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GENETIC AND BIOCHEMICAL EFFECTS OF
GINKGO BILOBA ON SOMATIC AND GERM CELLS OF MICE

**Abdulhakeem A. Al-Majed*, Abdulaziz A. Al-Yahya,
A.M. Al-Bekairi, O.A. Al-Shabanah and S. Qureshi**

يستخدم نبات الجنكة (Ginkgo biloba) شعبياً في علاج مرض ألزهايمر وخرف الشيخوخة. وتتناول هذه الدراسة التأثيرات الوراثية والبيوكيميائية لهذا النبات، على ضوء التقارير عن الآثار المسرطنة للمواد الغذائية المحتوية على نبات الجنكة، واحتمالية التسمم الخلوي والتحور الأحيائي لمكوناته وندرة ما نشر عن سميته الجينية. وقد شمل بروتوكول المعالجة الفموية للفئران بجرعات مختلفة (100، 200، 400 مغ/كغ) من نبات الجنكة لمدة سبعة أيام متتالية. وتم إجراء التجارب التالية: (i) دراسات خلوية على اختبار النواة الدقيقة، (ii) التحليل الخلوي لشذوذ الحييات المنوية، (iii) تقدير البروتينات والأحماض النووية في الخلايا الكبدية والخلايا الخصوية. (iv) تقدير مادة مالونالدهيد ومادة سلفهيدريل اللابروتيني في الخلايا الكبدية والخلايا الخصوية. وقد أظهرت نتائج هذه الدراسة بصورة جلية عدم وجود أي تأثير لنبات الجنكة على عدد النويات الدقيقة، وعلى نسبة الكريات الحمراء متعددة اللون/ الكريات الحمراء سوية اللون، وعلى شذوذ الحييات المنوية وعلى التراكيز الكبدية للأحماض النووية، بينما استنفذت مستويات الحمض النووي في الخلايا الخصوية بصورة معنوية، وقد فشلت نتائج الأحماض النووية الخصوية في التوافق مع بياناتنا عن شذوذ الحييات المنوية. وقد يرجع عدم التوافق إلى دور جهاز القناة الخارجية (excurrent duct) في التخلص من المنويات المشوهة أثناء نقلها من الخصية إلى القناة الدافقة ووعاء التخزين الذيلي. أما بشأن مالونالدهيد ومادة سلفهيدريل فقد تبين أن نبات الجنكة قد تسبب في توليد البيروكسيدات الشحمية. تدل هذه الدراسة أن التغيرات الملحوظة في الخلايا الجسدية والجرثومية لا تعتمد على توليد البيروكسيدات الشحمية المستحثة بواسطة نبات الجنكة. وقد تكون هذه التغيرات في نبات الجنكة مرتبطة بتأثيرها على زيادة مادة مالونالدهيد ونضوب مادة سلفهيدريل. وعلى ضوء الأكسدة الملحوظة، فإن دراستنا هذه توصي بالاستعمال الحذر لنبات الجنكة.

Ginkgo biloba has immense folkloric significance in the treatment of Alzheimer's disease and age-related dementia. The present study on the genetic and biochemical effects of Ginkgo biloba was undertaken, in view of the reports on the carcinogenic effects of the dietary supplements containing Ginkgo biloba, the cytotoxic and mutagenic potentials of its constituents and a paucity of literature on the genotoxicity. The protocol included the oral treatment of mice with different doses (100, 200 and 400 mg/kg) of Ginkgo biloba for 7 consecutive days. The following experiments were conducted: (i) cytological studies on micronucleus test, (ii) cytological analysis of spermatozoa abnormalities, (iii) quantification of proteins and nucleic acids in hepatic and testicular cells and (iv) estimation of malondialdehyde (MDA) and nonprotein sulfhydryl (NP-SH) in hepatic and testicular cells. The results obtained in this study clearly demonstrate lack of any effect of Ginkgo biloba on the frequency of micronuclei, ratio of polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE), spermatozoa abnormalities and hepatic concentrations of nucleic acids, while the nucleic acid levels of testicular cells were significantly depleted. The results on testicular nucleic acids failed to reflect our data on spermatozoa abnormality. The discordance might be related to the role of excurrent duct system in eliminating morphologically abnormal spermatozoa during transit from testis to vas deferens and caudae

