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RESEARCH ARTICLE

Evaluation of *Smilax excelsa* L. Use in Experimentally Induced Nephrotoxicity [1]

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Summary

The protective effect of an aqueous extract of the shoots and leaves of *Smilax excelsa* L. against acute carbon tetrachloride (CCl_4)-induced toxicity and the changes in antioxidative defense activities in kidney of rats were investigated. Female Wistar rats were supplied with *S. excelsa* shoots and leaves aqueous extract once a day for 9 days (orally at a dose of 100, 200 and 400 mg/kg of body weight) prior to renal injury induction through intraperitoneal injection with a single dose of CCl_4 (1 ml/kg body wt, in a 20 % v/v olive oil solution) on the 10th day. 24 h after CCl_4 intoxication serum and tissue biochemical and hispathological analyses were undertaken after sacrification under anesthesia. Administration of the extract reversed the antioxidant parameters which were impaired in CCl_4 group, in a dose dependent manner and at a dose of 400 mg/kg of body weight the levels of almost all the parameters were almost back to normal Control group. Nevertheless, the extract did not completely improve the CCl_4-induced degenerative changes observed microscopically in kidney tissue. The results of this study suggest that *S. excelsa* could protect the kidney tissue against CCl_4-induced nephrotoxicity in rats, probably by increasing antioxidative defense activities.

Keywords: *Smilax excelsa, Renal damage, Carbon tetrachloride, Antioxidant effect, Nephrotoxicity*

Deneysel Olarak Oluşturulmuş Böbrek Hasarı Üzerine *Smilax excelsa* L. Kullanımının Değerlendirilmesi

Özet

Bu çalışmada *Smilax excelsa* L.'nın genç sürgün ve yapraklarının sulu ekstresinin sıçanlarda karbon tetraklorür (CCl_4) ile deneysel olarak oluşturulmuş böbrek hasarına olan etkisi ve antioksidan savunma sistemindeki değişiklikleri incelendi. Wistar albino dişi sıçanlara, 9 gün süre ile, *S. excelsa* extresinin 100, 200 ve 400 mg/kg dozunda oral olarak uygulanmasının ardından, 10. gününde CCl_4'ün %20'lik zeytin yağındaki solüsyonunun 1ml/kg intraperitoneal verilmesiyle böbrek harabiyeti oluşturuldu. CCl_4 uygulanmasından 24 saat sonra, sıçanlar anestezi altında öldürüldü, ardından serum ve böbrek dokusunda biyokimyasal ve histopatolojik analizler yapıldı. CCl_4 grubunda bozulan tüm değerlerin, ekstrenin verilmesiyle doza bağlı olarak düzeldiği ve 400 mg/kg dozunda aşağı yukarı normal Kontrol grubu değerlerine ulaştığı gözlemlendi. Buna karşın, ekstre CCl_4 ile oluşturulan, böbrek dokusunda mikroskobik olarak gözlenen dejeneratif değişiklikleri tam olarak iyileştirmedi. Bu çalışmanın sonucunda, *S. excelsa*'nın sıçanlarda böbrek dokusunu CCl_4 ile oluşturulan hasardan koruduğu ve bu etkisini organizmanın antioksidan savunma sistemlerini arttırarak gösterdiği ileri sürülebilir.

Anahtar sözcükler: *Smilax excelsa, Böbrek hasarı, Karbon tetraklorür, Antioksidan etki, Nefrotoksisite*

INTRODUCTION

Traditional medicines and extracts from medicinal plants have been extensively used as alternative medicine for better control and management of kidney diseases.

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Plants from the genus *Smilax* are used to treat syphilis, acute bacillary dysentery, acute and chronic nephritis, eczema, dermatitis, cystitis, and mercury and silver poisoning [1]. Rhizomes of several *Smilax* species from this genus possess a variety of bioactivities including anti-cancer [2,3], anticonvulsant [4], antiinflammatory and anti-nociceptive [5-7], hepatoprotective [8-10], antihyperuricemic and nephroprotective [11] actions. A survey of the literature showed that several Smilax species contain phenyl-propanoid glycosides [12], anthocyanins [13], flavonoid glucosides [2,14] and steroid saponins [15,16]. The antioxidant potential of *Smilax* species has predominantly been derived from *in vitro* and *in vivo* studies on the rhizomes of the plant [17-19].

Smilax excelsa L. (sarsaparilla, *Liliaceae*) is a climbing scrub up to 20 m, known as "Melocan, Melvocan, Silcan, Diken otu, Mamula (Rize), Melevcen, Sıraca (Mersin), Kırçan and Çıtırgı" in Turkey. It occurs in the deciduous forests, the scrubs and the roadsides. The shoots of the plant are consumed as vegetables [20] and it is known as a medical and economic plant, as well [21]. *Smilax excelsa* is used in folk medicine for the treatment of breast cancer, stomach pain and bloating [22]. The plant used here has no scientific proof of its use in renal disorders.

As the evidence of earlier studies shows that the leaves and shoots of *Smilax excelsa* possess flavonoids and anthocyanins, which are the major chemical constituents responsible for exhibiting antioxidant activity [23] the present study has been undertaken to evaluate the protective effects of water extract of the shoots and leaves of *Smilax excelsa* in kidney tissues in CCl_4-induced oxidative stress.

MATERIAL and METHODS

Plant Material

S. excelsa L. shoots and leaves were collected in September from Istanbul, Turkey and identified by Prof. Dr. Kerim Alpinar from the Faculty of Pharmacy, Istanbul University. The shoots and leaves were separated from the other parts, washed in running tap water and dried at room temperature. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE); Herbarium code number: ISTE 81928. The dried leaves were manually ground to a fine powder before extraction.

