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Attenuation of tamoxifen-induced hepato-renal toxicity by cumin (*Cuminum cyminum* L.) in rats

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ABSTRACT

With the widespread therapeuticand emerging prophylactic use of TAM, there has been much discussion about side-effects of the drug, particularly its organ toxicity. The present study was designed to evaluate the toxic effects of tamoxifen (TAM) on the liver and kidney of experimental rats and the possible protective effects of an aqueous methanolic extract of cumin (*Cuminum cyminum* L), which is rich in phenolics and flavonoids against tamoxifen toxicity. Thirty two female adult rats were used in the study and divided into four groups. The first group was designed as controls. The second group was intraperitoneally injected with TAM (10 mg/kg body weight) daily for 10consecutive days. The third group was intraperitoneally pre-injected with cumin extract (10 mg/kg) daily for 20 consecutive days, starting 10 days before the TAM-injection. The fourth group was intraperitoneally injected with cumin extract (10 mg/kg) daily for 20 consecutive days. The level of lipid peroxidation of serum as well as the histology of liver and kidney of the rats can be adversely affected by TAM-treatment. Cumin administration decreased the levels of lipid peroxidation and normalized the histological structure of the studied organs. In conclusion, the administration of cuminprior to tamoxifen resulted in amelioration of the toxicity to the organs from tamoxifen.

KEYWORDS

Tamoxifen;
Cumin extract;
Liver;
Kidney;
Lipid peroxidation;
Rat.

INTRODUCTION

Tamoxifen, a triphenylethylene derivative, is used in the treatment and prevention of all stages of hormone dependent breast cancer[6]. With the widespread use of Tamoxifen, attention has been focused on its adverse effects, particularly toxicity to various organs[7]. reported cases of non-alcoholic steatohepatitis with cirrhosis in women receiving tamoxifen as adjuvant treatment for breast cancer. Tamoxifen-induced fatty liver has been observed in more than 30% of breast cancer patients who received adjuvant tamoxifen treatment[10]. In addition[22], reported that TAM-induced hepatorenal toxicity. Recent evidence suggested that the generation of reactive oxygen species (ROS) and oxidative stress may also play a role in the TAM toxicity[15].

With the widespread use of herbal medicines increasing worldwide, several natural antioxidants have been evaluated for their chemoprotective effects in various pathological states[29]. Cumin (*Cuminum cyminum* L.) is a small annual herbaceous plant belonging to the Apiaceae family and is a popular spice regularly used as a food flavoring agent. It is cultivated in Arabia, India, China and in the countries bordering the Mediterranean Sea[30]. Cumin seeds have been used as drugs and spice for about a thousand years[3]. The pharmacological effects of many traditional drugs may be ascribed to the presence of phenolic and flavonoid compounds[23]. Cumin seeds contain 14 flavonoid groups[17] and have been used therapeutically for disorders of the gastrointestinal, gynecological and respiratory (asthma and dyspnea) systems as described in ancient Iranian medical texts[33].

As the metabolism of TAM in humans appears to be qualitatively similar to its metabolism in rodents[24], the present study was undertaken to gain insight into the tamoxifen induced toxicity of organs in female albino rats and its amelioration by administration of cumin (*Cuminum cyminum*).

MATERIALS AND METHODS

Chemicals

Tamoxifen citrate (Acdima International, Giza, Egypt) was kindly provided by the Medical Union Pharmaceuticals Company (MUP), Cairo, Egypt. Kit of malondialdehyde was purchased from Biodiagnostic Co., Cairo, Egypt. Cumin aqueous methanolic extract was prepared at the laboratory and all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland).

Extraction

Cumin seeds obtained from a local market were ground to powder and exhaustively extracted with 80% methanol and filtered. The filtrate was vacuum-dried and the extract was kept in refrigerator until use in the experiment.

Determination of total phenolics and flavonoids

The total concentration of phenolics in the aqueous cumin extract was determined according to the Folin-Ciocalteu method[21] with gallic acid (GA) (El Gomhouria Pharm. Co., Cairo, Egypt) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract[14]. Ten milligram extract was dissolved in one milliliter aq. methanol to prepare a stock solution, which was diluted and 1 ml was mixed with 1ml of Folin-Ciocalteu reagent and vortexed for 5 s. Then, 1ml of a 10% (w/w) sodium carbonate aqueous solution was added to the mixture. The mixture was incubated at room temperature for 1 h, after which colorimetric measurements were made at 700 nm.