Department of Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Kingdom of Saudi Arabia

* To whom correspondence should be addressed.
  E-mail: majed107@hotmail.com

epididymis. The observation on MDA and NP-SH showed Ginkgo biloba to cause genesis of lipid peroxides. This study indicates that the changes observed in the somatic and germ cells were independent of the Ginkgo biloba-induced genesis of lipid peroxides. The observed changes of Ginkgo biloba might be related to its effect on the increase of MDA and depletion of NP-SH. In view of the observed oxidant potentials, our study warrants careful use of Ginkgo biloba.

Key words: Ginkgo biloba, genotoxicity, somatic cells, germ cells, nucleic acids, lipid peroxides, nonprotein-sulfhydryl groups.

Introduction

The seed and leave extracts of Ginkgo biloba are most commonly used as a folk medicine in China and Europe (1). Ginkgo biloba is claimed to sharpen memory by increasing blood circulation and oxygenation to the brain. Some studies have suggested Ginkgo extracts to exhibit therapeutic activity against a variety of health disorders. These include Alzheimer's disease, age-related dementia, poor cerebral and ocular blood flow, fatigue, anxiety, depressed mood, congestive symptoms of pre-menstrual syndrome and the prevention of altitude sickness (1-4).

Ginkgo biloba has been suggested to possess antioxidant properties as revealed by a number of studies. It is reported to cause significant elevations in DT-diaphorase activity and increased the levels of hepatic glutathione (5). Ozenirler *et al*. (6) found Ginkgo biloba to protect against CCl_4-induced changes in the hepatic levels of MDA, glutathione and hydroxyproline in rat. It is shown to inhibit the cardiotoxic effects of doxorubicin in mice as evidenced by lowered myocardial lipid peroxidation, normalization of antioxidant enzymes, reversal of ECG changes and minimal ultrastructural damage of the heart (7). Boulieu *et al*. (8) found the extract to inhibit the triethyltin-induced cerebral edema and elevation of MDA concentration in brain rat.

Ginkgo biloba is a complex mixture, which contains both the antioxidant and oxidant constituents. Flavonoid glycosides, terpene lactones, non-flavone fraction are responsible for its antioxidant activity, as demonstrated by a number of studies on protection against proven oxidants (4,9-14). In contrast, a group of alkylphenols (Ginkgolic acids, cardanols, biflavones and cardols) have been reported to possess oxidant, allergenic, mutagenic, embryotoxic, and cytotoxic potentials (2,4,12, 15-17).

A large number of papers were published on the claimed therapeutic uses of Ginkgo biloba and toxicity of its constituents. However, there is a paucity of literature on the toxicity of Ginkgo biloba (as a whole product), except a few reports. Although, it was found to protect against the radiation-induced chromosomal damage (18), dietary supplements containing Ginkgo biloba have been found to increase the incidence of colorectal and lung cancers (19).

The present study was undertaken in view of the paucity of reports on the genetic and biochemical toxicity of Ginkgo biloba, as a whole product. The rationale of this study was based on (i) reported cytotoxic and mutagenic potentials of the different constituents of Ginkgo biloba, (ii) observed antioxidant potentials of different constituents of Ginkgo biloba and (iii) known toxicity of antioxidants and (iv) carcinogenic potentials of Ginkgo biloba containing dietary supplement.

Materials and Methods

1.   *Test herbal product:*

Ginkgo biloba was used as the test herbal product in the present study. It is marketed by General Nutrition Corporation, (USA) in form of tablets. Each tablet contains 50 mg of Ginkgo biloba leaf extract and 12 mg of Ginkgo flavone glycosides. The other contents are dicalcium phosphate and cellulose.