Preparation of the Extract

80 g of ground dried shoots and leaves were extracted with boiling water (2.000 ml) for 15 min while stirring. The extracts were filtered and evaporated to dryness under reduced pressure at 40°C. The yield was 25 g. Appropriate dilutions were made before each experiment.

Animals

The experimental protocol described in the present study was approved on 19.06.2007 by the Animal Assays Ethics Comittee of Istanbul University. Female Wistar rats weighing 200-240 g were supplied from Istanbul University, Institute of Experimental Medicine (DETAE). Animals were acclimatized to their environment for one week prior to experimentation. The animals were housed in a room with a 12 h light/dark cycle at about 22°C and fed on standard diet with *ad libitum* access to drinking water.

Induction of Renal Injury

The animals were divided into 5 groups each containing 6 animals. The treatment was as follows:

Group 1 (Control), which served as normal control, received water and basal diet for 10 days.

Group 2 (CCl_4) which served as toxin control, received water and basal diet for 10 days and was treated i.p. with CCl_4 (1 ml/kg body weight in 20% olive oil, v/v) on the 10th day.

Group 3, 4 and 5 ($S_{100+CCl_4}$, $S_{200+CCl_4}$ and $S_{400+CCl_4}$) were separately treated with 100, 200 and 400 mg/kg body weight of the extract of *S. excelsa* shoots and leaves once daily for 9 days 24 h prior to CCl_4 intraperitoneal administration on the 10th day.

24 h after CCl_4 intoxication, the rats were lightly anesthetized and sacrificed. Blood samples were taken from each rat through direct intracardiac intervention and centrifuged at 3.000 x g for 10 min to separate the sera. Urea, creatinine levels, paraoxonase/aryl esterase (ARE) activity and the extent of LPO measured as MDA were determined in serum. Immediately after collecting the blood samples, the kidneys were excised, rinsed in ice-cold normal saline solution followed by ice-cold 0.15 M potassium phosphate buffer, pH 7.4, blotted, dried, and weighed. Part of kidney tissues were used for histopathological examination. With another part of kidney tissues, 10% w/v homogenates were prepared in ice-cold 0.15 M potassium phosphate buffer using Art-MICCRA D-1 homogenator and centrifuged at 13.000 rpm for 5 min at 4°C (Megafuge Hereaus 1.0R). The supernatants, thus obtained were used for the estimation of antioxidant parameters like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), myelo-peroxidase (MPO). Lipid peroxidation (LPO) reduced (GSH) and oxidized (GSSG) glutathione, GSSG/GSH, protein carbonyl content (PCC), carbonic anhydrase (CA) activity, were also estimated. All biochemical assays were done in triplicate using different homogenates. Serum and kidney samples were aliquoted and stored in a freezer (-80°C) for use in biochemical analyses.

Biochemical Assays

Shimadzu Spectrophotometer UV-(1800) was used for

all spectrophotometric measurements.

Assessment of serum biochemical parameters: Serum creatinine and urea levels were evaluated by Jaffe reaction [24], and diacetylmonooxime method [25], respectively.

Serum samples were assayed for paraoxonase/arylesterase activity using 1.0 mM phenylacetate as substrate and 0.9 mM $CaCl_2$ in 20 mM Tris/HCl, pH 8.0. The reaction was initiated by the addition of the serum sample, and the increase in the absorbance at 270 nm was recorded over a 90-s period. Enzymatic activity was calculated from the molar extinction coefficient 0.00131 $mM^{-1}cm^{-1}$. A unit of arylesterase activity was defined as 1µmol phenylacetate hydrolyzed per min under the above assay conditions [26].

Assessment of antioxidant status via antioxidant enzymes in kidney homogenates: CAT activity was measured by the ethod of Aebi [27] and expressed as mmol H_2O_2/mg protein.

SOD activity was assayed by the method described by Aruoma et al.[28]. Results were expressed as U/mg protein. One unit of SOD inhibits the rate of increase in absorbance at 560 nm by 50% under the conditions of the assay.

The activity of glutathione peroxidase (GPx) was measured using a coupled enzyme assay system linked with glutathione reductase (GR) as described by Lawrence and Burk [29].

GR activity was determined by following the oxidation of NADPH at 340 nm as described by Carlberg and Mannervik [30]. GR and GPx activities were expressed as mmol NADPH oxidized/min/mg protein using the molar extinction coefficient for NADPH at 340 nm of 6.22 mM^{-1}/ cm^{-1}.

GST activity using 1-chloro-2,4-dinitrobenzene as substrate was assayed spectrophotometrically as described by Habig and Jakoby [31]. Specific activity was expressed as mmol conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 $mM^{-1}cm^{-1}$.

Tissue MPO levels were measured according to Hillegass et al.[32]. Results were expressed as units of MPO per gram of protein of supernatant as determined by method of Lowry.

The formation of LPO products was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) levels on the basis of MDA reaction with thiobarbituric acid at 532 nm according to Buege and Aust [33]. The values of TBARS were calculated using an extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1} and expressed as nmol of MDA/g wet weight.

The levels of GSH and GSSG were measured in kidney tissue by the enzymic recycling procedure using 5,5´-dithiobis-(2-nitrobenzoic acid) (DTNB) and glutathione reductase (DTNB-GSSG reductase recycling assay) as described by Anderson [34].

The PCC was assayed by the modification of the procedure described by Reznick & Packer [35], using dinitrophenylhydrazine (DNPH) dissolved in HCl, accompanied by blanks in HCl alone. Results were expressed as nmol of protein carbonyl per mg of protein (determined on the HCl blank pellets using a BSA standard curve in 6 M guanidine-HCl and reading the absorbance at 280 nm) using a molar extinction coefficient of 22.000 $M^{-1}cm^{-1}$ for DNPH.