Total content of flavonoids was determined by using a colorimetric method according to[34]. Briefly, test tubes containing 20 µg extract or different concentrations of Quercetin standard solution (10-80 µg/mL) (Nacalai Tesque Inc., Kyoto, Japan) were mixed with 75 µL 5% (w/v) $NaNO_2$. After 6 min, 150 µl of a 10% (w/v) $AlCl_3.6H_2O$ solution was added and the mixture was allowed to stand for a further 5 minutes before 0.5 mL of 1M NaOH was added. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer (Genesys 5, ThermoSpectronic, Rochester, NY, USA). The results were expressed as the mean ± SD mg of Quercetin equivalents per gram of extract.

Animals and experimental design

Thirty two adult female albino rats (4 months old) weighing (120-140g) obtained from the Animal House, The Egyptian Company for Vaccines production in Helwan, were used in the present study. The animals were kept in a controlled light room with a photoperiod of 12 hours dark and 12 hours light at a temperature of 28±2 °C. The experimental design was approved by the Animal Care and Use Committee, University of Aswan. The rats were randomly divided into four groups (Cont, TAM, TAM+ Cum and Cum), 8 rats each. Control group (Cont) was injected daily intraperitoneally (i.p.) with normal saline for 20 successive days. TAM-group was injected daily i.p. with normal saline for 10 consecutive days starting from day 1 to 10, followed by daily i.p. injection of tamoxifen starting from day 11 to 20 at a dose of 10 mg/kg in normal saline. TAM + Cum-group was treated daily i.p. with 10 mg/kg of cumin for 20 successive days and

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injected i.p. with 10 mg/kg of TAM for 10 successive days starting at day 11 to 20. Cum-group was treated daily i.p. with 10 mg/kg of cumin in normal saline for 20 successive days. 24 hours after the last injection, rats were sacrificed by decapitation.

Measurements of lipid peroxidation (LPO) in the serum

Blood samples were collected in non-coated serum-separating tubes. The blood was centrifuged at 3000 r.p.m. for 15 mins to separate the serum. Sera were used for measurement of MDA concentrations in both control and treated groups. Malondialdehyde (MDA) concentrations were expressed as nmol/ ml serum and assayed according to a modified method of[20]. The principle is based on the reaction of thiobarbituric acid with malondialdehyde in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm.

Histology

For histological examination, specimens from the liver and kidney were rapidly excised, and cut into small blocks, which were fixed in 10% neutral buffered formalin (pH 7.2), dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections 5 µm thick were cut and stained with Harris's hematoxlin and eosin[12].

Statistical analysis

Results of LPO were expressed as means ± SEM. Statistical differences were analyzed using a one-way ANOVA followed by the Student-Newman-Keuls t-test. Statistical significance was accepted at $p < 0.005$.

RESULTS

Phenolic and flavonoid contents

Quantitative analyses of total phenolics 82.68 mg GAE (gallic acid equivalent)/g extract and flavonoids content of 29.35 mg QE (Quercetin equivalent)/g extract, respectively are shown in Figure 1.

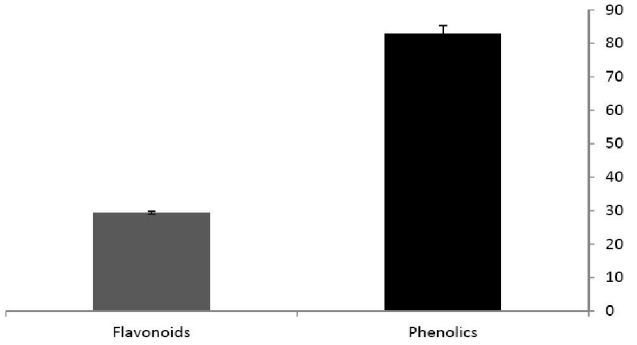

Figure 1 : Total phenolics (mg Gallic acid equivalent per gram extract) and flavonoids (mg Quercetin equivalent per gram extract) in dry Cumin aqueous methanolic extract. Data expressed as mean ± SD (n=3).