2.   *Animals:*

Male Swiss albino mice (SWR) aged 6-8 weeks and weighing 25-28 g were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia. The animals were fed on Purina chow diet and water ad libitum. They were maintained under standard conditions of humidity, temperature, and light (12 h light/12 h dark cycle). The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care

Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

3. *Dose, route and duration of treatment:*

The doses of Ginkgo biloba selected were 100, 200 and 400 mg/kg. These doses were determined corresponding to 1/16th, 1/8th and 1/4th, respectively, of the evaluated maximum tolerated dose, namely 1.6 g/kg) (20). Aqueous suspension of Ginkgo biloba tablet was administered by gavage and the concentration was adjusted such that each 100 g. body weight received 3 ml containing the required dose. The doses were administered once daily for seven consecutive days.

4. *Cytological studies on micronucleus test:*

The procedure of micronucleus test described by Schmid (21) was followed. The femoral cells were collected in fetal calf serum. After centrifugation, the cells were spread on slides and air-dried. Coded slides were fixed in methanol and stained in May-Gruenwald solution followed by Giemsa stain. The PCE were screened for micronuclei, and reduction of the mitotic index was assessed on the basis of the ratio of PCE/NCE.

5. *Cytological analysis of spermatozoa abnormalities:*

The mice were sacrificed 5 weeks after the last day of the seven day treatment (22-25). The study was further extended to assess the sensitivity of spermatozoa to stages of spermatogenesis. Experiments were undertaken to obtain spermatozoa at different times to exclude any difference in sensitivity to treatment. In these experiments, mice were treated with the high dose (400 mg/kg/day) of Ginkgo biloba for seven days and sacrificed after 7 days (spermatozoa stage), 21 days (spermatid stage) and 35 days (spermatocyte stage) corresponding to late, mid- and early spermatogenesis, respectively. The spermatozoa were obtained by making small cuts in caudae epididymis and vas deferens and placed in 1 ml of modified Krebs' Ringer-bicarbonate buffer (pH 7.4). The sperm suspension obtained was stained with 0.05% of eosin-Y; smears were made on slides, air-dried and made permanent. The spermatozoa morphology was examined by bright-field microscopy with an oil immersion lens. The different spermatozoa abnormalities (amorphous, banana shaped, swollen achrosome, triangular head, macrocephali and rotated head) screened were those found in all the slides (22, 26-28).

6. *Biochemical analysis:*

The frozen samples of liver and testes were used for estimation of proteins, RNA, DNA, MDA and NP-SH levels.

6.1. *Estimation of total proteins and nucleic acids:*

Total proteins were estimated by the modified Lowry method of Schacterle and Pollack (29). Bovine serum albumin was used as standard. The method described by Bregman (30) was used to determine the levels of nucleic acids. The sample tissues were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol. The levels of DNA were determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

6.2. *Determination of MDA concentrations:*

The method described by Ohkawa *et al*. (31) was followed. The sample tissues were homogenized in TCA and the homogenate was suspended in thiobarbituric acid. After centrifugation the optical density of the clear pink supernatant was read at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard.

6.3. *Quantification of the NP-SH levels:*

The method described by Sedlak and Lindsay (32) was followed to determine the levels of NP-SH. The sample tissues were homogenized in ice cold 0.02 M ethylene-di-aminetetraaceticacid disodium (EDTA) and mixed with TCA. The homogenate was centrifuged at 3000g. The supernatant was suspend-ded in Tris buffer, 5-5'-dithiobis-(2 nitrobenzoic acid) and red at 412 nm against reagent blank with no homogenate.

7. *Statistical analysis:*

The different studies undertaken were statistic-cally analyzed by one way ANOVA and Post hoc Tukey-Kramer multiple comparison test.

Results

1. Genetic toxicity:

1.1. *Effect of Ginkgo biloba on the frequency of micronuclei and the PCE/NCE ratio:*

Treatment with Ginkgo biloba (100, 200 and 400 mg/kg/day did not induce any significant changes in the frequency of micronuclei in male mice. The ratio of PCE/NCE was also not affected (Table 1).

