

The influence of *Ginkgo biloba* on birth weight and histomorphometric characteristics of neonatal kidneys in albino rats

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ABSTRACT

The purpose of this study was to elucidate the effects of *Ginkgo biloba* extract administered during pregnancy on the fetal growth in terms of weight and renal histogenesis. Twenty-eight pregnant female albino rats were divided into four groups: A, B, C and D (n = 7). *Ginkgo biloba* was administered orally 3.5, 7 and 14 mg/kg/day to groups A, B, and C, respectively from the 8th to the 20th day of gestation. Group D served as the control group (no medicine was given). The pregnant females were weighed weekly and observed for any signs of toxicity during pregnancy. After parturition, various morphological features of neonates were measured. The neonates were euthanized and the kidneys collected. The length, width, weight, and the cortical and medullary thickness of the neonatal kidneys were recorded. Renal sections were prepared by the paraffin tissue technique followed by hematoxylin and eosin staining to observe tissue degenerative alterations. Data revealed that average weight gain was non-significant in dams, although a significant (P<0.01) weight reduction was witnessed in neonate weight. The weight of the neonatal kidneys showed a highly significant (P<0.01) increase in the treated group in comparison to the controls, in a dose-dependent manner. Interstitial edema, inflammation, tubular degenerations, and hemorrhages were observed in renal sections. This experimental data suggested that extensive use of *Ginkgo biloba* during the second and third trimester of pregnancy has a negative effect on renal genesis, however, no changes were recorded in maternal weight gain nor did it produce any noxious effect on the mothers' health, thus, proving its safety to mothers during pregnancy, but deleterious effects on renal histogenesis.

Key words: *Ginkgo biloba*; birth weight; renal genesis; herbal medicine; reproductive toxicity; organogenesis

Introduction

Ginkgo biloba (Family: Ginkgoaceae) has been used in China and its native countries as an oriental cuisine, folkloric medicine and nutritional

supplement (BRENNER et al., 2005) for over 5000 years. *Ginkgo Biloba* was introduced to Europe and America in 1980 as a part of the herbal repertoire.

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It is among the highest selling herbal tonics. The uncertainty it holds in the treatment of various diseases has created an unprecedented interest in determining its biological activities. Its embryotoxic or fetotoxic effects are likely to remain unrecognized as it has only been tested in a few randomized controlled trials.

Clinically, *Ginkgo biloba* extracts are used in the treatment of vascular and avascular dementia (TAN et al., 2015; WEINMANN et al., 2010) including Alzheimer's disease (MANCUSO et al., 2012), cerebrovascular diseases such as stroke (ZENG, et al., 2005), peripheral vascular diseases such as intermittent claudication (NICOLAI et al., 2013), migraine (ALLAIS et al., 2013; ESPOSITO and CAROTENUTO, 2011), erectile dysfunction (WU et al., 2015; YEH et al., 2008), and antidepressant induced sexual dysfunctions (ASHTON et al., 2000; KANG, et al., 2002). It is very popular in women for improving the symptoms associated with menopause or premenstrual syndrome (OZGOLI et al., 2009). It has strong anti-ageing properties (DONG et al., 2004; HUANG et al., 2010).

The standardized dried leaves extract contains 24 percent flavonoids (Quercetin, Kaempferol and Isorhamnetin in large quantities) U.S Pharmacopeia and 6 percent terpenoids (ginkgolides and bilobalides). These are believed to account for *Ginkgo biloba*'s beneficial health effects. Terpenes improves circulation while flavonoids are neuroprotective (WOLLSCHLAEGGER, 2003; SMITH and LUO, 2004). Its toxicity can be attributed to its constituents which include ginkgolides, bilobalides, biflavones, cardols, cardanols and quercetin (Al-YAHYA, et al., 2006). *Ginkgo biloba* is excreted either through kidneys (21%) or expired through the lungs (16%). Its flavonoids can cross the placenta and enter the fetus where their concentration in tissues have been found to be greater than in the mother (SCHRÖDER-VAN et al., 1998). Colchicine was also found in the placental blood of women using *Ginkgo biloba*. Colchicine is linked to Down's syndrome and inhibits cell division, therefore it is strongly contraindicated in pregnancy (PETTY et al., 2001). No specific literature regarding the teratogenic effect of *Ginkgo biloba* is available, despite its wide

medicinal usage. So, the purpose of this study was to assess the effects of *Ginkgo biloba* on fetal growth in terms of weight and histomorphometric changes in neonatal kidneys after maternal ingestion during the gestation period from day 8 to day 21.

