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The Effects of Curcumin Nanoparticles on an Experimental Diabetes Model: Reproductive Hormones, Lipid Profile and Immunohistochemical and Immunofluorescence Markers

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Summary

Diabetes affects many organs and causes serious problems for reproductive health. There is still no effective treatment but a range of new strategies are being used to treat and to reduce the possible complications of diabetes. One of the most promising of them relies on the use of nanoparticles. We have evaluated the effects of curcumin and curcumin nanoparticles on blood glucose, lipid profile, reproductive hormones and histopathological, immunohistochemical and immunofluorescent parameters in experimental diabetic conditions. We created eight study groups (control group, diabetes group, curcumin group, Mg-curcumin nanoparticle group, diabetes+curcumin group, diabetes+Mg-curcumin nanoparticle group, encapsulated Mg-curcumin nanoparticle group, diabetes+encapsulated Mg-curcumin nanoparticle group), allocating rats to the groups at random. Triglyceride, cholesterol and LDL levels were high in the diabetes group, NOP10 and PI3K levels were intense and reproductive hormone levels were low. Curcumin and curcumin nanoformulations administered to the diabetes groups led to significant improvements in these parameters. The testosterone level, which was low in the diabetes group, increased significantly after the application of encapsulated Mg-curcumin. Curcumin nanoformulations were more effective than curcumin at counter-

Zusammenfassung

Effekte von Curcumin Nanopartikeln in einem experimentellen Diabetesmodell: Fortpflanzungshormone, Lipidprofil, immunhistochemische Marker und Immunfluoreszenzmarker

Diese Studie wurde durchgeführt, um die Auswirkungen von Curcumin und Curcumin-Nanopartikeln auf den Blutzuckerspiegel, das Lipidprofil, die Fortpflanzungshormone, histopathologische, immunhistochemische und Immunfluoreszenz-Parameter unter experimentellen Diabetesbedingungen zu untersuchen. Diabetes, der viele Organe beeinträchtigt, verursacht auch ernsthafte Probleme in Bezug auf die reproduktive Gesundheit. Heutzutage werden neue Strategien angewandt, um Diabetes zu behandeln, für den es noch immer keine wirksame Therapie gibt, und um mögliche Komplikationen zu verringern. Eine der vielversprechendsten dieser Strategien ist die Verwendung von Nanopartikeln. In der Studie wurden 8 Gruppen (Kontrollgruppe, Diabetes-Gruppe, Curcumin-Gruppe, Mg-Curcumin-Nanopartikel-Gruppe, Diabetes + Curcumin-Gruppe, Diabetes + Mg-Curcumin-Nanopartikel-Gruppe, eingekapselte Mg-Curcumin-Nanopartikel-Gruppe, Diabetes + eingekapselte Mg-Curcumin-Nanopartikel-Gruppe) erstellt. Die Anzahl der in der

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ing the diabetes-impaired lipid profile and improving the levels of reproductive hormones, the histopathological findings and PI3K and NOP10 expression.

Abbreviations: FSH = follicle stimulating hormone; HDL = high density lipoprotein(s); LDL = low density lipoprotein(s); LH = luteinizing hormone; Mg = magnesium; NOP10 = nucleolar protein 10; PI3K = phosphatidylinositol 3-kinase; ROS = reactive oxygen species; STZ = streptozotocin; VLDL = very low density lipoprotein(s)

Studie verwendeten Ratten betrug 56, und die Verteilung auf die Gruppen erfolgte nach dem Zufallsprinzip. In der Diabetesgruppe waren die Triglycerid-, Cholesterin- und LDL-Werte hoch, die NOP10- und PI3K-Expressionswerte hoch und die Reproduktionshormonwerte niedrig. Curcumin und Curcumin-Nanoformulierungen, die den Diabetes-Gruppen verabreicht wurden, führten zu signifikanten Verbesserungen bei diesen Parametern. Der Testosteronwert, der vor allem in der Diabetesgruppe signifikant niedrig war, stieg nach der Verabreichung von verkapseltem Mg-Curcumin signifikant an. In Anbetracht aller Ergeb. nisse lässt sich vermuten, dass Curcumin-Nanoformulierungen bei Diabetes wirksamer als Curcumin auf ein gestörtes Lipidprofil, reproduktive Hormone, PI3K- und NOP10-Expression wirken und auch histopathologisch feststellbaren Veränderungen entgegenwirken.

Introduction

Diabetes mellitus is a metabolic disease characterized by insufficient insulin secretion or insulin resistance (Hakim et al. 2008; Woldu & Lenjisa 2014). Diabetes patients experience testicular damage that seriously affects their quality of life (Kushwaha & Jena 2013). Testicular biopsy score, number of Sertoli cells, tubular diameter and germinal epithelial height decrease in diabetes and there are reports of increased DNA damage and deterioration of the germinal epithelium (Kushwaha & Jena 2013; Sonmez et al. 2015).

Trace metals such as magnesium (Mg) and zinc affect the maintenance of blood glucose (Chausmer 1998; Wells 2008). The Mg^{2+} ion is involved in more than 300 enzymatic reactions including those involved in glycolysis (Pasternak et al. 2010) and is a necessary cofactor for the tyrosine kinase activity of the insulin receptor. Mg deficiency impairs tyrosine kinase activity, leading to insulin resistance (Saris et al. 2000; Barbagallo & Dominguez 2007) and decreased sensitivity to insulin. As insulin sensitivity decreases, the body needs more insulin to process the same amount of glucose (Barbagallo et al. 2003). Magnesium supplementation is thought to improve insulin sensitivity and the lipid profile (Olatunji & Soladoye 2007). Magnesium oxide nanoparticles may reduce insulin resistance in type 2 diabetes and thus may be an antidiabetic agent (Jeevanandam et al. 2015).