1.2. *Effect Of Ginkgo biloba on epididymal spermatozoa morphology:*

Treatment with Ginkgo biloba (100, 200 and 400 mg/kg/day) failed to induce morphological abnormalities in epididymal spermatozoa (amorphous, banana shaped, swollen achrosome, triangular head, macrocephali and rotated head). The total percent sperm abnormalities were also not affected. (Table 2). The high dose of Ginkgo biloba (400 mg/kg/day) failed to cause abnormalities in the individual and/or the total percent spermatozoa at different stages (spermatozoa, spermatids and spermatocytes) of spermatogenesis (Table 3).

2. Biochemical toxicity:

2.1. *Effect of Ginkgo biloba on proteins and nucleic acids in hepatic cells:*

Ginkgo biloba decreased the hepatic levels of RNA, however these changes were statistically insignificant. The treatment also failed to affect the proteins and DNA contents in the liver (Table 4).

Table 1 : Effect of Ginkgo biloba on the frequency of micronuclei and the ratio of polychromatic to normochromatic erythrocytes in femoral cells of Swiss albino mice after sub-acute treatment.

Sl. No.	Treatment and dose (mg/kg/day)	Total PCE screened	PCE (%) (Mean ± S.E.)	Total NCE screened	PCE/NCE ratio (Mean ± S.E.)
1.	Control (0.3 ml tap water/mouse)	6025	0.27±0.04	5320	1.13±0.04
2.	Ginkgo biloba (100)	5250	0.30±0.03	5235	1.01±0.05
3.	Ginkgo biloba (200)	5250	0.34±0.04	5105	1.03±0.04
4.	Ginkgo biloba (400)	5100	0.29±0.05	5170	0.99±0.03

n = 5, Insignificant at P>0.05 (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).

Table 2: Effect of Ginkgo biloba on epididymal spermatozoa in Swiss albino mice after sub-acute treatment.

Sl. No.	Different Spermatozoal abnormalities screened/Total	Treatments and dose (mg/kg/day)/percent sperm abnormalities (Mean ± S.E.)			
		Control (tap water, 0.3 ml/mouse/day)	Ginkgo biloba (100)	Ginkgo biloba (200)	Ginkgo biloba (400)
1.	Amorphous	0.45±0.06	0.33±0.08	0.66±0.15	0.66±0.10
2.	Banana shaped	0.44±0.05	0.29±0.10	0.55±0.14	0.44±0.13
3.	Swollen achrosome	0.33±0.07	0.31±0.08	0.49±0.06	0.34±0.06
4.	Triangular head	0.27±0.03	0.25±0.06	0.34±0.04	0.46±0.11
5.	Macrocephali	0.48±0.15	0.16±0.10	0.65±0.11	0.42±0.04
6.	Rotated head	0.16±0.05	0.23±0.08	0.17±0.04	0.09±0.03
7.	Total abnormalities	1.98±0.09	1.58±0.28	2.91±0.44	2.51±0.38
8.	Total sperms screened	5145	5140	5243	5169

n = 5, Insignificant at P>0.05 (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).

Table 3: Effect of Ginkgo biloba on different stages of spermatogenesis in Swiss albino mice after sub-acute treatment.