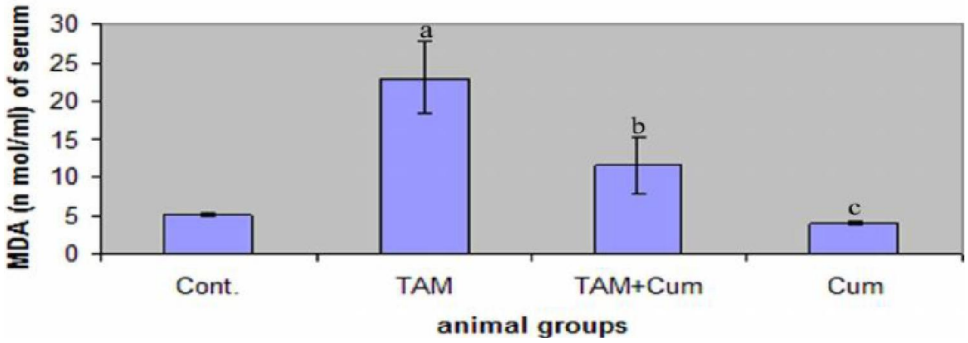

Figure 2 : The influence of cumin on malondialdehyde levels in tamoxifen treated rats. alues are expressed as mean ± SEM. [a] Highly significant difference from the corresponding control group at P < 0.005. [b]Highly significant difference from corresponding Tamoxifen-treated group at P < 0.005. [c]Non-significant difference from the corresponding control group at P > 0.05 .

Biochemical assays

Lipid peroxidation (LPO) measurements

Serum LPO levels (as indicated by malondialdehyde [MDA] concentrations of the control and treated groups) are shown in Figure 2. Treatment of animals with TAM resulted in a highly significant enhancement in the levels of serum MDA (P<0.005) when compared with control group. In TAM + Cum group, the levels of LPO were highly significant decreased (P<0.005) vs. the TAM-treated animals. The cumin group showed a

non-significant difference (P>0.05) in the levels of serum MDA when compared to control rats.

Histopathological results

Liver

Upon microscopic examination, liver sections of the control (Figure 3A) and cumin (Figure 3B) groups showed no histopathological changes in hepatic cells, central veins and blood sinusoids. Liver sections of TAM-treated rats showed severely damaged hepatocytes with congested central veins. Also, mild infiltration of inflammatory cells with vacuolar degeneration and hemorrhage were observed (Figure 3C). Liver sections in the TAM + Cum group showed a normal histological appearance of all contents of liver lobule including hepatic cells and central veins as well as blood sinusoids (Figure 3D).

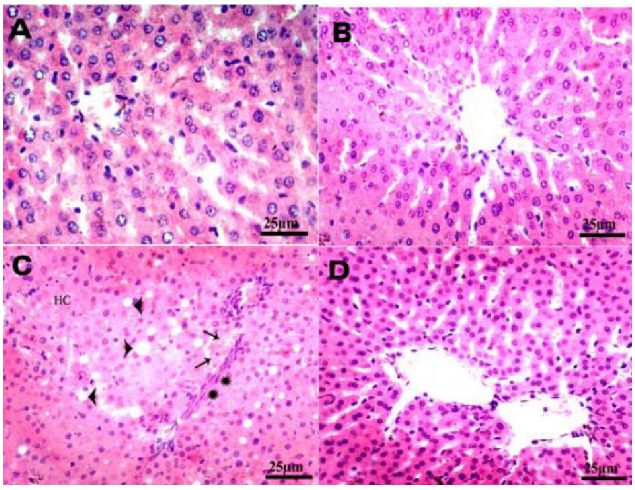

Figure 3 : Effect of cumin (Cum) pretreatment on tamoxifen (TAX) induced liver damage in rats. (A) and (B) Liver sections of the control and cumin groups respectively, show normal histological pattern of hepatic lobule. (C) Liver section from rats treated with tamoxifen shows mild infiltration of inflammatory cells (*), vacuolar degeneration (arrowhead), hemorrhage (arrows), severely damaged hepatocytes (HC) and damaged central veins. (D) Liver section of rats treated with tamoxifen + cumin shows normal hepatic cells and central veins as well as normal blood sinusoids. (H&E, Scale bar =25µm).