Materials and methods

A total of twenty-eight healthy female Albino rats weighing 200-250 g were used in this study. These animals were acclimatized for 15 days under optimally maintained room temperature, (humidity and a 12-hour light/dark cycle. After mating the females with male albino rats at the ratio of 3:1, the pregnant females were separated and divided into four groups (A, B, C, and D) containing seven biological replicates each. The first three groups, A, B and C were experimental groups, while D was the control. Standardized extract of *Ginkgo biloba*, in a 120mg/5ml liquid preparation, was purchased from Trimax Pharmaceuticals, Pakistan. The highest dose for humans is 240 mg/day, which is equivalent to 3.5 mg/kg/day in an adult human being. In this study, an aqueous solution of 3.5, 7 and 14 mg/kg/day was administered in a total 1ml of water volume to Groups A, B, and C, respectively, while only water was given to Group D. The medicine was given once daily via oral gavage from the 8th to the 21st day of pregnancy. The mothers were weighed every seventh day to look for weight gain. Daily water and food intake, piloerection, any locomotor alterations, vaginal bleeding and maternal death were also observed. After delivery, neonates were weighed using an electrical balance, while Crown-rump length (cm), head circumference (cm), and abdominal circumference (cm) were measured by measuring tape. They were examined for any gross congenital abnormalities. Each neonate was euthanized and a midline abdominal incision was made to expose the abdominal viscera. The viscera were inspected for any visible deformities; the color and consistency of the organs were checked and recorded. The neonatal kidneys were collected and cleared of fat and fascia. The kidneys were weighed on an electric balance in grams. Renal length (mm), width (mm), cortical and medullary thickness (mm) were measured with the help of a Vernier's caliper. The fetal tissues were fixed in 10% buffered formalin for 72 hours, and they were

processed later in an automatic tissue processor and embedded in paraffin. Labeled blocks were kept for fifteen minutes in a freezer before cutting. Five micron thin sections were obtained using a rotary microtome (Leica RM 2125), floated in warm water at 45 °C, and transferred to precleaned albumenized glass slides. Hematoxylin and eosin (H&E) were used for general histology, and the periodic acid Schiff (PAS) technique was used for demonstration of the basement membranes. The sections were studied under a light microscope (Leica DM 1000) at $\times 200$. Diameter (μm) of glomeruli, and Bowman's capsule, proximal and distal convoluted tubules (μm), were measured using an automated image analysis system, Image J[®] version 1.49v. All measurements were made with a standardized ocular micrometer.

Statistical analysis. Descriptive statistics were applied for each parameter studied, with the help of Microsoft Excel computer software. The means of the parameters were compared by one-way analysis of variance (ANOVA), and the least significance difference (LSD) test helped to compare the group means at 1% and 5% level of significance.

Results

The gain in maternal weight during pregnancy was compared with that of control group D (Table 1). Non-significant ($P > 0.05$) changes were observed in the body weight of the gravid rats.

The morphological pictures of the neonatal kidneys from groups A, B and C after the mother was treated with *Ginkgo biloba* extract (A 3.5, B 7 and C 14 mg/kg/day) respectively from the 8th to 20th day of pregnancy, compared with the control Group (D) is shown in Fig. 1.

The histological picture of the neonatal kidneys from Group D, whose mothers served as the control group showed normal interstitial space with no inflammation or hemorrhage (Fig. 2). The glomeruli appeared normal and well defined. The tubular epithelium (T) was well defined, with normal cell height in both proximal and distal convoluted tubules. The tubular epithelial cells showed no changes, with the loss of nuclei and a decrease in the height of the epithelial cells. The lumen (L) appeared to be dilated. Early fibrotic changes were also seen in the proximal tubules.

The histological picture of the neonatal kidneys in Group A, whose mothers were exposed to *Ginkgo biloba* 3.5 mg/kg/day, showed mild to moderate interstitial edema, with few foci of inflammation (In) and hemorrhage. The glomeruli (G) were well defined with normal Bowman's space (Fig. 2). Tubular epithelium (T) was intact. Mild congestion could also be visualized in the blood vessels of the medulla. No fibrosis or tubular damage could be seen. The cortex appeared to be unremarkable. The histological picture of the neonatal kidneys from Group B, whose mothers were exposed to *Ginkgo biloba* 7 mg/kg/day (Fig. 2) showed moderate interstitial edema, with multiple foci of inflammation (In) and hemorrhages. The glomeruli (G) were well defined with normal Bowman's space. The tubular epithelium (T) was foamy in appearance, and more prominent in the distal convoluted tubules. The tubular epithelium showed degenerative changes with foci of mild fibrosis. The cortex appeared to be unremarkable but slight thinning could be observed.