Curcumin, which is obtained from *Curcuma longa*, is thought to be effective against diabetes complications (Sanidad et al. 2019). It has many biological impacts, such as antidiabetic, antiapoptotic, antioxidant and anti-inflammatory (Mahmoudi et al., 2022; Tsao et al. 2022). Curcumin is used in the cosmetics industry, in dietary supplements and in many other areas. Due to its conjugated double bonds, it suppresses the formation of reactive oxygen species (ROS) (Esatbeyoglu et al. 2012) and can prevent oxidative damage (Chikara et al. 2018). Although safe and effective from a pharmacological point of view, curcumin is not widely used due to a number of reasons such as low bioavailability, rapid metabolism and poor solubility (Dulbecco & Savarino 2013).

Curcumin nanoformulations have been developed to increase its solubility and inhibiting its metabolism, thereby prolonging its residence in the systemic circulation (Chauhan et al. 2018; Karthikeyan et al. 2020). Similarly, insulin and other sugar-lowering drugs have been loaded into nanoparticles (Woldu & Lenjisa 2014; Siddiqui et al. 2020). The therapeutic power of nanocurcumin is better than that of curcumin alone (Chauhan et al. 2018; Sarawi et al. 2022). Chitosan-encapsulated nanocurcumin exhibits enhanced antihyperglycaemic function (Chauhan et al. 2018). Nanocurcumin is more effective than curcumin in reducing the lipid profile (cholesterol, triglycerides, low density lipoproteins (LDL), and very low density lipoproteins (VLDL); Shamsi-Goushki et al. 2020). We now report an evaluation of the effects of curcumin and curcumin nanoparticles on lipid profile, reproductive hormones, blood sugar and histopathological findings in experimental diabetes, as well as on the activities of NOP10 and PI3K.

Material and methods

Chemicals

Streptozotocin (STZ) was purchased from Sigma Aldrich (Germany) and curcumin from Alfa Aesar, Ward Hill, Massachusetts (USA). Encapsulated nanoparticles and curcumin nanoparticles were prepared as described (Kaya & Taskin 2016). FSH (00380740014810, Ireland), LH (00380740003920,

Ireland), total testosterone (0038074017764, UK) and cholesterol (00380740158132, Ireland) kits were purchased from Abbott/Architect, while the triglyceride (8681812526227, Turkey) kit was purchased from Archem. Recombinant anti-NOP10 antibody (ab134902, UK), recombinant anti-PI3 kinase antibody (ab225720, UK) and FITC-conjugated goat anti-mouse IgG (ab6785) were supplied by Abcam.

Experimental animals and design of the study

Fifty-six male rats (Wistar albino, average weight 300–370 g, 14–15 weeks old) were randomly divided into eight equally sized groups. Their body weights were recorded and clinical examinations performed. The National Institutes of Health guidelines for the care and use of laboratory animals were followed in all experimental practices. Before we started the study, we obtained approval (decision dated 29.12.2022 and numbered 2022/13 - 04) from the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University. Experimental interventions were carried out in the same centre. Rats were fed drinking water and pellets (Purina, Bursa, Turkey) containing 21 % crude protein.

The study groups were as follows.

Group 1 (control group): saline was administered orally at a dose of 0.5 ml/kg daily.

Group 2 (diabetes group): STZ 45 mg/kg (i.p.) was administered as a single dose.

Group 3 (curcumin group): curcumin was administered orally once a day at a dose of 10 mg/kg.

Group 4 (Mg-curcumin nanoparticle group): Mg-curcumin nanoparticles were administered orally once a day at a dose of 10 mg/kg.

Group 5 (diabetes+curcumin group): administration of curcumin started 72 hours after administration of STZ 45 mg/kg (i.p.). Curcumin was administered orally once a day at a dose of 10 mg/kg.

Group 6 (diabetes+Mg-curcumin nanoparticle group): administration of Mg-curcumin nanoparticles started 72 hours after a single dose of STZ 45 mg/kg (i.p.). Mg-curcumin nanoparticles were administered once a day at a dose of 10 mg/kg.

Group 7 (encapsulated Mg-curcumin nanoparticle group): encapsulated Mg-curcumin nanoparticles were administered orally once a day at a dose of 10 mg/kg.

Group 8 (diabetes+encapsulated Mg-curcumin nanoparticle group): administration of encapsulated Mg-curcumin nanoparticles started 72 hours after STZ 45 mg/kg (i.p.) was administered. Encapsulated Mg-curcumin nanoparticles were administered orally once a day at a dose of 10 mg/kg.

Curcumin, Mg-curcumin nanoparticles and encapsulated Mg-curcumin nanoparticles were each administered for 15 days.

Induction of experimental diabetes

Before starting the experiment, we measured the fasting blood glucose levels of the rats in groups 2, 5, 6 and 8. We induced diabetes in these groups by administering STZ (45 mg/kg i.p.) prepared in 0.1 M citrate buffer with pH of 4.2 to rats fasted overnight and then gave the rats sugar water (20% glucose solution) for 24 hours. The blood glucose levels were measured with a glucometer (IME-DC, Hof, Germany) 72 hours after administration of STZ (Naghibi et al. 2022).