Kidney

Microscopic examination of control (Figure 4A) and cumin (Figure 4B) groups showed normal histological pattern of kidney structure as Malpighian corpuscles and renal tubules. Histopathological examination of the renal tissue of the TAM-group (Figure 4C) revealed severe lesions characterized by evident necrosis in some distal renal tubules with dilatation and congestion in the peritubular capillaries. Compared with the TAM-group, these lesions were absent in the TAM + Cum (Figure 4D).

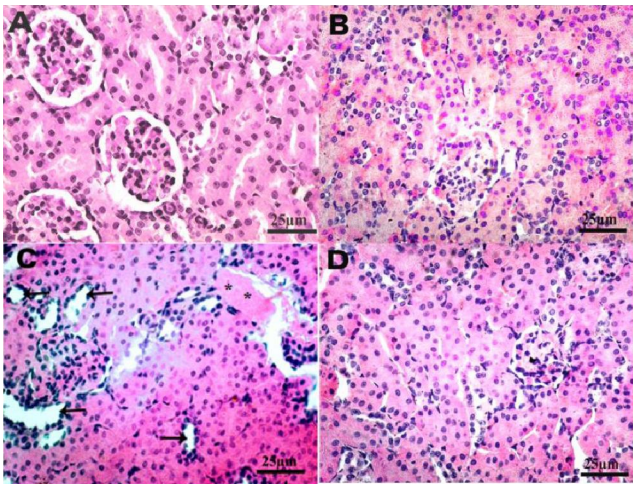

Figure 4 : Effect of cumin (Cum) pretreatment on tamoxifen (TAX) induced kidney damage in rats. (A) and (B) Kidney sections of the control and cumin groups respectively, show normal histological structure of the kidney. (C) Photomicrograph of kidney section of TAX-treated rats reveals evident necrosis (arrows) in some distal renal tubules with dilatation and congestion (*) in the peritubular capillaries . (D) Kidney section of TAX + Cum group reveals normal architecture of the renal tubules as well as renal corpuscles. (H&E, Scale bar =25µm)

DISCUSSION

With the widespread therapeutic use of tamoxifen (TAM), there has been considerable discussion about side-effects of this drug, particularly regarding organ toxicity. Therefore, strategies to ameliorate organ toxicity induced by tamoxifen treatment are of clinical interest. In the present study, a repeated dose of TAM significantly enhanced serum LPO levels when compared to the control group. Our results are supported by observations of[22] who observed a significant increase in the levels of lipid peroxides in hepatic and renal tissues of TAM-treated mice[28]. found that treatment of mice with TAM caused increased LPO in hepatic mitochondria, which was accompanied by increased production of superoxide radicals. Our results are in agreement with the findings of[9] and[2] who reported that TAM treatment resulted in depletion of antioxidant enzymes and accumulation of

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oxidant enzymes and lipid peroxidation. The interpretation of our results may be attributed to overproduction of oxygen radicals during TAM metabolism[8,16]. In addition, our study showed that tamoxifen induced severe histopathological damage in the hepato-renal tissues[19] found that the histopathological changes may be due to the production of reactive oxygen species (ROS), which could damage the cellular elements. Hepatic and renal toxicity induced by TAM in the present work may be result from TAM being initially metabolized in the liver with subsequent accumulation of some metabolites such as 4-hydroxytamoxifen, 4-hydroxy-N-desmethyltamoxifen and N-desdimethyltamoxifen in various tissues[5]. Moreover, LPO, mediated by oxygen free radicals, is believed to be an important cause of destruction and damage to cell membranes and has been suggested to be a contributing factor to the development of TAM-mediated tissue damage[27].