The *p*-nitrophenylacetate esterase activity of CA was measured by the method of Verpoorte et al.[36]. One unit of enzyme activity was expressed as mmol nitrophenol formed per minute at 0°C using a molar extinction coefficient of 5 mM^{-1}/ cm^{-1}.

Histopathological Assay

Dissected kidney tissues were taken immediately and fixed in Bouin's solution for histopathological examinations. The tissues were dehydrated and embedded in paraffin. The paraffin sections of 5 µm thickness were stained with Masson's trichrome stain (Masson) and Periodic Acid-Schiff stain (PAS). All sections were examined under Olympus-CX 41 light microscope.

Statistical Analysis

Biochemical results were evaluated using an unpaired *t*-test and ANOVA variance analysis using the NCSS statistical computer package. The values were expressed as mean ± SD. Analysis between control and experimental groups was performed using the Mann-Whitney test. $P<0.05$ was considered as significant.

RESULTS

Assessment of Serum Biochemical Parameters

Serum urea levels were significantly raised in the CCl_4 group compared to the control group ($P<0.005$). Treatment with *S. excelsa* leaf extract at 400 mg/kg dose only, restored urea levels to control values ($P_{ANOVA}=0.0001$). Thus, pretreatment of rats with aqueous extract of *S. excelsa* attenuated the CCl_4-induced rise in serum urea level confirming the protective effect of the extract on serum kidney parameters. Nevertheless no significant change in serum creatinine levels was observed ($P_{ANOVA}=0.487$; *Table 1*).

The serum TBARS (expressed as MDA) concentration in the CCl_4 treated group was significantly higher ($P<0.0001$) than that of the normal controls. Treatment with *S. excelsa* aqueous extract with 100, 200 and 400 mg/kg doses, prior to the CCl_4 administration decreased the enchanced MDA level significantly ($P<0.005$, $P<0.05$ and $P<0.0001$ respectively compared to CCl_4 group). Reduction in the levels of MDA in the group treated with 400 mg/kg aqueous extract was almost close to normal group ($P_{ANOVA}=0.0001$; *Table 1*).

CCl_4 did not produce a significant change in serum ARE levels *(Table 1)*. Whereas treatment with *S. excelsa* extracts at 200 (P<0.005 *versus* Control group) and 400 mg/kg (P<0.05 *versus* Control group) increased significantly serum ARE activity (P_{ANOVA}=0.001).

Assessment of Antioxidant Status in Kidney Homogenates

Antioxidant enzyme activities (CAT, SOD, GPx, GR, GST and MPO) in kidney of Control and tested groups are shown in *Table 2*.

No significant change in CAT activity of all the experimental groups (P>0.05) was recorded (P_{ANOVA}=0.647).

SOD activity was significantly decreased in the CCl_4 group (P<0.05 *versus* Control group), administration of the extracts at the three doses reversed this effect (P_{ANOVA}= 0.002).

Although no significant difference was seen in the CCl_4 group, the activities of GPx, GR, and GST were significantly enhanced by pre-treatment of CCl_4-intoxicated rats with *S. excelsa* extracts at 100 mg/kg, 200 mg/kg, 400 mg/kg doses compared with those of CCl_4 group (P_{ANOVA}=0.0001, 0.002, 0.008 respectively).

In the control CCl_4 treated group and the group pretreated with 100, 200 and 400 mg/kg aqueous extract of *S. excelsa* renal MPO levels were significantly (P<0.05) higher than those of the normal Control group. A significant (P<0.05) reduction was observed in the group treated with 400 mg/kg of aqueous extract of *Smilax excelsa* in comparison with those observed in the control CCl_4 treated group and the group pretreated with 100 and 200 mg/kg. However, MPO levels remained greater than control (P_{ANOVA}=0.011; *Table 2*).

Table 1. *Effect of pretreatment with Smilax excelsa L. aqueous extract after CCl_4 intoxication, on serum urea, creatinine and MDA levels and aryl esterase (ARE) activity in rats*

Tablo 1. *Sıçanlarda CCl_4 ile oluşturulmuş böbrek hasarında Smilax excelsa L. sulu ekstresinin serum üre, kreatinin ve MDA düzeyleri ile arilesteraz (ARE) aktivitesi üzerine etkisi*

Group	Urea mg/dl	Creatinine mg/dl	MDA mmol/l	ARE kU/l
Control	25.2±4.3	0.76±0.4	1.4±0.3	29.6±9.4
CCl_4	44.2±13.5[a]	1.00±0.4	3.4±0.4[d]	29.7±19.4
$S_{100+CCl_4}$	43.3±11.4[a]	0.78±0.4	2.5±0.4[d,e]	36.1±27.6
$S_{200+CCl_4}$	44,3±9.3[b]	0.81±0.2	2.7±0.6[c,e]	79.7±25.2[a,g]
$S_{400+CCl_4}$	28.6±7.9[c]	0.77±0.2	1.6±0.4[f]	64.3±21.8[c,e]
P_{ANOVA}	0.000	0.487	0.0001	0.001

[a] *P<0.005 vs. Control;* [b] *P<0.001 vs. Control;* [c] *P<0.05 vs. CCl_4;* [d] *P<0.0001 vs. Control;* [e] *P<0.05 vs. Control;* [f] *P<0.0001 vs. CCl_4;* [g] *P<0.005 vs. CCl_4*

Table 2. *Effect of pretreatment with Smilax excelsa L. aqueous extract after CCl_4 intoxication, on activies of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and myeloperoxidase (MPO) in kidney tissue of rats*