| Sl. No | Different spermatozoal abnormalities | *Different stages of spermatogenesis | | | | | |
| | | Spermatozoa (7 days) | | Spermatids (21 days) | | Spermatocytes (35 days) | |
		Control	G. biloba (400 mg/kg)	Control	G. biloba (400 mg/kg)	Control	G. biloba (400 mg/kg)
1.	Amorphous	0.34± 0.09	0.40± 0.06	0.42± 0.06	0.58± 0.12	0.44± 0.04	0.67± 0.17
2.	Banana shaped	0.38± 0.08	0.39± 0.06	0.43± 0.07	0.68± 0.12	0.48± 0.04	0.81± 0.23
3.	Swollen achrosome	0.44± 0.06	0.47± 0.03	0.49± 0.06	0.74± 0.17	0.50± 0.40	0.88± 0.21
4.	Triangular head	0.49± 0.07	0.43± 0.03	0.59± 0.06	0.83± 0.16	0.50± 0.05	0.79± 0.14
5.	Macrocephali	0.38± 0.08	0.39± 0.05	0.30± 0.20	0.53± 0.04	0.44± 0.06	0.56± 0.12
6.	Rotated head	0.28± 0.15	0.28± 0.18	0.07± 0.02	0.07± 0.01	0.13± 0.02	0.17± 0.03
7.	Total abnormalities	2.01± 0.22	2.19± 0.18	2.32± 0.21	3.45± 0.56	2.61± 0.15	3.90± 0.87
8.	Total sperms screened	5020	5500	5350	5270	5250	5150

n = 5. Insignificant, $P>0.05$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters). *Results are expressed as mean±S.E.

Table 4: Effect of Ginkgo biloba on proteins and nucleic acids contents in hepatic tissue of Swiss albino mice after sub- acute treatment.

Sl. No	Treatment (Dose, mg/kg/day)	Proteins (mg/100 mg tissue) [Mean ± S.E]	RNA (µg/100mg tissue) [Mean ± S.E]	DNA (µg/100mg tissue) [Mean ± S.E]
1.	Control (tap water, 0.3 ml/mouse)	12.69±0.44	754.64±38.64	95.24±9.92
2.	Ginkgo biloba (100)	12.74±0.37	677.52±16.65	102.08±4.85
3.	Ginkgo biloba (200)	12.90±0.11	636.56±30.15	98.00±7.03
4.	Ginkgo biloba (400)	12.90±0.23	624.24±24.26	97.76±4.01

n = 5, Insignificant at $P>0.05$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for male, female and different parameters).

Table 5: Effect of Ginkgo biloba on proteins and nucleic acids contents in testicular tissue of Swiss albino mice after sub-acute treatment.

Sl. No	Treatment (Dose, mg/kg/day)	Proteins (mg/100 mg tissue) [Mean ± S.E]	RNA (µg/100mg tissue) [Mean ± S.E]	DNA (µg/100mg tissue) [Mean ± S.E]
1.	Control (tap water, 0.3 ml/mouse)	9.79±0.05	597.5±11.23	229.64±6.73
2.	Ginkgo biloba (100)	9.04±0.39	483.2±40.72*	198.72±18.16
3.	Ginkgo biloba (200)	9.84±0.13	455.04±11.11**	177.56±3.97**
4.	Ginkgo biloba (400)	10.15±0.09	498.56±31.25	203.02±3.68

n = 5, Significant at *$P<0.05$; **$P<0.01$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for male, female and different parameters).

Table 6: Effect of Ginkgo biloba on MDA contents in the hepatic and testicular tissue of Swiss albino mice after sub-acute treatment.

Sl. No.	Treatment and dose (mg/kg/day).	Malondialdehyde concentrations (nmol/g wet tissue) [Mean ± S.E]	
		Hepatic tissue	Testicular tissue
1.	Control (tap water, 0.3 ml/mouse)	179.52±14.91	194.83±22.37
2.	Ginkgo biloba (100)	238.93±12.63	223.88±12.07
3.	Ginkgo biloba (200)	315.88±31.17*	245.68±13.80
4.	Ginkgo biloba (400)	324.87±16.48**	336.58±31.16**

n = 5, Significant at *P<0.05; **P<0.01 (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done.

Table 7: Effect of Ginkgo biloba on NP-SH concentrations in the hepatic and testicular tissue of Swiss albino mice after sub-acute treatment.