Given the growing interest and attention on the roles of polyphenols and antioxidants in human health, the presence of these in some spices has been the subject of several reports in the literature[4,25]. In addition, attention has been paid to the protective effects of natural antioxidants against anticancer drug-induced toxicities involving free radical-mediated oxidative stress and tissue injury[11]. Flavonoids are a diverse group of low molecular weight polyphenolic compounds that are widely distributed in nature. Many health-related properties, including anticancer, antiviral, anti-inflammatory activities, antioxidant properties and an ability to inhibit human platelet aggregation, have been ascribed in particular to phenolics[26]. In the present study, the cumin treatment significantly decreased serum LPO levels when compared to the TAM-group. Our results are in accordance with results that suggested that cumin has the capability to neutralize free radicals and prevent unsaturated fatty acid oxidation[30]. Also, caffeic acid phenethyl ester is a small, lipid soluble flavonoid-like compound that protects rats against TAM-toxicity by preserving cellular integrity, preventing oxidative stress and lipid peroxidation, enhancing antioxidant enzymes activities and inhibition of inflammation [2,28] found that pretreatment of mice with catechin, an antioxidant flavonoid, endowed significant protection as demonstrated by marked attenuation of increased oxidative stress parameters such as LPO and superoxide production. It also restored the decreased non-enzymatic and enzymatic antioxidants of mitochondria. Previous studies confirmed that flavonoids inhibit lipid peroxidation *in vitro* at the initiation stage by acting as scavengers of superoxide anions and hydroxyl radicals[1,31]. It has been proposed that flavonoids terminate chain radical reactions by donating hydrogen atoms to the peroxy radical forming a flavonoid radical[1]. The flavonoid radical in turn reacts with free radicals thus terminating the propagating chain[31]. Moreover, flavonoids have the potential to scavenge reactive oxygen species (ROS),

chelate metal ions, act as a chain-breaking antioxidant by scavenging lipid peroxyl radicals, or partition into the lipid bilayer to prevent lipid damage[32,35]. Furthermore, we found in our study that cumin administration ameliorated the histopathological changes in specific organs. Our results are accordance with[18] who reported that the aqueous extract of cumin seeds provided protective effects against gentamycin-induced nephrotoxicity[22] reported that flavonoids such as catechins, which are non-cytotoxic plant polyphenolic constituents, may prevent tamoxifen-induced hepato-renal toxicity in mice. The interpretation of our results may be attributed to the fact that the diverse interaction between flavonoids and cell proteins or enzymes protects the cells against drug toxicity[13].

In conclusion, the results of the present study indicate that the administration of cumin may prevent tissues toxicity-induced by tamoxifen in rats and may provide therapeutic potential in alleviating the side-effects of chemotherapy. The protective effects of cumin are probably due to a counteraction of free radicals by its antioxidant nature. If this protective function is confirmed in patients, cumin may have an important clinical significance as an adjuvant therapy in tamoxifen drug treatment.

REFERENCES

[1] I.B.Afanas'ev, A.I.Dorozhko, A.V.Brodskii, V.A.Kostyuk, A.I.Potapovitch; Biochem. Pharmacol., **38**, 763-9 **(1989)**.

[2] A.A.Albukhari, H.M.Gashlan, H.A.El-Beshbishy, A.A.Nagy, A.B.Abdel-Naim; Food and Chemical Toxicol., **47**, 1689-95 **(2009)**.

[3] V.Agarwal, V.Gupta, K.Nepali, O.P.Suri, K.L.Dhar; J.Nat.Conscientia, **1**, 148-56 **(2010)**.

[4] A.Ardestani, R.Yazdanparast; Food Chem., **104**, 21-9 **(2007)**.

[5] P.Carthew, B.M.Nolan, R.E.Edwards, L.L.Smith; Cancer Lett., **106**, 163-9 **(1996)**.

[6] P.Desai, S.Nallani, R.Sane, L.Moore, B.Goodwin, D.Buckley, A.Buckley; Drug Metab.Dispos., **30**, 608-12 **(2002)**.

[7] X.Dray, M.H.Tainturier, P.De La Lande, O.Marty, L.Mallet; Gastroenterol Clin.Biol., **24**, 1122-3 **(2000)**.

[8] H.El-Beshbishy; J.Biochem.Mol.Biol., **38**, 300-6 **(2005)**.

[9] H.A.El-Beshbishy; Asian J.Trad.Med., **2**, 175-88 **(2007)**.