The histological section of the neonatal kidneys from Group C, whose mothers were exposed to

Table 1. Mean values of live weight of pregnant females of groups A, B, C treated with *Ginkgo biloba* 3.5, 7 and 14 mg/kg/day respectively, as compared to group D on Days 1, 8, 15, 21 of pregnancy

Groups	Days of pregnancy			
	Day 1	Day 8	Day 15	Day 21
Group A	208.00 ^A	217.86 ^B	227.86 ^C	243.43 ^D
Group B	205.63 ^A	216.63 ^B	226.50 ^C	241.50 ^D
Group C	211.43 ^A	220.29 ^B	230.71 ^C	241.71 ^D
Group D	211.43 ^A	220.71 ^B	231.29 ^C	242.71 ^D

Different superscripts in each row/column indicate significant differences at 1 percent level.



Fig. 1. Morphological pictures of neonatal kidneys from groups A, B and C after the mothers were treated with *Ginkgo biloba* extract (A 3.5, B 7 and C 14 mg/kg/day) respectively from the 8th to 20th day of pregnancy, compared with control Group (D).

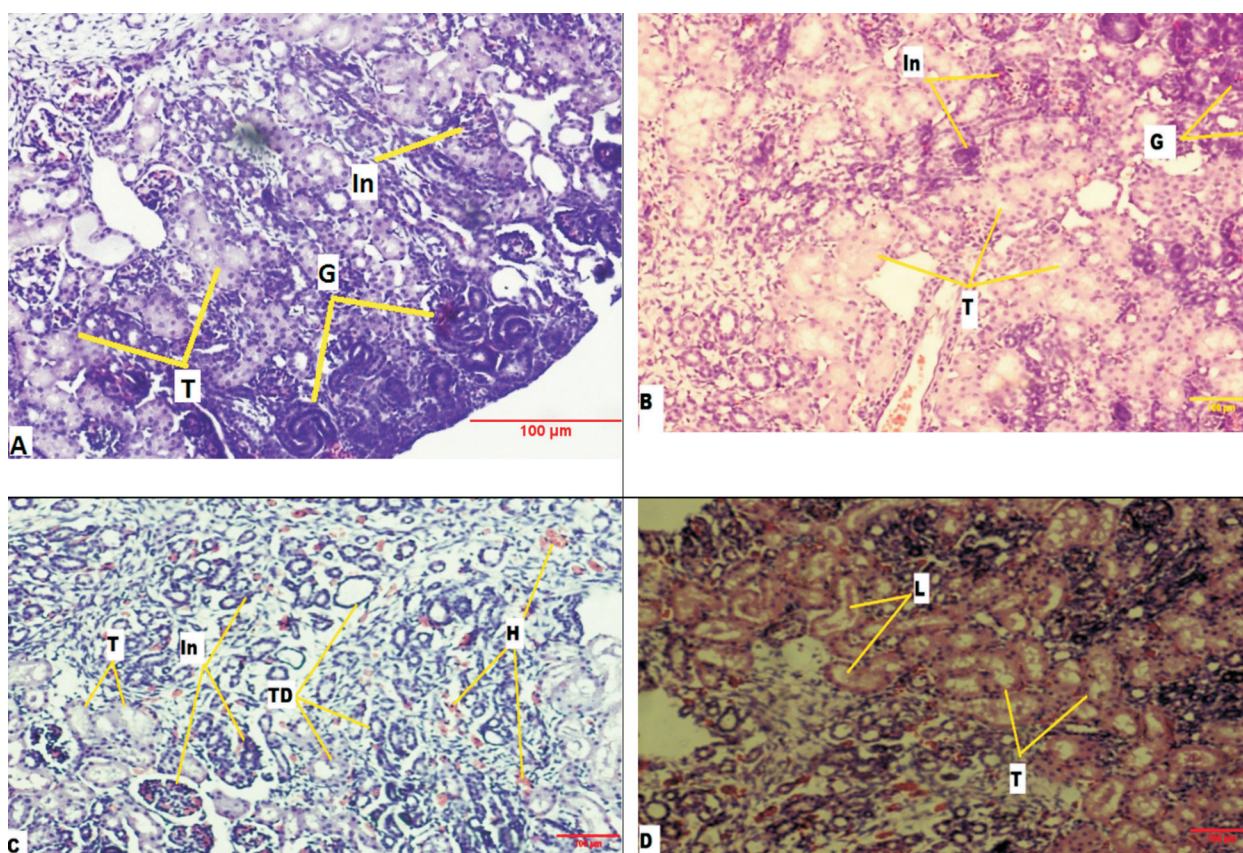


Fig. 2. Histomicrograph of neonatal kidneys of Groups A, B, C and D after the mothers were treated with *Ginkgo biloba* extract 3.5, 7, 14mg/kg/day respectively. A: kidneys showed mild interstitial edema, well defined Glomeruli (G) with normal Bowman's space, a few foci of inflammation (In) and intact cellular structure of Tubules (T). B: Renal histograph demonstrating moderate interstitial edema, multiple foci of inflammation (In), degenerative and foamy tubular epithelium (T) with normal glomerular (G) structure. C: Renal micrograph showing severe interstitial edema, hemorrhages (H), degenerative and atrophied tubular epithelium (T), more foamy tubular appearance (T) and fibrotic changes as well as Distal Tubule (DT). D: renal histology exhibited no inflammation, hemorrhages or tubular (T) degenerations, but to some extent dilated lumen (L).

Mean values of the morphological parameters of the neonates from the experimental groups were compared with the control group D (Table 2). Mean birth weight and crown-rump length in neonate albino rats fed at different doses of *Ginkgo biloba*

extract was significantly lower ($P < 0.01$) than in the control group. The average weight, length and width of the kidneys of the albino neonates revealed a significant ($P < 0.01$) increase, in a dose-dependent manner (Table 3).