Tablo 2. *Sıçanlarda CCl_4 ile oluşturulmuş böbrek dokusu hasarında Smilax excelsa L. sulu ekstresinin katalaz (CAT), süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx), glutatyon redüktaz (GR), glutatyon peroksidaz (GST) ve miyeloperoksidaz (MPO)aktiviteleri üzerine etkisi*

Group	CAT U/mg protein	SOD U/mg protein	GPx U/mg protein	GR U/mg protein	GST U/mg protein	MPO U/g tissue
Control	81.7±14.8	2.5±0.2	523.9±71.6	90.6±14.6	92.1±23.4	0.18±0.1
CCl_4	76.9±14.1	2.1±0.3[a]	553.7±31.8	86.0±10.0	89.2±24.9	0.99±0.5[d]
$S_{100+CCl_4}$	88.3±10.6	2.6±0.3[b]	723.8±53.9[c]	106.0±27.0	124.8±9.3[a]	0.76±0.2[c]
$S_{200+CCl_4}$	85.0±13.9	3.0±0.5[b]	759.3±97.5[d]	124.0±24.3[a]	133.9±37.5[a]	1.22±0.3[c]
$S_{400+CCl_4}$	86.0±14.2	2.5±0.0[b]	759.5±23.5[c]	124.4±11.1[d]	128.7±20.5[a]	0.50±0.1[c,b]
P_{ANOVA}	0.647	0.002	0.0001	0.002	0.008	0.0001

[a] *P< 0.0 5 vs. Control;* [b] *P< 0.05 vs. CCl_4;* [c] *P< 0.001 vs Control;* [d] *P< 0.005 vs. Control*

Table 3. *Effect of pretreatment with Smilax excelsa L. aqueous extract after CCl_4 intoxication, on lipid peroxidation (LPO), reduced (GSH) and oxidized glutathione (GSSG) levels, GSSG/GSH ratio, protein carbonyl content (PCC), carbonic anhydrase (CA) activity in kidney tissue of rats.*

Tablo 3. *Sıçanlarda CCl_4 ile oluşturulmuş böbrek dokusu toksisinde Smilax excelsa L. sulu ekstresinin lipit peroksidasyonu (LPO), indirgenmiş (GSH) ve oksitlenmiş (GSSG) glutatyon düzeyleri, GSSG/GSH oranı, protein karbonil miktarı (PCC), ve karbonik anhidraz (CA) aktivitesi üzerine etkisi.*

Group	LPO nmol MDA/g tissue	GSH mmol/g tissue	GSSG mmol/g tissue	GSSG/ GSH	PCC nmol/mg protein	CA U/mg protein
Control	24.1±7.2	0.58±0.1	0.05±0.01	0.10±0.01	1.1±0.4	168.8±24.5
CCl_4	38.6±9.4[a]	0.31±0.1[b]	0.06±0.04	0.18±0.07[a]	2.9±0.3[f]	230.3±21.7[b]
$S_{100+CCl_4}$	36.0±9.5[a]	0.58±0.1[d]	0.05±0.03	0.11±0.02[c]	2.9±1.4[b]	216.7±29.1[a]
$S_{200+CCl_4}$	38.9±8.4[b]	0.53±0.1[e]	0.09±0.03[a]	0.18±0.08[a]	2.6±1.7[a]	238.3±24.6[b]
$S_{400+CCl_4}$	27.1±6.9[c]	0.67±0.2[e]	0.04±0.01	0.09±0.01[c]	1.5±0.6[g]	176.5±22.0[e]
P_{ANOVA}	0.011	0.003	0.046	0.004	0.002	0.0001

[a] *P< 0.05 vs. Control;* [b] *P< 0.005 vs. Control;* [c] *P< 0.05 vs. CCl_4;* [d] *P< 0.001 vs CCl_4;* [e] *P< 0.005 vs CCl_4;* [f] *P< 0.0001 vs. Control;* [g] *P< 0.0001 vs CCl_4*

The content of GSH was significantly decreased in the CCl_4-treated rats compared with normal control (P<0.005). In the group of rats pretreated with 100, 200 and 400 mg/kg aqueous extract of *S. excelsa*, GSH levels were comparable to those of normal Control group (P>0.05). The differences between all the groups was found significant (P_{ANOVA}=0.003). There was no significant difference between the kidney GSSG levels of all the experimental groups (P_{ANOVA}=0.046; *Table 3*). The GSSG/GSH ratio which was significantly increased in the CCl_4 group (P<0.05 versus Control group), was decreased to normal Control group levels by administration of *S. excelsa* extract at 400 mg/kg dose (P_{ANOVA}=0.004; *Table 3*).

The significant raise in the PCC content, in the kidney of CCl_4 treated group was only significantly reduced with pretreatment with *S. excelsa* leaf extract at 400 mg/kg (P<0.05 *versus* CCl_4 group), thus restoring it back to the controls range (P_{ANOVA}=0.002; *Table 3*).

The CA activity in the CCl_4 treated group and the group pretreated with 100 and 200 mg/kg aqueous extract of *S. excelsa* was also observed to be highly significant (P<0.005, P<0.05 and P<0.005 respectively) as compared to that in the normal Control group. Pretreatment with 400 mg/kg *S. excelsa* brought the activity near to that of the Control rats (P<0.05 *versus* CCl_4 group; P_{ANOVA}=0.0001; *Table 3*).