Determination of lipid profile and levels of glucose and reproductive hormones

Reproductive hormones, glucose and lipid profiles were measured in serum obtained by centrifuging blood samples taken from the rats after they were sacrificed. An Abbott Architect I4000 SR device was used to measure serum testosterone levels with the chemiluminescent microparticle immunological method. An Abbott Architect I6200 SR device was used to measure serum glucose, lipid profile (triglyceride, cholesterol, high density lipoproteins (HDL), LDL), follicle stimulating hormone (FSH) and luteinizing hormone (LH). Related measurements were made with the chemiluminescent microparticle immunological method. We used an appropriate calibrator and kit, as well as controls for comparison, for all measurements.

Histopathological examination

Testicular tissue samples fixed in formaldehyde (10 % buffered) solution and placed in paraffin blocks by passing through alcohol-xylene series were stained with haematoxylin and eosin and histopathological changes evaluated. We examined a total of 56 tissue samples, from 7 animals in each group, under a light microscope for the absence or severity of lesions.

Immunohistochemical examination

Tissue sections on adhesive (poly–L-lysine) slides for immunoperoxidase examination were deparaffinized and dehydrated and the primary antibody NOP10 (Dilution Ratio: 1/100) was dripped onto the tissues and incubated according to the instructions for use. The sections were then examined under a light microscope (Zeiss Axio Germany) and evaluated (Belhan et al. 2020).

Immunofluorescence examination

Testicular tissue sections on adhesive (poly–L-lysine) slides for immunofluorescence examination were deparaffinized and dehydrated and the primary antibody PI3K (Dilution Ratio: 1/100) was dripped onto the tissues. FITC-conjugated goat anti-mouse IgG (Dilution

Ratio: 1/1000) was used as a secondary antibody and the slide left in the dark for 45 minutes before dripping DAPI with mounting medium (D1306, Dilution Ratio: 1/200) onto the sections leaving them in the dark for another 5 minutes. Finally, the sections were covered with a coverslip and the tissues evaluated under a fluorescent microscope (Zeiss Axio Germany) (Kokturk et al. 2022).

Statistical analysis

We used the Shapiro-Wilk test to determine whether the glucose, lipid profile and reproductive hormone data were normally distributed. The biochemical data in the groups were normally distributed, so we determined significant differences between the groups with the one-way ANOVA test. We used the post-hoc (Tukey HSD) test following ANOVA to determine which group caused the differences for biochemical analysis. To determine the differences between groups, we used the Kruskal Wallis test (non-parametric) for immunohistochemical and immunohistopathological evaluations before applying the Bonferroni-adjusted Mann Whitney U test to determine which group caused the differences. To determine the positive staining intensity, we evaluated five randomly selected areas in each image (from immunohistochemical and immunofluorescent staining) with the ZEISS Zen Imaging Software program. Immunohistochemical and immunohistopathological findings were converted into semi-quantitative data. Following Kruskal Wallis analysis, we performed the Bonferroni-adjusted Mann Whitney U test to determine which group caused the differences. We used the SPSS 25.0 software for the statistical analysis of the histopathological and biochemical evaluations considering $p<0.05$ as significant.

Results

The triglyceride levels in the diabetic groups were higher than those in the control group ($p<0.001$). The encapsulated Mg-curcumin group had the lowest triglyceride levels ($p<0.001$). Cholesterol levels were not affected in the diabetes group but the diabetes+curcumin group had the lowest levels ($p<0.001$) and the levels in the diabetes+curcumin, diabetes+Mg-curcumin and diabetes+encapsulated Mg-curcumin groups were considerably lower than those in the diabetes group ($p<0.001$). The results for LDL levels were similar: they were higher in the diabetes group than in the control, diabetes+curcumin, encapsulated Mg-curcumin and diabetes+encapsulated Mg-curcumin groups ($p<0.001$). Interestingly, LDL levels in the curcumin and Mg-curcumin groups were greater than in the control group ($p<0.001$). HDL levels did not differ from the control group ($p>0.05$) and no groups were statistically different from the controls. HDL levels in the diabetes+curcumin and diabetes+encapsulated Mg-curcumin groups were similar to those in the diabetes group ($p>0.05$) (Table 1).

Glucose values in serum samples were highest in the diabetes and diabetes+encapsulated Mg-curcumin groups ($p<0.001$) and lowest in the Mg-curcumin group ($p<0.001$). Table 2 gives the detailed results.

The testosterone level in the diabetes group was significantly lower than in the other groups apart from the diabetes+curcumin and diabetes+Mg-curcumin groups. The testosterone levels only rose in the group of diabetic rats that also received encapsulated Mg-curcumin. The levels of LH and FSH were lowest in the diabetes group ($p<0.001$). Table 2 shows further results.