Table 1. Mean values of live weight of pregnant females of groups A, B, C treated with *Ginkgo biloba* 3.5, 7 and 14 mg/kg/day respectively, as compared to group D on Days 1, 8, 15, 21 of pregnancy

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Different superscripts in each row/column indicate significant differences at 1 percent level

Table 2. Mean \pm SEM values of morphological parameters of neonates of albino rats delivered by mothers treated with *Ginkgo biloba* extract (A) 3.5, (B) 7, and (C) 14 mg/kg/day from the 8th to 20th day of pregnancy as compared to Group (D)

Parameter	Group A	Group B	Group C	Group D
Birth weight (gm)	4.57 \pm 0.05 ^A	4.39 \pm 0.043 ^B	4.20 \pm 0.05 ^C	4.69 \pm 0.04 ^A
Crown rump length (cm)	6.50 \pm 0.03 ^B	6.48 \pm 0.06 ^B	6.07 \pm 0.03 ^C	6.78 \pm 0.01 ^C
Head circum-ference (cm)	3.23 \pm 0.01 ^A	3.23 \pm 0.01 ^B	2.90 \pm 0.03 ^C	3.23 \pm 0.01 ^A

Different superscripts in each row indicate significant differences at 1 percent level

Table 3. Mean \pm SEM values of renal morphological parameters of neonates delivered by mothers treated with *Ginkgo biloba* extract (A) 3.5, (B) 7 and (C) 14 mg/kg/day from the 8th to 20th day of pregnancy as compared to Group (D)

Parameter	Group A	Group B	Group C	Group D
Weight (mg)	20.84 \pm 0.02 ^D	22.62 \pm 0.05 ^B	23.30 \pm 0.05 ^A	21.75 \pm 0.04 ^C
Length (mm)	3.68 \pm 0.06 ^D	4.98 \pm 0.05 ^C	6.00 \pm 0.03 ^A	5.27 \pm 0.10 ^B
Width (mm)	2.95 \pm 0.04 ^C	3.68 \pm 0.060 ^B	3.70 \pm 0.06 ^B	2.89 \pm 0.10 ^A

Different superscripts in each row indicate significant differences at 1 percent level

Ginkgo biloba 14 mg/kg/day, (Fig. 2) showed severe interstitial edema with multiple foci of inflammation (In) and hemorrhage (H). The glomeruli appeared to be poorly defined with a tuft of capillaries irregularly arranged. Bowman's space seemed to be increased due to flattening of the lining epithelium and edema around the capillaries. The tubular epithelium was foamy, and more prominent in the distal convoluted tubules. The tubular epithelial cells showed marked

atrophic changes, with the loss of nuclei and the height of the epithelial cells was lower. The lumen appeared to be dilated. Early fibrotic changes were also seen in the proximal tubules. The cortex was, however, preserved, with a slight reduction in its thickness.