Histopathological Assessment

Under light microscope, the cortex in the kidneys of Control group animals has a large number of glomeruli that proximal and distal tubules located around them *(Fig. 1 A)*. CCl_4 caused moderate degenerative changes primarily in proximal tubules, in addition to less in distal tubules in the renal tissues of rats. The renal injury induced by CCl_4 consisted of histopathological changes such as cytoplasmic debris and desquamated nuclei in the widened lumens of proximal and distal tubules, shortening and rupturing at the brush border of proximal tubular cells, vacuolisation and hypertrophy in the proximal and distal tubular cells, hyperemia in the interstitial vascular areas. Besides these histopathological changes, necrotic areas were observed in the kidney tissues of some animals given CCl_4 *(Fig. 1 B)*. However, the degenerative changes were partially apparent in the kidneys of rats received *Smilax excelsa* extract at the 100 mg/kg *(Fig. 1 C)*, 200 mg/kg *(Fig. 1 D)* and 400 mg/kg *(Fig 1. E)* dose together with CCl_4. PAS positive reaction in brush border and basal membrane of proximal tubular cells in the kidney tissue of all groups were similar.

DISCUSSION

In recent years, the search for herbal and natural drugs with antioxidant activity has gained importance as the

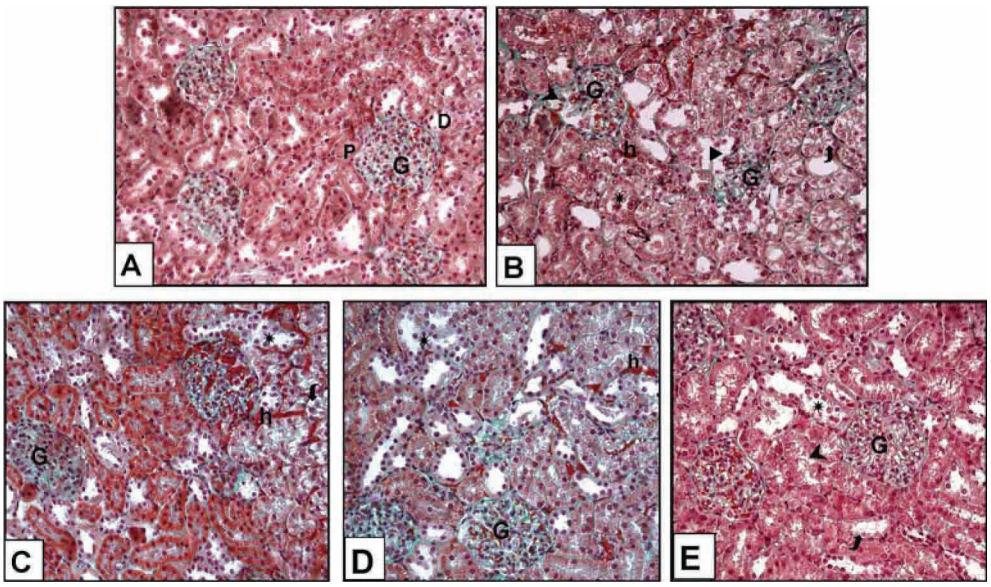

Fig 1. Light microscopic appearance of kidney tissue of a control animal (A). The kidney section of rats given CCl_4 (B), light micrographs of *S. excelsa* (100 mg/kg)-treated CCl_4 group (C), of *S. excelsa* (200 mg/kg)-treated CCl_4 group (D), and of *S. excelsa* (400 mg/kg)-treated CCl_4 group (E). Proximal tubule (P), distal tubule (D) and glomerulus (G). Cytoplasmic debris and desquamated nuclei (✶) in the widened lumens of proximal and distal tubules, shortening (→) and the rupturing (➤) at the brush border of proximal tubular cells, hypertrophy (➥) in tubular cells, hyperemia (h), necrotic area (▶). Masson. Original magnification x400

Şekil 1. Kontrol hayvanın böbrek dokusunun ışık mikroskobik görünümü (A). CCl_4 verilen sıçana ait böbrek kesiti (B), *S. excelsa* (100 mg/kg) verilen CCl_4 grubuna ait (C), *S. excelsa* (200 mg/kg) verilen CCl_4 grubuna ait (D) ve *S. excelsa* (400 mg/kg) verilen CCl_4 grubuna ait (E) ışık mikrografları. Proksimal tübül (P), distal tübül (D) ve glomerulus (G). Proksimal ve distal tübüllerin genişlemiş lümeninde sitoplazma artıkları ve dökülmüş nukleuslar (✶), proksimal tübül hücrelerinin fırça kenarlarında kısalma (→) ve kopma (➤), tübül hücrelerinde hipertrofi (➥), hiperemi (h), nekrotik alan (▶). Masson. Orijinal büyütme x400

dietary intake of antioxidants obtained from natural sources is considered to be relatively safe and involves no side effects. *Smilax excelsa* shoots and leaves constitutes a particularly interesting source of biologically active phytochemicals as it contains a variety of phenolic compounds with substantial *in vitro* antioxidant activity [1]. However, until recently, there have been few studies about the pharmacological effects of the leaves of *Smilax excelsa*. *In vitro* antioxidant properties of leaf extracts were shown in one study on *Smilax china* [37] after our research [23]. Nephroptotective activity of extracts obtained from *S. china* rhizomes in hyperuricemic animals was demonstrated in a recent study without histopathological observation [38]. Despite the many beneficial biological properties of *Smilax excelsa*, its protective effect against CCl_4 nephrotoxicity has not so far been explored. The current study was aimed at identifying biochemical and renal histopathological abnormalities that occur with the evolution of nephrotoxicity in rats and to appreciate their possible reversal after the treatment with the water extract of *Smilax excelsa* shoots and leaves.

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to drugs or chemical reagents. Several medicinal plants are reviewed for their nephroprotective activities [39]. CCl_4 is commonly used in rat experimental models to investigate the oxidative stress induced in various organs. In addition to its hepatic toxicity, it was reported that CCl_4 also causes disorders in kidneys.

The elevation in blood urea, and the observed histopathological alterations recorded in this work indicated that CCl_4 caused moderate impairment in renal function along with significant oxidative stress in the kidneys which is consistent with other studies [40].

