African Journal of Pharmacy and Pharmacology Vol. 6(37), pp. 2664-2669, 8 October, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.319
ISSN 1996-0816 © 2012 Academic Journals

Full Length Research Paper

Antidiabetic and renoprotective effects of water extract of *Rosmarinus officinalis* in streptozotocin-induced diabetic rat

Najla O. Ayaz

Department of Biochemistry, Faculty of Girls Science, King Abdul-Aziz University, Jeddah, Saudi Arabia.
E-mail: kheffha@yahoo.com. Tel: 00966551291289.

Accepted 11 September, 2012

The antidiabetic effect of water extract of *Rosmarinus officinalis* was studied in streptozotocin (STZ)-induced diabetes in rats. The efficacy of the extract was also evaluated for protective effects on renal functions and histology in diabetic rats. Oral administration of extract to diabetic rats (200 mg/kg body weight per day for a period of 21 days) produced significant (P <0.001) fall in fasting blood glucose (FBG). Treatment with the extract showed significant improvement in body weight and serum insulin and C-peptide when compared with diabetic control. Also, the extract improved the kidney function and reduced lesions associated with STZ induced diabetes. Thus, our study indicates that the water extract of rosemary (200 mg/kg) exhibits protective effect on these organs, significantly improves the homeostasis of glucose and proves its potentials as an antidiabetic agent. The results of this study serve as valuable data for health professionals in proper menu planning for diabetes mellitus.

Key words: Diabetes mellitus, rosemary, streptozotocin, medicinal plant.

INTRODUCTION

Diabetes mellitus (DM) is a syndrome that affects more and more people in all countries over the world. It is well known that diabetes mellitus is the commonest endocrine disorder that, according to the World Health Organization (WHO, 2004), affects more than 176 million people worldwide. From an ethno-pharmacological perspective, it is important to understand that this disease is one at the interface of conventional biomedical and local (or traditional) treatment. Diabetes mellitus (DM) does not only lead to hyperglycemia, but also causes many complications such as hyperlipidemia, hypertension and atherosclerosis (Bakırel et al., 2008). In the recent years, there is growing interest in herbal medicine all over the world (Lie and Crawford, 2004).

Rosemary, *Rosmarinus officinalis* L. (Labiatae) is an evergreen perennial shrub grown in many parts of the world. It has been reported to possess a number of therapeutic applications in folk medicines in curing or managing wide range of diseases, such as diabetes mellitus, respiratory disorders, stomach problems and inflammatory diseases. Rosemary has long been recognized as having antioxidant molecules, such as rosmarinic acid, carnasol and rosmaridiphenol (Huang and Zheng, 2006; Bakirel et al., 2008). High total phenolic content and flavonoid content are responsible for its antioxidant activity.

In our previous study, we have reported that rosemary has a high radical scavenging and antioxidant activity. The percent inhibition of linoleic acid peroxidation of plant extract was 85% (Khalil et al., 2011). The purpose of this study was to evaluate the protective effect of water extract of Rosemary leaves after oral administration in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Plant material and preparation of extract

Leaves of Rosemary were obtained from the local herbal market of Kingdom Saudi Arabia. Voucher specimens from plant material

were deposited at the Herbal Museum, Department of Pharmacology, Faculty of Science, King Abdul-Aziz University of Medical Sciences, for identification. The fresh leaves of plant material (5 g), after 1 h stirring, were soaked in 50 ml of boiled water at room temperature overnight. The supernatant was decanted and the residue was macerated two more days with distilled water. The pooled supernatants were combined and filtered.

Experimental animals

Adult male albino Wistar rats (weighing 150 – 200 g) were obtained from the Central Animal House in Jeddah, Saudi Arabia. The animals were housed in acrylic cages in standard conditions of temperature prior to the experiments for 1 week in order to adapt to the laboratory condition. They were fed with commercial diet and water *ad libitum.* The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Saudi Arabia, Jeddah. All experimental procedures were conducted in accordance to the ethical guidelines of International Association for the Study of Pain (Zimmermann et al., 1983).

Induction of experimental diabetes

A freshly prepared solution of streptozotocin (STZ) 45 mg/kg body weight in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally into overnight fasted rats. The STZ injected animals exhibited hyperglycemia within 48 - 36 h (Siddiqui et al., 1987). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

Experimental procedure

The experiment was carried out on 5 groups of five rats in each group to study the effect of plant water extract on STZ-induced diabetes and changes in renal function as follows: Group 1: healthy control rats; Group 2: diabetic control rats; Group 3: normal rats administered rosemary extract (200 mg/kg body weight) using gastric cannula; Group 4: protective group received water extract of Rosemary for two weeks before STZ injection; Group 5: diabetic treatment rats received water extract of Rosemary for 3 weeks after 36 h STZ injection. The control rats received distilled water and treated rats received Rosemary in 1 ml of distilled water. The treatment with rosemary was given daily for a period of 3 weeks using gastric cannula (Makino et al., 2002). Blood samples were obtained from hearts of overnight fasted rats using micro-capillary technique and allowed to clot for 20 min in laboratory temperature and then centrifuged at 10000 rpm for 10 min for serum separation.