Table 4. Mean \pm SEM values of morphological and histomorphometric parameters of neonatal kidneys delivered by mothers treated with *Ginkgo biloba* extract, A 3.5, B 7 and C 14 mg/kg/day) from the 8th to 20th day of pregnancy as compared to Group (D).

Parameter	Group A	Group B	Group C	Group D
Morphometric parameters				
Cortical thickness (mm)	0.88 \pm 0.02 ^C	1.21 \pm 0.01 ^A	1.06 \pm 0.01 ^B	0.80 \pm 0.01 ^D
Medullary thickness (mm)	1.80 \pm 0.01 ^D	2.50 \pm 0.01 ^B	2.81 \pm 0.01 ^A	1.89 \pm 0.012 ^C
Histometric parameters				
Glomerular diameter (μ m)	119 \pm 0.02 ^B	120 \pm 0.01 ^A	121 \pm 0.01 ^B	120 \pm 0.01 ^C
Bowman capsular area (μ m)	10.80 \pm 0.01 ^D	12.50 \pm 0.04 ^B	14.81 \pm 0.02 ^A	10.89 \pm 0.06 ^C
Dia. of proximal tubules (μ m)	59.93 \pm 0.10 ^B	59.71 \pm 0.18 ^B	55.18 \pm 0.10 ^C	62.22 \pm 0.29 ^A
Dia. of distal tubules (μ m)	30.11 \pm 0.074 ^A	30.09 \pm 0.07 ^A	27.47 \pm 0.18 ^B	30.27 \pm 0.12 ^A

Dia = Diameter. Different superscripts in each row indicate significant differences at 5% level

Discussion

Ginkgo biloba is top-ranked prescription medicine in western countries. It has been used traditionally to treat various reproductive and contraceptive diseases, both in humans and animals (DEKA and SARMA, 2011). To our knowledge, this is the first report to evaluate the effects of *Ginkgo biloba* on the histomorphometrical development of the kidneys in Albino rats.

In the present study, non-significant ($P > 0.05$) changes in the body weight of the rats were observed. Moreover, non-significant differences in the estimated food and water intake of rats were observed during this experiment. There were no clinical signs or symptoms of maternal toxicity (tremors, locomotive changes, piloerection, head flicking, convulsions or death) in the study animals. Even after delivery, no gross congenital malformations were seen in the neonates during macroscopic analysis.

Mean birth weight and crown-rump length of the neonates of albino rats fed different doses of *Ginkgo biloba* extract were significantly lower ($P < 0.01$) than the control group. These observations are in line with various studies (FARIA et al., 2008;

FERNANDES et al., 2010; PINTO et al., 2007), who claimed no effect on maternal body weight but intra-uterine retardation of the fetus. Macroscopic malformations were absent in neonates in the present study, in contrast to the observations recorded by ZEHRA et al., (2010) where the tendency for gross malformation in the fetuses increased when the pregnant mice were fed with *Ginkgo biloba* 100 mg/kg/day throughout their pregnancy.

The average weight, length and width of kidneys in the albino neonates revealed significant ($P < 0.01$) increases in a dose-dependent manner in the present study (Table 3).

Moreover, significant variations in the histomorphometric parameters of the kidneys *i.e.* the thickness of the cortex and medulla, the diameter of glomeruli, Bowman's capsule, proximal tubules and distal convoluted tubules, were observed in the study animals (Table 4). Probably the inhibition of iNOS expression by *Ginkgo biloba* extract in a "dose dependent manner" explains the renal congestion in the fetuses. Nitric oxide (NO), synthesized by the endothelial NO synthase, plays a vital role in stabilizing the renal microcirculation