The histopathological examination showed a normal appearance of the testicular tissues of the control, curcumin, Mg-curcumin nanoparticle and encapsulated Mg-curcumin nanoparticle groups (Figure 1). However, the diabetes group showed severe degeneration and necrosis of spermatocytes, along with severe thinning of the tubular wall, severe oedema in the intertubular spaces and hyperemic vessels (Figure 1). The diabetes+curcumin group exhibited moderate degeneration and necrosis of spermatocytes, thinning of the tubule wall, oedema between the tubules and hyperechoic

Tab. 1: Lipid profile levels in serum samples / Werte für die in Serumproben gemessenen Lipidprofile

Group	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group 1	47.86 ± 3.85^{cd}	58.80 ± 4.74^{ab}	41.73 ± 5.54^{abc}	6.59 ± 0.27^{bc}
Group 2	58.29 ± 5.56^{ab}	61.17 ± 3.66^{a}	36.73 ± 1.57^{c}	8.15 ± 0.75^{a}
Group 3	43.14 ± 2.48^{de}	58.29 ± 4.61^{ab}	$38.79 \pm 3.16b^{cd}$	7.93 ± 0.78^{a}
Group 4	49.00 ± 3.61^{c}	58.50 ± 5.32^{ab}	44.23 ± 4.92^{a}	7.71 ± 1.59^{a}
Group 5	58.14 ± 5.34^{ab}	47.86 ± 4.10^{d}	37.29 ± 4.72^{c}	6.47 ± 0.53^{bc}
Group 6	55.86 ± 4.63^{b}	52.00 ± 3.46^{cd}	36.20 ± 5.44^{cd}	7.23 ± 0.56^{ab}
Group 7	40.00 ± 3.90^{e}	50.86 ± 1.77^{cd}	43.22 ± 2.64^{ab}	5.98 ± 0.31^{c}
Group 8	62.50 ± 4.68^{a}	54.33 ± 1.31^{bc}	36.30 ± 1.15^{c}	6.62 ± 0.50^{bc}
p value	p<0.001	p<0.001	p<0.003	p<0.001

a,b,c,d: Values with different superscript letters in the same column are significantly different ($p<0.05$). In columns, the p values are the results of the Tukey HSD test following the one-way ANOVA test. / a,b,c,d: Werte mit unterschiedlichen hochgestellten Buchstaben in derselben Spalte sind signifikant verschieden ($p<0,05$). Die p-Werte in derselben Spalte sind die Ergebnisse des Tukey HSD-Tests nach Anwendung der One Way ANOVA.

vessels and was statistically different from the diabetic group. Degeneration and necrosis of spermatocytes and thinning of the tubule wall were mild in the diabetes+Mg-curcumin nanoparticle group and milder still in the diabetes+encapsulated Mg-curcumin nanoparticle group. When the diabetes+Mg-curcumin nanoparticle group and the diabetes+encapsulated Mg-curcumin nanoparticle group were compared with the diabetes group, the statistical difference was significant ($p<0.05$). Figures 2A–2D depict the thinning of the tubular wall in spermatocytes, the necrosis of the spermatocytes and the oedema in the intertubular spaces.

The level of NOP10 in spermatocytes was at a minor level in rats without diabetes but high in the diabetes group, moderate in the diabetes+curcumin group, mild in the diabetes+Mg-curcumin nanoparticle group and very mild in the diabetes+encapsulated Mg-curcumin nanoparticle group. NOP10 levels were significantly lower in diabetic rats given curcumin and Mg formulations ($p<0.05$). Figure 3 shows the immunohistochemical images from the groups and Table 3 presents a statistical representation of the findings of the immunohistochemical staining of the testicular tissues.

Figure 4 shows the PI3K levels and Table 3 gives a statistical representation of the findings of the immunofluorescence staining of the testicular tissues. We found high PI3K levels in spermatocytes in the diabetes group but no detectable PI3K in spermatocytes of the control, curcumin, Mg-curcumin nanoparticle and encapsulated Mg-curcumin nanoparticle groups.

Tab. 2: Glucose and reproductive hormones measured in serum samples / In Serumproben gemessene Glukose- und Fortpflanzungshormonwerte

Group	Glucose (mg/dl)	Testosterone (nmol/l)	LH (mIU/dl)	FSH (mIU/dl)
Group 1	160.57 ± 11.75^{c}	5.84 ± 0.41^{b}	11.13 ± 0.83	2.19 ± 0.17^{ab}
Group 2	620.29 ± 27.32^{a}	1.21 ± 0.06^{e}	7.99 ± 0.41*	1.49 ± 0.10^{d}
Group 3	157.71 ± 13.88^{c}	6.83 ± 0.56^{a}	11.41 ± 0.45	1.78 ± 0.14^{c}
Group 4	148.86 ± 10.25^{c}	7.17 ± 0.75^{a}	11.37 ± 0.96	2.27 ± 0.18^{ab}
Group 5	499.14 ± 32.01^{b}	1.78 ± 0.22^{e}	10.90 ± 0.85	2.11 ± 0.13^{b}
Group 6	606.14 ± 58.94^{a}	1.39 ± 0.15^{e}	11.44 ± 0.42	2.32 ± 0.17^{a}
Group 7	162.50 ± 9.89^{c}	4.49 ± 0.21^{c}	11.02 ± 0.84	2.08 ± 0.25^{b}
Group 8	620.50 ± 18.37^{a}	3.09 ± 1.39d	11.02 ± 0.79	1.75 ± 0.19c
p value	p<0.001	p<0.001	p<0.005	p<0.001

a,b,c,d: Values with different superscript letters in the same column are significantly different ($p<0.05$). In columns, the a,b,c,d and *p values are the results of the Tukey HSD test following the one-way ANOVA test. / a,b,c,d: Werte mit unterschiedlichen Buchstaben in derselben Spalte sind signifikant verschieden ($p<0,05$). Die p-Werte in derselben Spalte sind die Ergebnisse des Tukey HSD-Tests nach Anwendung der One Way ANOVA.