Because, generally, the effect of CCl_4 is observed after 24 h of its administration and withdrawal of blood and excising of kidneys were carried out after 24 h of CCl_4 intoxication, serum creatinine levels remained normal 24 h after the induction of renal toxicity in rats, which is in accordance with the observation reported by Tirkey at al.[41] who failed to observe any increase in BUN nor serum creatinine levels after CCl_4 administration.

Our histopathological findings were in agreement with the degenerative structural changes reported to occur in kidney tissues after the application of CCl_4 [42,43]. Our results showed that CCl_4 leads to moderate degenerative changes mainly necrosis, hyperemia, and vacuolar degeneration in the kidney of rats. It was observed that pretreatment with the aqueous extracts of *Smilax excelsa* leaves did not completely diminish CCl_4-induced degenerative injury in kidney tissue, morphologically. Thus, the improvement in antioxidant enzymes system seem to have been incapable of neutralizing increased CCl_4 toxicity seen in the histopathology of kidney cells.

There was a significant increase in serum MDA levels, which is an indirect measure of lipid peroxidation that suggests the posibility of enhanced production of free radicals as reported by Manna et al.[40] and Tirkey at al.[41]. In recent years paraoxonase/arylesterase activities have been used as oxidative stress markers. It has been suggested that they may play a protective role under oxidative stress. Paraoxonase was found to use efficiently not only lipoprotein-associated peroxides (including cholesteryl linoleate hydro-peroxides), but also hydrogen peroxide (H_2O_2) [44]. In this study the induction of paraoxonase/arylesterase activity may merely be a manifest of antioxidant response to the increased oxidative stress. It was reported that flavonoids can act as potent inhibitors of LDL oxidation via preserving or increasing serum paraoxonase/arylesterase activity thus promoting hydrolisis of LDL-associated lipid peroxides [45]. The presence of high quantity of flavonoids in *Smilax excelsa* leaves could be responsible for the protective effect against the oxidative stress of CCl_4 in kidneys of rat.

CAT, SOD, GR, GPx, GST and GSH were evaluated as an index of antioxidant status of kidney tissues. SOD generates hydrogen peroxide as a metabolite, which must be scavenged by catalase or GPx. In this study the small degree of changes in renal CAT and SOD activities did not attain statistical significance between CCl_4 treated control and groups pretreated with *S. excelsa*. May be CAT and SOD are easily inactivated by lipid peroxides, which are scavenged by GPx. GPx faciliate the conjugation of hydrogen peroxide to reduced glutathione (GSH) leading to generation of water and oxidized glutathione (GSSG), which is then reduced to GSH by the NADPH-dependent GR. The increase in GR and GPx activities may be due to the elevated GSH content. Some of the changes in glutathione antioxidant system may possibly reflect an inter-organ antioxidant response to a generalized increase in tissue oxidative stress associated with intoxication, possibly through the export of hepatic glutathione for the subsequent uptake by extrahepatic tissues. The absence of a change in the GSSG/GSH ratio, a potent inhibitor of tissue oxidative stress in the group pre-treated with 400 mg kg of *S. excelsa* leaves is in accordance with the suggestion that free radical damage is attenuated. The increase in GST activity which is involved in the detoxification of the lipid peroxidation products may also be an early adaptive response to oxidative stress. It may be concluded that administration of *Smilax excelsa* leaves protected the antioxidant status of the kidney as revealed by the enhanced level of GR, GPx, GST and GSH in this experiment. Earlier studies have also shown that different plant extracts comprehensively ameliorated the renal injuries induced through CCl_4 intoxication by increasing antioxidant enzyme activities [40,41].

In the present study, the administration of CCl_4 resulted in a significant elevation in renal PCC and MDA levels, indicating increased protein and lipid oxidation,

respectively, leading to tissue damage and failure of the antioxidant mechanisms to prevent the production of excessive free radicals. Interestingly, pretreatment of 400 mg/kg *S. excelsa* leaves markedly reduced the extent of protein and lipid oxidation by decreasing the PCC and MDA levels, which confirms the nephroprotective effect of *S. excelsa* leaves against the renal protein oxidation and lipid peroxidation induced by CCl_4.

Increased MPO enzyme activity is an indicator of inflammation. This enzyme is highly enriched in the azurophilic granules of polymorphonuclear leukocytes (PMNs) recruited to injured tissue to mediate the acute phase of the inflammatory response [46]. Reduced MPO levels in the group pretreated with 400 mg/kg *S. excelsa* extract indicate that the extract may cause significant supression of neutrophil infiltration. These results were in accordance with those of Tsumbu et al.[47] who reported that plant polyphenols could decrease the MPO activity released by the neutrophils.

Carbonic anhydrases (CAs) are key enzymes that regulate acid-base homeostasis in both normal and pathological conditions. The fact that CA III in humans as well as in other species is abundantly expressed in skeletal muscle and some other tissues [48] make it a physiologically significant pool of reactive sulfhydryls that function as oxyradical scavengers. The concentration of CA III in these cells could reach the same order of magnitude as that of glutathione. Thus it may provide an important physiological mechanism of protecting tissues against oxidative damage [49]. Increased CA activity in the CCl_4 treated group and the group pretreated with 100 and 200 mg/kg aqueous extract of *S. excelsa* observed in this study indicate that CA may have a direct role in cellular response to oxidative damage.

The present study confirmed and extended the results of other studies showing that oxidative stress occurs depending on the increase of reactive oxygen species production in different diseases. The administration of antioxidants could conceivably protect tissues from the effects of free radicals and lipid peroxidation and thereby retard the progress of many diseases [44,50-52].