and protects the kidney from oxidative injury (NANJI et al., 1995; NISHIDA et al., 1994; WANG and ABDEL-RAHMAN, 2005). There is evidence that ginkgolide A, B, and bilobalide may have a selective inhibitory effect on iNOS expression, by inhibiting the transcriptional activity of nitric oxide synthase (DEFEUDIS et al., 2003). A similar trial revealed that after treatment with *Ginkgo biloba* extract (50 mg/mL), NO metabolites released by the endothelial cells were reduced (CHEUNG et al., 1999); This inhibition of NOS metabolites resulted in decreased blood flow to the kidneys thus affecting renal agenesis. The statistically significant renal damage observed in the present study, may have been caused by a similar mechanism. Consumption of plants containing terpenoids can cause fatal proximal tubular necrosis (OBATOMI and BACH, 1998). Since terpenoids are one of the major components of Ginkgo extract, this supports our results. *Ginkgo biloba* extract also contains 24% phytoestrogens, which are responsible for its hormone replacement potential (OH and CHUNG, 2006). There is evidence suggesting the harmful effects of phytoestrogens on fetal outcomes (PADILLA-BANKS et al., 2012). The teratogenic effects of Ginkgo extract have also been previously reported, where Chan (CHAN, 2006) stated that Ginkgo glide compounds in *Ginkgo biloba* halt early post-implantation embryonic development. The early degenerative changes in the renal parenchyma, in the present study, may either be due to the vasodilator and anticoagulant properties of *Ginkgo biloba*, or its metabolites. However adulteration with herbal medicines, such as the addition of colchicine, may also be responsible for these atrophic and degenerative changes (DUGOUA et al., 2006). As renal cells are immature their exposure to toxic substances can lead to destruction. There were more deleterious effects on the interstitium, tubules (proximal and distal) and blood vessels. However, Bowman's capsules were preserved as they are somewhat resistant to toxicity. Clinicians and patients should also be concerned about the interactions that may occur between ginkgo and numerous other medications, particularly anticoagulant and antiplatelet drugs. This issue has greater significance when exposure

or toxicity may lead to malformations in fetuses and neonates. The traditional use of *Ginkgo biloba* has not indicated any substantive risks of taking this herb during pregnancy and lactation. This is the first report to evaluate the more comprehensive effects of feeding *Ginkgo biloba* to albino mice during gestation, and its toxic effects on renal genesis in fetuses. Since animal studies are accepted as human risks, it may be generalized that products containing *Ginkgo biloba* pose a risk to consumers. Nonetheless, rigorous and well-controlled research is needed to assess the toxic effects of *Ginkgo biloba* and other herbal medicines on maternal and fetal body systems.

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SAŽETAK

Cilj ovoga istraživanja bio je pokazati učinke ekstrakta ginkgo bilobe primijenjenog tijekom gravidnosti na rast ploda s obzirom na porođajnu masu i bubrežnu histogenezu. Ukupno 28 gravidnih ženki štakora albino podijeljeno je u četiri jednake skupine: A, B, C i D. Ekstrakt *Ginkgo biloba* primijenjen je peroralno u dozi od 3,5 mg/kg po danu u skupini A, 7 mg/kg u skupini B i 14 mg/kg u skupini C, od 8. do 20. dana gravidnosti. Skupina D bila je kontrolna (nije primala ekstrakt ginkgo bilobe). Gravidne ženke tjedno su vagane te su praćeni znakovi toksičnosti. Nakon porođaja izmjerena su različita morfološka svojstva novorođenih štakora te su nakon njihove eutanazije uzorkovani bubrezi. Izmjerene su dužina, širina, masa te kortikalna i medularna debljina bubrega. Uzorci bubrega uklopljeni su u parafin, rezani i obojeni hematoksilinom i eozinom kako bi se uočile degenerativne promjene tkiva. Podaci pokazuju da prosječan prirast u gravidnih ženki nije bio znakovit, no zabilježeno je znakovito smanjenje ($P < 0,01$) porođajne mase novorođenih štakora. Porođajna masa bubrega novorođenih štakora pokazala je znakovit porast ($P < 0,01$) mase u pokusnim skupinama u usporedbi s kontrolnom, što je ovisilo o dozi. U mikroskopskim preparatima bubrega istraženi su intersticijski edem, upala, tubularna degeneracija i krvarenja. Ovo je istraživanje pokazalo da primjena *Ginkgo biloba* u većoj količini u drugom i trećem tromjesečju gravidnosti negativno utječe na formiranje bubrega, dok promjene u prirastu mase gravidnih ženki nisu zabilježene niti je bilo štetnih učinaka na njihovo zdravlje. Time je dokazana sigurnost upotrebe za vrijeme gravidnosti za majke, no istodobno i štetan utjecaj na bubrežnu histogenezu mladunčadi.

Ključne riječi: *Ginkgo biloba*; porođajna masa; formiranje bubrega; biljna medicina; reproduktivna toksičnost; organogeneza