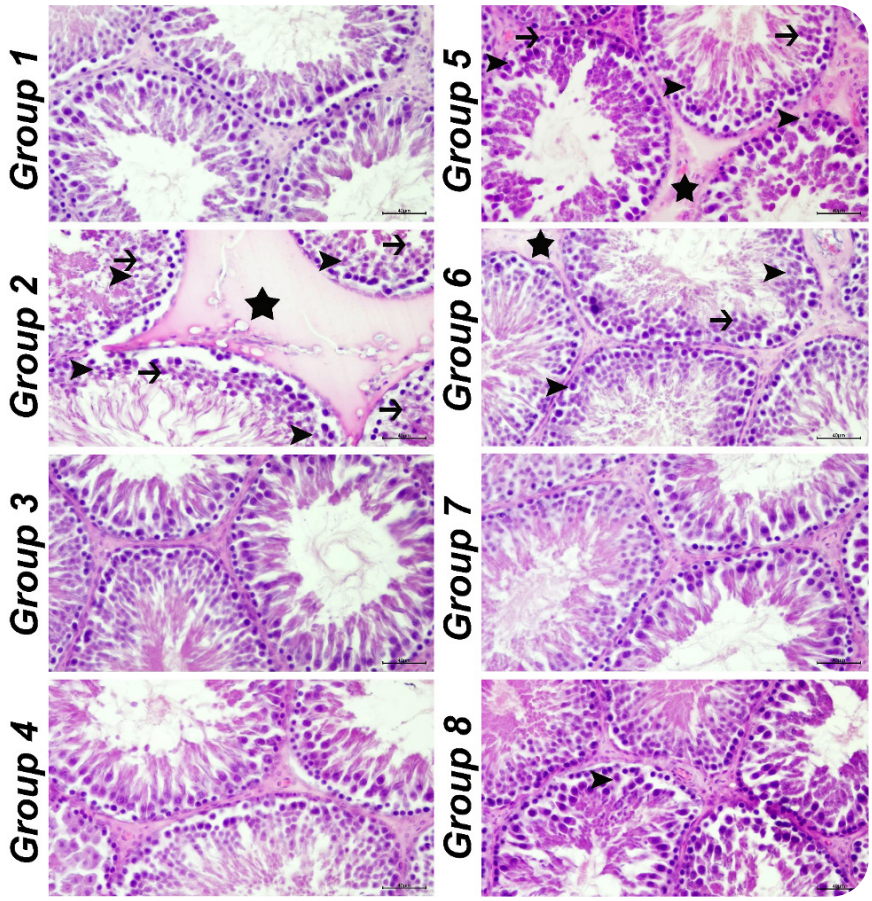

Fig. 1: Testicular tissue, degeneration of spermatocytes (arrowheads), necrosis (arrows), thinning of the tubular wall, oedema in the intertubular spaces, haematoxylin-eosin staining, Bar: 40 μm / Hodengewebe, Degeneration der Spermatozyten (Pfeilspitzen), Nekrose (Pfeile), Ausdünnung der Tubuluswand, Ödem in den Tubulusräumen, Hämatoxylin-Eosin Färbung, Balken: 40 μm