The biochemical findings of the present study revealed that pretreatment with 400 mg/kg of body weight *S. excelsa* leaves of CCl_4-treated rats ameliorated the toxic effects of CCl_4 by restoring the markers mentioned above to normal levels.

In conclusion, our results indicated the protective role of *S. excelsa* leaves against CCl_4-induced nephrotoxicity. The mechanisms of protection include the inhibition of protein and lipid oxidation processes and the increase in antioxidant enzymes activities, which results in the recovery only of biological parameters but not contribute to the integrity of kidney histological aspects.

REFERENCES

1. Ivanova A, Mikhova B, Kostova I, Evstatieva L: Bioactive chemical constituents from *Smilax excelsa*. *Chem Nat Compd*, 46, 295-297, 2010.

2. Li YL, Gan GP, Zhang HZ, Wu HZ, Li CL, Huang YP, Liu YW, Liu JW: A flavonoid glycoside isolated from *Smilax china* L. rhizome *in vitro* anticancer effects on human cancer cell lines. *J Ethnopharmacol*, 113, 115-124, 2007.

3. Wu LS, Wang XJ, Wang H, Yang HW, Jia AQ, Ding Q: Cytotoxic polyphenols against breast tumor cell in *Smilax china* L. *J Ethnopharmacol*, 130, 460-464, 2010.

4. Vijayalakshmi A, Ravichandiran V, Anbu J, Velraj M, Jayakumari S: Anticonvulsant and neurotoxicity profile of the rhizome of *Smilax china* Linn. in mice. *Indian J Pharmacol*, 43, 27-30, 2011.

5. Reanmongkol W, Itharat A, Bouking P: Evaluation of the anti-inflammatory, antinociceptive and antipyretic activities of the extracts from *Smilax corbularia* Kunth rhizomes in mice and rats (*in vivo*). *Songklanakarin J Sci Technol* 29 (Suppl. 1): 59-67, 2007.

6. Shu XS, Gao ZH, Yang XL: Anti-inflammatory and anti-nociceptive activities of *Smilax china* L. aqueous extract. *J Ethopharmacol*, 103, 327-332, 2006.

7. Khan I, Nisar M, Ebad F, Nadeem S, Saeed M, Khan H, Samiullah Khuda F, Karim N, Ahmad Z: Anti-inflammatory activities of Sieboldogenin from *Smilax china* Linn.: Experimental and computational studies. *J Ethopharmacol*, 121, 175-177, 2009.

8. Rafatullah S, Mossa JS, Ageel AM, Al-Yahya MA, Tariq M: Hepato-protective and safety evaluation studies on Sarsaparilla. *Int J Pharmacogn,* 29, 296-301, 1991.

9. Chen T, Li J, Cao J, Xu Q, Komatsu K, Namba T: A new flavanone isolated from rhizoma *Smilacis glabrae* and the structural requirements of its derivatives for preventing immunological hepatocyte damage. *Planta Med*, 65, 56-59, 1999.

10. Mandal SC, Jana GK, Das S, Sahu R, Venkidesh R, Dewanjee S: Hepatoprotective and antioxidant activities of *Smilax chinensis* L. root. *Pharmacologyonline,* 2, 529-535, 2008.

11. Chen L, Yin H, Lan Z, Ma S, Zhang C, Yang Z, Li P, Lin B: Anti-hyperuricemic and nephroprotective effects of *Smilax china* L. *J Ethnopharmacol*, 135, 399-405, 2011.

12. Chen T, Li JX, Xu Q: Phenylpropanoid glycosides from *Smilax glabra*. Phytochemistry, 53, 1051-1055, 2000.

13. Longo L, Vasapollo G: Extraction and identification of anthocyanins from *Smilax aspera* L. berries. *Food Chem*, 94, 226-231, 2006.

14. Chen L, Yin Y, Yi H, Xu Q, Chen T: Simultaneous quantification of five major bioactive flavonoids in *Rhizoma Smilacis Glabrae* by high-performance liquid chromatography. *J Pharm Biomed Anal*, 43, 1715-1720, 2007.

15. Bernardo RR, Pinto AV, Parente JP: Steroidal saponins from *Smilax officinalis*. *Phytochemistry*, 43, 465-469, 1996.

16. Ivanova A, Serly J, Dinchev D, Ocsovszki I, Kostova I, Molnar J: Screening of some saponins and phenolic components of *Tribulus terrestris* and *Smilax excelsa* as MDR modulators. *In Vivo,* 23, 545–550, 2009.

17. Lee SE, Ju EM, Kim JH: Free radical scavenging and antioxidant enzyme fortifying activities of extracts from *Smilax china* root. *Exp Mol Med*, 33, 263-268, 2001.

18. Cox SD, Jayasinghe KC, Markham JL: Antioxidant activity in Australian native sarsaparilla (*Smilax glyciphylla*). *J Ethnopharmacol,* 101, 162-168, 2005.

19. Ivanova A, Marinova E, Toneva A, Kostova I, Yanishlieva N: Antioxidant properties of *Smilax excelsa*. *La Rivista Italiana Della Sostanze Grasse,* 83, 124-128, 2006.

20. Baytop T: Türkiyede Bitkiler ile Tedavi. Pp. 367-368, İstanbul Üniversitesi Yayınları No. 3255-Eczacılık Fakültesi No. 40, İstanbul, 1984.

21. Özbucak TB, Ergen Akçin Ö, Yalçın S: Nutrition contents of the some wild edible plants in Central Black Sea region of Turkey. *IJNES*, 1, 11-13, 2007.

22. Yeşilada E, Sezik E, Honda G, Takaishi Y, Takeda Y, Tanaka T: Traditional medicine in Turkey IX: Folk medicine in north-west Anatolia. *J Ethnopharmacol*, 64, 195-210, 1999.