Sl. No.	Treatment and dose (mg/kg/day)	NP-SH concentration (nmol/100 g wet tissue) Mean ± S.E	
		Hepatic tissue	Testicular tissue
1.	Control (tap water, 0.3 ml/mouse)	105.55±8.28	102.22±9.43
2.	Ginkgo biloba (100)	61.10±8.87**	63.88±5.62`**
3.	Ginkgo biloba (200)	66.10±3.55**	57.77±6.93**
4.	Ginkgo biloba (400)	51.66±6.83***	72.77±3.76*

n = 5, Significant at *P<0.05; **P<0.05; ***P<0.01, (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for male, female and different parameters)

2.2. *Effect of Ginkgo biloba on proteins and nucleic acids in testicular cells*:

The testicular concentrations of RNA were reduced at both 100 and 200 mg/kg/day of Ginkgo biloba treatment. The DNA contents were reduced significantly only at the medium dose (200 mg/kg/day), while the protein concentrations were not affected (Table 5).

2.3. *Effect of Ginkgo biloba on MDA concentrations in hepatic cells:*

The hepatic levels of MDA were increased after the treatment with Ginkgo biloba. The increase was significant at 200 mg/kg/day. On increasing the dose to 400 mg/kg/day, there was further increase in the MDA concentrations. This elevation was statistically significant as compared to the values obtained in the control group (Table 6).

2.4. *Effect of Ginkgo biloba on MDA concentrations in testicular cells:*

In testicular tissue, there was a dose-dependent increase in the levels of MDA after treatment with the different doses (100, 200 and 400 mg/kg/day) of Ginkgo biloba. However, the increase was significant at the highest dose, as compared to the values obtained in the control group (Table 6).

2.5. *Effect of Ginkgo biloba on NP-SH levels in hepatic cells*:

Ginkgo biloba treatment depleted the liver contents of NP-SH. The depletion was significant at doses of 100, 200 and 400 mg/kg/day, as compared to the values observed in the control group (Table 7).

2.6. *Effect of Ginkgo biloba on NP-SH levels in testicular cells:*

The testicular concentrations of NP-SH were reduced at the different doses of Ginkgo biloba, the depletion was significant at the doses of 100, 200 and 400 mg/kg. body weight, as compared to the values obtained in the control group (Table 7).

Discussion

The treatment with Ginkgo biloba failed to

affect the frequency of micronucleated-PCE and the ratio of PCE/NCE, indicating lack of any clastogenic and cytotoxic activity. There is a paucity of literature on the genotoxic and/or cytotoxic potentials of Ginkgo biloba, except a report on the protection against radiation-induced chromosomal damage (18). However, the results of the present study showed lack of any effect on the hepatic levels of nucleic acids. These data did not confirm reports on the mutagenicity and cytotoxicity of the phyto-constituents of Ginkgo biloba. Alkylphenols, Ginkgolic acids, cardinals and cardols have been recognized as compounds with suspected mutagenic and carcinogenic properties (2,4,15,17). Hecker *et al*. (4) found Ginkgolic acids and related alkyl phenols (cardanols and cardol) to possess cytotoxic potentials on human keratinocyte cell line and Rhesus monkey kidney tubular epithelial cell line. Ginkgolic acids have been reported to cause death of cultured chick embryonic neurons in a concentration-dependent manner. These deaths showed features of apoptosis as chromatin condens-ation, shrinkage of nucleus and necrosis (16).

A comparison of our results with the reports on clastogenic potential of the constituents revealed that the activity of Ginkgo biloba observed in the somatic cells of mice is independent of its constituents. There is no definite reason except to conclude that the influence of Ginkgo biloba and its constituents on cytochrome P450 (CYP) might have interfered with the pathways of metabolism and resulted in the discordance (33). The oxidant activity of some of the constituents of Ginkgo biloba might be related to CYP-dependent reactions. A recent study also confirm that the genesis of lipid peroxides, nitric oxide and the activity of glutathione are CYP-dependent reactions (34). Kuo *et al*. (35) have also confirmed that the Ginkgo biloba-induced inhibition of the hepatic microsomal CYP1A activity in rodents is independent of its constituents (ginkgolides A, B, C or J, bilobalide, kaempferol, quercetin, iso-rhamcetin, quercetin, isorhamnetin or respective flavonol monoglycosides or diglycosides). On the other hand, the quercetin components of Ginkgo biloba were found to retard the activities of CYP2C9 or CYP3A4 in human liver microsomes (33).