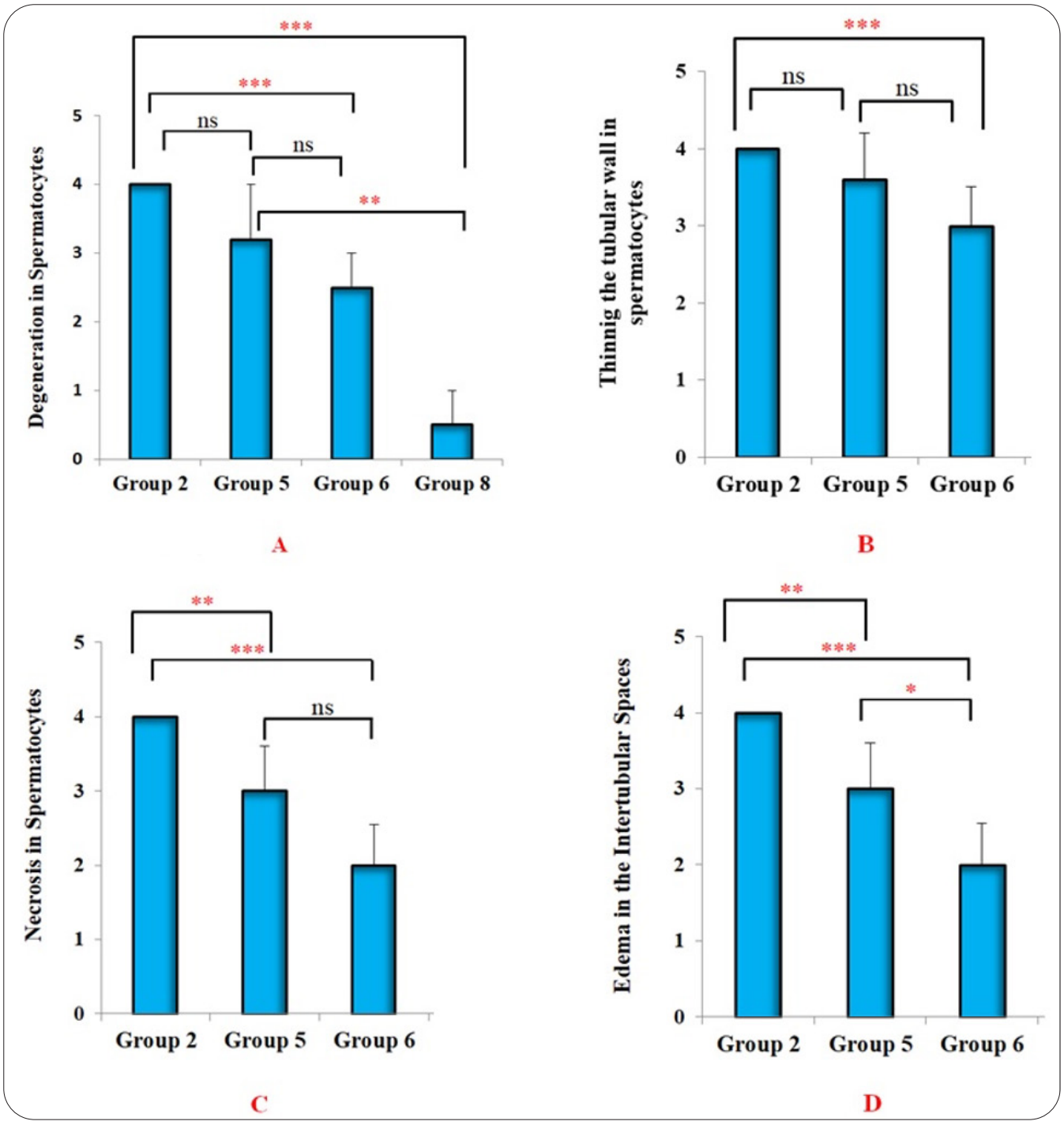

Fig. 2: Histopathological findings. A: Degeneration of spermatocytes, B: Thinning of the tubular wall in spermatocytes, C: Necrosis in spermatocytes, D: Oedema in the intertubular spaces) * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, ns = no significant difference / Histopathologische Befunde. A: Degeneration der Spermatozyten, B: Ausdünnung der tubulären Wand bei Spermatozyten, C: Nekrose in Spermatozyten, D: Ödeme in intertubulären Räumen) * = $p<0,05$, ** = $p<0,01$, *** = $p<0,001$, ns= kein signifikanter Unterschied

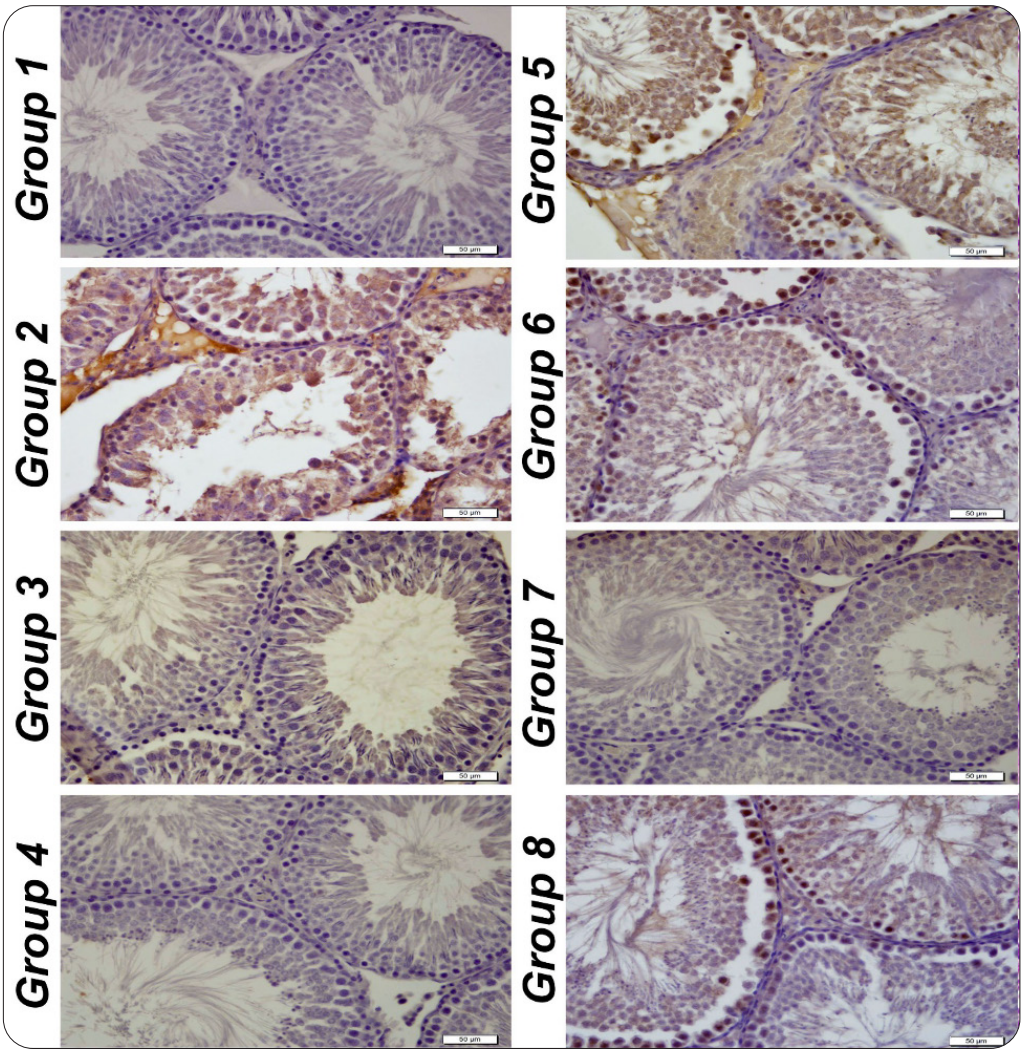

Fig. 3: Testicular tissue, NOP10 in spermatocytes, IHC - P, Bar: 50 μm / Hodengewebe, NOP10 in Spermatocyten, IHC - P, Balken: 50 μm