23. Ozsoy N, Can A, Yanardag R, Akev N: Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food Chem,* 110, 571-583, 2008.

24. Bonsness RW, Taussky HH: On the colorimetric determination of creatinine by the Jaffe reaction. *J Biol Chem*, 158, 581, 1945.

25. Barker SB: The direct colorimetric determination of urea in blood and urine. *J Biol Chem*, 152, 453, 1944.

26. Gan KN, Smolen A, Eckerson HW, La Du BN: Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. *Drug Metab Dispos*, 19, 100-106, 1991.

27. Aebi H: Catalase *in vitro*. *Method Enzymol*, 105, 121-126, 1984.

28. Aruoma OI, Halliwell B, Hoey BM, Butler J: The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med*, 6, 593-597, 1989.

29. Lawrence RA, Burk RF: Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun*, 71, 952-958, 1976.

30. Carlberg I, Mannervik B: Glutathione reductase. *Methods Enzymol,* 113, 484-490, 1985.

31. Habig WH, Jakoby WB: Assays for differentation of glutathione-S-transferases. *Methods Enzymol*, 77, 398-405, 1981.

32. Hillegass LM, Griswold DE, Brickson B, Albrightson-Winslow C: Assessment of myeloperoxidase activity in whole rat kidney. *J Pharmacol Methods*, 24, 285-295, 1990.

33. Buege JA, Aust SD: Microsomal lipid peroxidation. *Methods Enzymol,* 52, 302-310, 1978.

34. Anderson ME: Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol,* 113, 548-555, 1985.

35. Reznick AZ, Packer L: Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. *Methods Enzymol,* 233, 357-363, 1994.

36. Verpoorte JA, Mehta S, Edsall JT: Esterase activities of human carbonic anhydrases B and C. *J Biol Chem*, 242, 4221-4229, 1967.

37. Seo HK, Lee JH, Kim HS, Lee CK, Lee SC: Antioxidant and antimicrobial activities of *Smilax china* L. leaf extracts. *Food Sci Biotechnol*, 21, 1723-1727, 2012.

38. Chen L, Yin H, Lan Z, Ma S, Zhang C, Yang Z, Li P, Lin B: Anti-hyperuricemic and nephroprotective effects of *Smilax china* L. *J Ethnopharmacol*, 135, 399-405, 2011.

39. Mohana Lakshmi S, Usha Kiran Reddy T, Sandhya Rani KS: A review on medicinal plants for nephroprotective activity. *Asian J Pharm Clin Res,* 5, 8-14, 2012.

40. Manna P, Sinha M, Sil PC: Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Compl. Alternative Med*, 6, 33-42, 2006.

41. Tirkey N, Pilkhwal S, Kuhad A, Chopra K: Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacology*, 5, 2-10, 2005.

42. Jaramillo-Juárez F, Rodríguez-Vázquez ML, Rincón-Sánchez AR, Martínez MC, Ortiz GG, Llamas J, Posadas FA, Reyes JL: Acute renal failure induced by carbon tetrachloride in rats with hepatic cirrhosis. *Ann Hepatol,* 7, 331-338, 2008.

43. El Denshary ES, Al-Gahazali MA, Mannaa FA, Salem HA, Hassan NS, Abdel-Wahhab MA: Dietary honey and ginseng protect against carbon tetrachloride-induced hepatonephrotoxicity in rats. *Exp Toxicol Pathol*, 64, 753-760, 2012.

44. Turunc V, Kontas Aşkar T: The determination of oxidative stress by paraoxonase activity, heat shock protein and lipid profile levels in cattle with theileriosis. *Kafkas Univ Vet Fak Derg*, 18, 647-651, 2012.

45. Fuhrman B, Aviram M: Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Curr Opin Lipidol*, 12, 41-48, 2001.

46. Kothari N, Keshari RS, Bogra J, Kohli M, Abbas H, Malik A, Dikshit M, Barthwal MK: Increased myeloperoxidase enzyme activity in plasma is an indicator of inflammation and onset of sepsis. *J Crit Care*, 26, 435. e1-e7, 2011.

47. Tsumbu CN, Deby-Dupont G, Tits M, Angenot L, Frederich M, Kohnen S, Mouithys-Mickalad A, Serteyn D, Franck T: Polyphenol content and modulatory activities of some tropical dietary plant extracts on the oxidant activities of neutrophils and myeloperoxidase. *Int J Mol Sci*, 13, 628-650, 2012.

48. Dodgson SJ, Contino LC: Rat kidney mitochondrial carbonic anhydrase. *Arch Biochem Biophys,* 260, 334-341, 1988.

49. Räisänen SR, Lehenkari P, Tasanen M, Rahkila P, Härkönen PL, Väänänen HK: Carbonic anhydrase III protects cells from hydrogen peroxide-induced apoptosis. *FASEB J*, 13, 513-522, 1999.

50. Bozukluhan K, Atakisi E, Atakisi O: Nitric oxide levels, total antioxidant and oxidant capacity in cattle with foot-and-mouth-disease. *Kafkas Univ Vet Fak Derg*, 19, 179-181, 2013.

51. Pekmezci D, Çenesiz S, Çakıroğlu D, Çiftci G, Çıra A, Gökalp G: Status of lipid peroxidation, cell destruction and the antioxidant capacity in foals with lower respiratory tract disease. *Kafkas Univ Vet Fak Derg*, 18, 157-160, 2012.

52. Bozkurt Y, Fırat U, Atar M, Sancaktutar AA, Pembegul N, Soylemez H, Yuksel H, Alp H, Bodakci MN, Hatipoglu NK, Buyukbas S: The protective effect of ellagic acid against renal ischemia-reperfusion injury in male rats. *Kafkas Univ Vet Fak Derg*, 18, 823-828, 2012.