The cytological studies in germ cells showed lack of any effect on the spermatozoa abnormality. However, the testicular concentrations of nucleic acids were depleted. Although, there are no parallel studies available for a possible comparison, the data on testicular nucleic acids failed to reflect our observations on spermatozoa morphology. The discordance between the results on nucleic acids and the lack spermatozoa abnormality might be related to the role of the excurrent duct system in eliminating morphologically abnormal spermatozoa during transit from testes to vas deferens and caudae epididymis. Previous studies (36, 37) have shown that the transit of spermatozoa through the extragonadal passage produces and/or eliminate morphologically abnormal spermatozoa by the excurrent duct system. The mechanism of these changes is yet a dilemma.

The results of our study on the biochemical changes induced by Ginkgo biloba clearly demonstrated the genesis of lipid peroxides in both liver and the testes as revealed by the respective increase and decrease of MDA and NP-SH. There appears to be a sharp difference in the results of biochemical activity and the cytological observations both in somatic and germ cells. The contradiction might be related to the differences in the developmental processes leading to development of the micro-nucleated polychromatic erythrocytes of the somatic cells and also the spermatozoa from the germ cells. There are two possibilities during the developmental process, which are either interference in the cell divisions or the influence of free radicals on DNA modification.

Our data on the oxidant potentials is not in agreement with several reports in the literature that confirm the protective and/or antioxidant activity of Ginkgo biloba and its constituents. However, a comparison of our investigation with others revealed differences in the conduct of experiments. Our protocol included in vivo treatment of seven repeated doses. Some of the reports in the literature involved in vitro techniques (4, 38-42), while others used a single dose treatment (43, 44). The discrepancies between the single and multi-doses regimen are obvious. Furthermore, the controversies on the in vitro protocol and the validity of the in vivo procedure are difficult to be dispensed with. The discrepancy in these protocols may be due to the exposure of the drug to pharmacokinetic and pharmacodynamic processes involved in the whole organ, which is different from the spatial heterogeneity of cells in vitro (45,46). Furthermore, the procedures involved in the in vitro studies are known to cause rearrangement and alteration of CYP

systems responsible for the genesis of free radicals (47).

Nevertheless, there are some important observations on the antioxidant and/or protection activity of pre-treatment with repeated doses of Ginkgo biloba in experimental animals. These studies involved the protective activity of Ginkgo biloba against CCl4-induced changes in hepatic cells (6), gentamicin-induced nephrotoxicity (48), doxorubicin-induced cardiotoxic effects (7), cisplatin-induced peripheral neuropathy (49), bleomycin-induced lung fibrosis (50), benzo(a) pyrene-induced forestomach carcinogenesis and doxorubicin-induced cardiotoxicity (51). The discordance between the results of our study on hepatic and testicular cells and those of the cells from the dorsal root ganglia, kidney, lung and stomach appears to be due to the differences in the cell sensitivity to the source of exposure. Previous reports have suggested that cell sensitivity to the source of exposure may reflect differences in the metabolism and the intracellular concentrations of enzymes and mediators of various target biochemical processes or repair mechanisms (52,53). Furthermore, the protocol used in most of these studies is the continuos pre-loading of Ginkgo biloba before the exposure of the tissues to the oxidant injury. The difference in duration and frequency of exposures might have determined the proportion of antioxidant/oxidant ratio and the related outcome.

Taken together, the observed changes of Ginkgo biloba might be related to its effect on the increase of MDA and depletion of NP-SH. The exact mechanism of action is not known, however, it appears to be related with the influence of Ginkgo biloba on CYPs, resulting in the accumulation of free radical species. Given that increasing number of people are exposed to herbal preparations that contain many constituents with potential of CYP modulation, our observation on the related oxidative potentials warrants careful use of Ginkgo biloba.

Acknowledgement

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