Tab. 3: Statistical analysis of immunohistochemistry and immunofluorescent staining in testicular tissues / Statistische Darstellung der Ergebnisse der Immunhistochemie und der Immunfluoreszenzfärbung im Hodengewebe

Groups	NOP10	PI3K
Group 1	23.42±0.43^{a}	25.56±0.28^{a}
Group 2	105.49±3.81^{b}	113.57±3.33^{b}
Group 3	22.98±0.6^{a}	25.57±0.57^{a}
Group 4	23.31±0.45^{a}	25.86±0.59^{a}
Group 5	58.42±2.96^{c}	61.33±1.75^{c}
Group 6	37.16±2.57^{d}	43.39±1.25^{d}
Group 7	23.4±0.57^{a}	26.13±0.6^{a}
Group 8	32.79±2.33^{d}	25.59±0.63^{a}

a,b,c,d; Different letters in the same column represent statistically significant differences ($p<0.05$). / a,b,c,d; unterschiedliche Buchstaben in derselben Spalte stehen für statistisch signifikante Unterschiede ($p<0.05$).

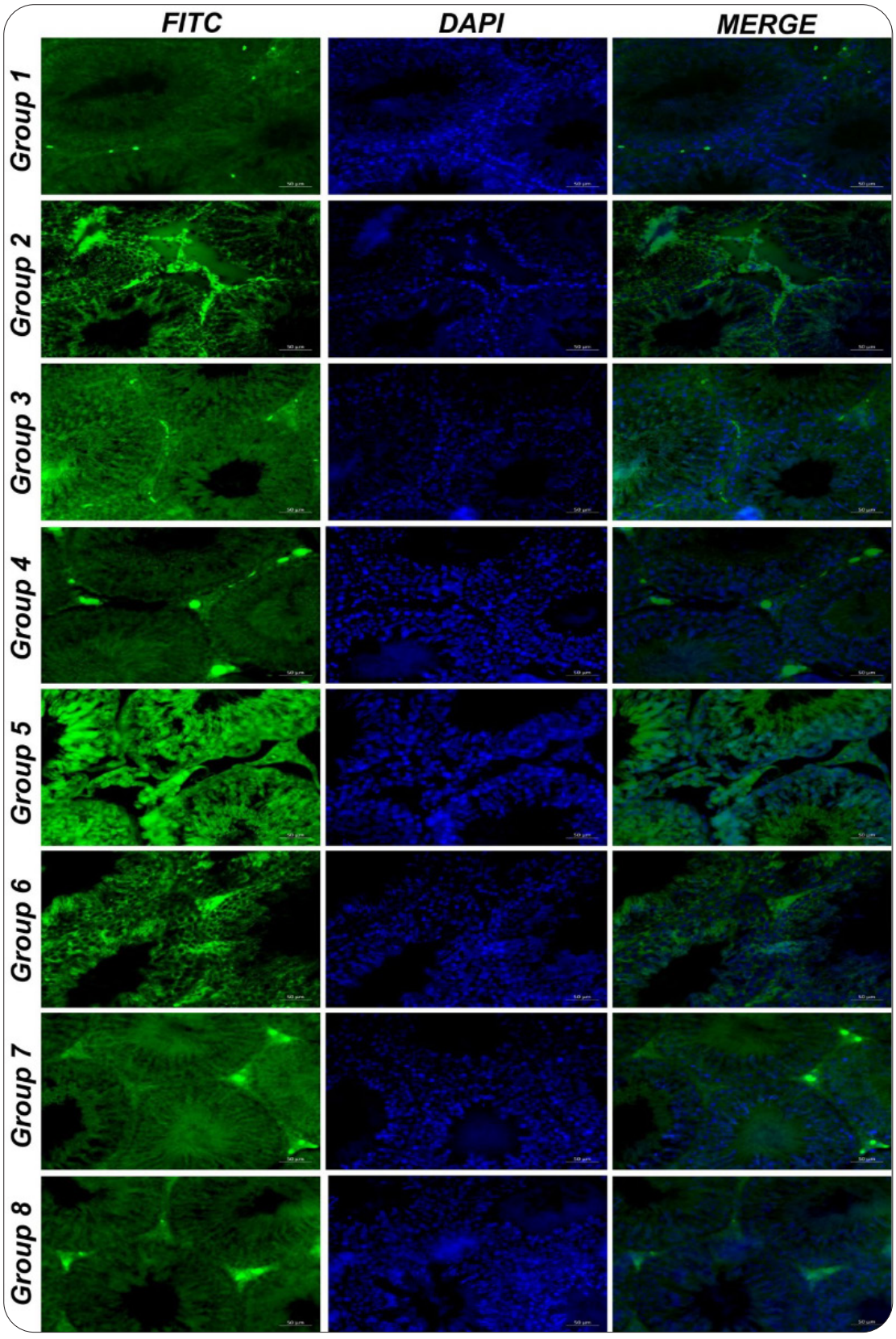

Fig. 4: Testicular tissue, PI3K in spermatocytes, Immunofluorescence, Bar: 50 μm / Hodengewebe, PI3K in Spermatocyten, Immunofluoreszenz, Balken: 50 μm

Discussion

Diabetes mellitus is a complicated metabolic disease that has adverse effects on many organs and progresses to hyperglycemia. Unfortunately, there is no effective treatment but the use of nanoparticles seems to hold promise. Curcumin has a number of beneficial effects but its low oral absorption and bioavailability limit its therapeutic efficacy. For this reason, we elected to work with nanocurcumin, which has larger surface area (Arozal et al. 2020). We also encapsulated curcumin nanoparticles with Mg: the decreased intake of Mg coupled with the increased urinary excretion in diabetes cause Mg deficiency, leading to insulin resistance by affecting tyrosine kinase activity. Mg supplementation may increase enzyme activity (Kostov 2019).