[10] I.S.Elefsiniotis, K.D.Pantazis, A.Ilias, L.Pallis, A.Mariolis, I.Glynou, et al.; Eur.J.Gastroenterol. Hepatol., **16**, 593-8 **(2004)**.

[11] B.Frei, J.V.Higdon; J.Nutr., **133**, 3275S-84S **(2003)**.

[12] M.Gabe; Histological techniques. New York, Heidelberg, Berlin/Paris, New York, Barcelona, Milan Springer-Verlag/Masson, **(1976)**.

[13] M.Galisteo, M.F.Garcý´a Saura, R.Jime´nez, I.C.Villar, A.Zarzuelo, F.Vargas, et al.; Mol.Cell Biochem., **259**, 91-9 **(2004)**.

[14] S.J.Hossein, M.El-Sayed, H.Aoshima; Orient. Pharm.Exp.Med., **9**, 217-24 **(2009)**.

[15] Y.Jin, Z.Desta, V.Stearns, V.Ward, T.Skaar, A.M.Storniolo, A.Araba; J.Nat.Cancer Inst., **97**, 30-9 **(2005)**.

[16] A.Karki, E.Mantyla, Y.Hirsimaki, S.Karlsson, S.Toikkanen, P.Hirsimaki; Arch.Toxicol., **74**, 249-56 **(2000)**.

[17] C.P.Khare; Indian medicinal plants. ISBN 978-0-387-70637-5 Springer-Verlag, Berlin/Heidelberg, **(2004)**.

[18] C.M.Mahesh, K.P.S.Gowda, A.K.Gupta; J.Pharmacy Res., **3**, 753-7 **(2010)**.

[19] F.A.Morsy, A.Gamal el Din, N.M.Shaffie, M.A.Badawi; Nature and Science, **8**, 1-15 **(2010)**.

[20] H.Ohkawa, N.Ohishi, K.Yagi; Anal.Biochem., **95**, 351-8 **(1979)**.

[21] C.S.Ough, M.A.Amerine; Methods for analysis of musts and wine.Wiley & Sons, New York, 196-221 **(1988)**.

[22] S.Parvez, H.Tabassum, H.Rehmana, B.Dev Banerjee, S.M.Athar, Raisuddin; Toxicology, **225**, 109-18 **(2006)**.

[23] P.G.Pietta; J.Nat.Prod., **63**, 1035-42 **(2000)**.

[24] G.K.Poon, Y.C.Chui, R.McCague, P.E.Lonning, M.G.Feng, M.G.Rowlands, et al.; Drug Metab. Dispos., **21**, 1119-24 **(1993)**.

[25] E.Sikora, E.Cieslik, T.Leszczynska, A.Filipiak-Florkiewicz, P.M.Pisulewski; Food Chem., **107**, 55-9 **(2008)**.

[26] G.Spignoli; Eur.Bull.DrugRes., 81-8 **(2000)**.

[27] L.A.Stanley, P.Carthew, R.Davies, F.Higginson, E.Martin, J.A.Stayles; Cancer Lett., **171**, 27-35 **(2001)**.

[28] H.Tabassum, S.Parvez, B.Dev Banerjee, S.Raisuddin; Toxicol.Lett., **211**, S194 **(2012)**.

[29] H.Tabassum, S.Parvez, H.Rahman, B.Banerjee, S.Raisuddin; Human Exp.Toxicol., **26**, 509-18 **(2007)**.

[30] N.B.Thippeswamy, K.A.Naidu; Eur.Food Res. Technol., **220**, 472-6 **(2005)**.

[31] J.Tore1, J.Cillard, P.Cillard; Phytochemistry, **25**, 383-5 **(1986)**.

[32] I.Urquiaga, F.Leighton; Biol.Res., **33**, 55-64 **(2000)**.

[33] A.Zargary; Medicinal plants. 5th Edition. Tehran University, **(1990)**.

[34] J.Zhishen, T.Mengcheng, W.Jianming; Food Chem., **64**, 555-9 **(1999)**.

[35] M.Zielinska, A.Kostrzewa, E.Ignatowicz; Folia Histochem.Cytobiol., **28**, 25-30 **(2000)**.