeup, 2008. Inhibition of microsomal lipid peroxidation and protein oxidation by extracts from plants used in Bamun folk medicine (Cameroon) against hepatitis. Afr. J. Trad. Comp. Alternative Med., 5: 278-289.

OCDE., 2002. Guidelines for the Testing of Chemicals / Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. http://www.oecdbookshop.org/oecd/display.asp?CID=&LANG=en&SF1=DI&ST1=5LMQCR2K7MZP.

Ognjanovic, B.I., S.Z. Pavlovic, S.D. Maletic, R.V. Zikic and A.S. Stajn *et al.*, 2003. Protective influence of vitamin E on antioxidant defense system in blood of rats treated with cadmium. Physiol. Res., 52: 563-570.

Pari, L. and N.A. Kumar, 2002. Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. J. Med. Food, 5: 171-177.

Rahmat, A., V. Kumar, L.M. Fong, S. Endrini and H.A. Sani, 2004. Determination of total antioxidant activity in three types of local vegetables shoots and the cytotoxic effect of their ethanolic extracts against different cancer cell lines. Asia. Pac. J. Clin. Nutr., 13: 308-311.

Shieh, Y.H., C.F. Liu, Y.K. Huang, J.Y. Yang, I.L. Wu, C.H. Lin and S.C. Lin, 2001. Evaluation of the hepatic and the renal protective effect of *Ganoderma lucidum* on mice. Am. J. Chin. Med., 29: 501-507.

Tafazoli, S., J.S. Wright and P.J. O'Brien, 2005. Prooxidant and antioxidant activity of vitamin E analogues and troglitazone. Chem. Res. Toxicol., 18: 1567-1574.

Traore, A., S. Ruiz, A.S. Baudrimont, S.D. Dano, G.J.F. Narbonne and E.E. Creppy, 2000. Combined effects of okadaic acid and cadmium on lipid peroxidation and DNA bases modifications (m5 dC and 8-(OH)-dG) in Caco-2 cells. Arch. Toxicol., 74: 79-84.

Winston, J.C., 1999. Health-promoting properties of common herbs. Am. J. Clin. Nut., 70: 491S-499S.

Yusup, A., H. Upur, I. Baudrimont, A. Umar, T. Kader, B. Begaud, E.E. Creppy and N. Moore, 2005. Cytotoxicity of abnormal Savda Munziq aqueous extract in human hepatoma (HepG2) cells. Fund Clin. Pharmacol., 19: 465-472.

Zhang, S.Z., M.M. Lipsky, B.F. Trump and I.C. Hsu, 1990. Neutral red (NR) assay for cell viability and xenobiotic-induced cytotoxicity in primary cultures of human and rat hepatocytes. Cell Biol. Toxicol., 6: 219-234.

Zhu, Q.Y., R.R. Holt, A.S. Lazarus, T.J. Orozco and C.L. Keen, 2002. Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical induced erythrocyte hemolysis. Exp. Biol. Med., 227: 321-329.