Evaluation of the lipid profile is very important for many diseases, especially cardiovascular diseases. Total cholesterol and triglyceride, LDL and HDL are the most important lipid profile parameters in routine biochemistry laboratories. VLDL is synthesized in the liver and released into the blood. Its triglyceride content is reduced by lipoprotein lipase in the vascular endothelium, which turns it into IDL and then LDL. HDL is synthesized primarily in the liver, with small amounts synthesized in the intestines, and released into the blood. There may be some degree of lipid or protein exchange lipoprotein particles in the circulation, where they develop into mature particles. While HDL carries cholesterol from the tissues to the liver for clearance, LDL releases cholesterol into the tissues. HDL is thus defined as “good” cholesterol and LDL as “bad” cholesterol (Mann et al. 2014; Lippincott’s Illustrated Reviews). Many diseases, such as fatty liver, diabetes and cardiovascular diseases, have been associated with changes in the lipid profile (Sabahelkhier et al. 2016; Shabana et al. 2020) and there is a significant increase in total cholesterol, triglyceride and LDL levels in type 2 diabetes mellitus (Sabahelkier et al. 2016). In the STZ-induced diabetes model, triglyceride, total cholesterol and LDL levels are higher in the diabetes group on the first and 14th days after STZ application, while HDL levels are dramatically lower (Fitri et al. 2022). We found higher triglyceride and LDL levels in the diabetes group but no change in total cholesterol and HDL levels, suggesting dyslipidemia. Dyslipidemia in the diabetes group may have resulted from insulin deficiency and hyperglycemia, which increases lipolysis (Goldberg 2001). Cholesterol levels in the diabetes+curcumin, diabetes+Mg-curcumin and diabetes+encapsulated Mg-curcumin groups were considerably lower than in the diabetes group, showing that curcumin is efficacious at lowering cholesterol levels. Our results on the effect of curcumin and nanocurcumin on the lipid profile are consistent with the findings of previous studies (Shamsi-Goushki et al. 2020).

The role of insulin levels in the regulation of the hypothalamic–pituitary–gonadal (HPG) axis in diabetes well known. We found significant changes in the hormonal profile. Testosterone levels may reduce due to damage to the number or function of Leydig cells (Ballester et al. 2004). The finding in the diabetes group is consistent with the results of previous studies (Belhan et al. 2020; Sheikh-Ahmad et al. 2022). Testosterone levels were only elevated in the group of diabetic rats that also received encapsulated Mg-curcumin. FSH is secreted by the gonadotropic cells of the pituitary and acts on the surface of target cells in the gonads of both males and females (Stamatiades & Kaiser 2018). In the male, this gonadotropin mediates testicular development and spermatogenesis (Huhtaniemi 2015). LH controls the production of testosterone by Leydig cells, the endocrine cells located in the interstitium of the testis. Testosterone is essential for male virilization and, in combination with FSH, triggers and maintains spermatogenesis. We found decreased levels of FSH and LH, as in studies of diabetes (Erol et al. 2002; Ali et al. 2022). This is because the HPG axis is suppressed in diabetes (Baccetti et al. 2002). FSH levels reverted to normal in all diabetic rats with the exception of the group receiving encapsulated Mg-curcumin. The composite formed by curcumin nanoformulations with various components increases FSH, LH and testosterone concentrations (Yousef et al. 2021; Abd Allah & Abd El Rahman 2022), which is compatible with the findings of the present study.

We found increased NOP10 levels with DNA damage caused by diabetes. The treatments may prevent apoptosis by preventing telomere shortening. As the shortening of the telomere in diabetes causes the loss of genetic information and, leading to aging and apoptosis of the cells, we expect high levels of NOP10 in the diabetes group. Our results are compatible with previous studies (Rashid & Sil 2015; Li et al. 2020). The high level of PI3K in the diabetes group suggests severe testicular damage. However, low levels in the curcumin groups are associated with the regulation of apoptosis-related pathways and PI3K/Akt signaling by curcumin: the antidiabetic and anti-apoptotic effects of curcumin stem from regulation of the PI3K/Akt signaling pathway (Xia et al. 2020). We found an increase in PI3K levels due to very intense cell damage in the diabetes group, while PI3K levels did not increase in the treatment groups due to the prevention of cell damage. As oxidative stress caused by *hyperglycemia* in diabetes suppresses the PI3K pathway and causes apoptosis, we expect high PI3K levels in the diabetes group.

In our experimental diabetes model, curcumin and curcumin nanoformulations have positive effects on cholesterol levels. Curcumin nanoformulations have more pronounced effects than curcumin on NOP10 and PI3K expression.

Fazit für die Praxis:
Curcumin und Curcumin Nanoformulierungen haben positive Auswirkungen auf den Cholesterinspiegel und die Expression von PI3K- und NOP10. Curcumin-Nanoformulierungen könnten zu den ersten potenziellen Therapeutika gehören, die die reproduktive Gesundheit bei Diabetes schützen können.

Conflict of interest statement
The authors declare no conflict of interest.

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