Biochemical assays

Starting from the 1st day (3rd day of STZ injection) of extract administration to diabetic rats, FBG (blood glucose) level was measured on every 7th day using glucometer. Serum insulin (Awareness Technologies, USA) and C-peptide (Packard, USA) levels were determined by the enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) methods, respectively. Blood urea nitrogen (BUN), serum creatinine (Scr) and uric acid were determined as markers of kidney function using the commercially available kits from Siemens Health Care Diagnostics according to their manufacturers.

Histological study

After blood sampling, the kidneys were removed and fixed in 10% neutral buffered formalin for 24 h. Washing was done in tap water and then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration.

Specimens were cleared in xylene and embedded in paraffin at 56° in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains (Carleton, 1979) for histopathological examination through the electric light microscope.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). The data were subjected to one-way analyses of variance (ANOVA) and student's t-tests using the statistical analysis program. P-value ≤ 0.05 was considered statistically significant.

RESULTS

No detectable irritation or restlessness was observed after extract administration. Also, no noticeable adverse effect (such as respiratory distress, abnormal locomotion or catalepsy) was observed in any animals after the extract administration. Throughout the experimental period, the body weight was monitored. At the end of 3 weeks treatment, all the animals were anesthetized (nesdonal 50 mg/kg, i.p.).

Biochemical results

Body weight

Diabetic rats grew poorly and had significantly ($P < 0.01$) lower body weight compared with healthy control (Table 1). On the other hand, the diabetic rats treated with the extract (200 mg/kg) had their body weight comparable to healthy control.

Antihyperglycemic activity

There was a significant decrease ($P<0.001$) in the levels of FBG in diabetic rats treated with water extract of rosemary (200 mg/kg) from day 3 to 21; diabetic control rats showed marked hyperglycemia throughout the experimental period (Table 2).

The levels of serum insulin and C-peptide were significantly decreased in diabetic rats when compared with normal rats. Administration of effective dose of rosemary extract to diabetic rats significantly reversed all

Table 1. Effect of rosemary (200 mg/kg) on body weight after 3 weeks treatment in diabetic rats.

Group	Initial body weight (g)	Final body weight (g)
Healthy control	185.1 ±3.4	199.8±5.3
Diabetic control	188.5 ± 3.5	161.7 ± 6.7[a]
Normal+ Rosemary (200 mg/kg)	184.5 ± 4.4	195.4 ±5.5[b]
Protective group	186.2 ± 4	198.6 ±5.5[b]
Diabetic treated group	187.5 ± 4.3	194.5 ± 6.3[b]

Values are given as mean ± SD from five rats in each group. [a]P<0.001 vs. healthy control; [b]P<0.001vs diabetic control.

Table 2. Effect of rosemary on changes in FBG, serum insulin and C-peptide of normal and experimental rats.

Group	FBG (mg/dl)	Insulin (µU/ml)	C-peptide (ng/ml)
Healthy control	126.4 ± 20	13.4 ± 0.72	4.2 ± 0.2
Diabetic control	329 ± 122[a]	4.5 ± 0.29[a]	1.6 ± 0.05[a]
Normal + Rosemary (200 mg/kg)	117 ± 19[b]	14.5 ± 0.87[b]	4.7 ± 0.21[b]
Protective group	132 ± 20[b]	11.7 ± o.7[ab]	2.3 ± 0.1[ab]
Diabetic treated group	131 ± 79[b]	12.4 ± 0.59[b]	4.1 ± 0.24[b]

FGB: Fasting blood sugar. Values are given as mean ± SD from five rats in each group. [a]P<0.001 vs. healthy control; [b]P<0.001vs. diabetic control.

Table 3. Effect of rosemary on changes in kidney functions in normal and experimental rats.

Group	BUN (mmol/L)	Scr (µmol/L)	Uric acid (µmol/L)
Healthy control	8.2 ± 0.85	39 ± 5.5	59 ± 16.2
Diabetic control	19.3 ± 1.7[a]	47 ± 7.8	77.5 ± 32[a]
Normal + Rosemary (200 mg/kg)	8.2 ± 1.26[b]	43 ± 8.2	65 ± 20[ab]
Protective group	9.2 ± 1.7[b]	45 ± 7	68 ± 20[ab]
Diabetic treated group	8.2 ± 0.92[b]	40 ± 6.8[b]	64 ± 30[ab]

BUN, Blood urea nitrogen; Scr, serum creatinine. Values are given as mean ± SD from five rats in each group. [a]P<0.001 vs. healthy control; [b]P<0.001vs diabetic control.

these changes to near normal levels and produced no adverse effect on the general behavior. All the rats survived during the experimental period.

Effect of rosemary extract on kidney function

BUN, serum creatinine and uric acid as markers of kidney function were significantly (P <0.05) increased in the untreated diabetic group in comparison with the control group. Treatment of the diabetic animals with 200 mg/kg/d rosemary significantly inhibited the increase of BUN, serum creatinine and uric acid in comparison to the untreated diabetic animals. Treatment with rosemary extract 2 weeks before STZ induction prevented the increase in the kidney function parameters tested. The results of renal functional determinations are summarized in Table 3.

Histological results

Kidney sections of diabetic rat showed tubular damage, coagulative necrosis in the lining epithelium and focal hemorrhage. Hemorrhage was seen within the Bowman's space due to glomerular damage. However, no histopathological alteration was detected in control group (Figure 1). Treatment with rosemary extract 2 weeks

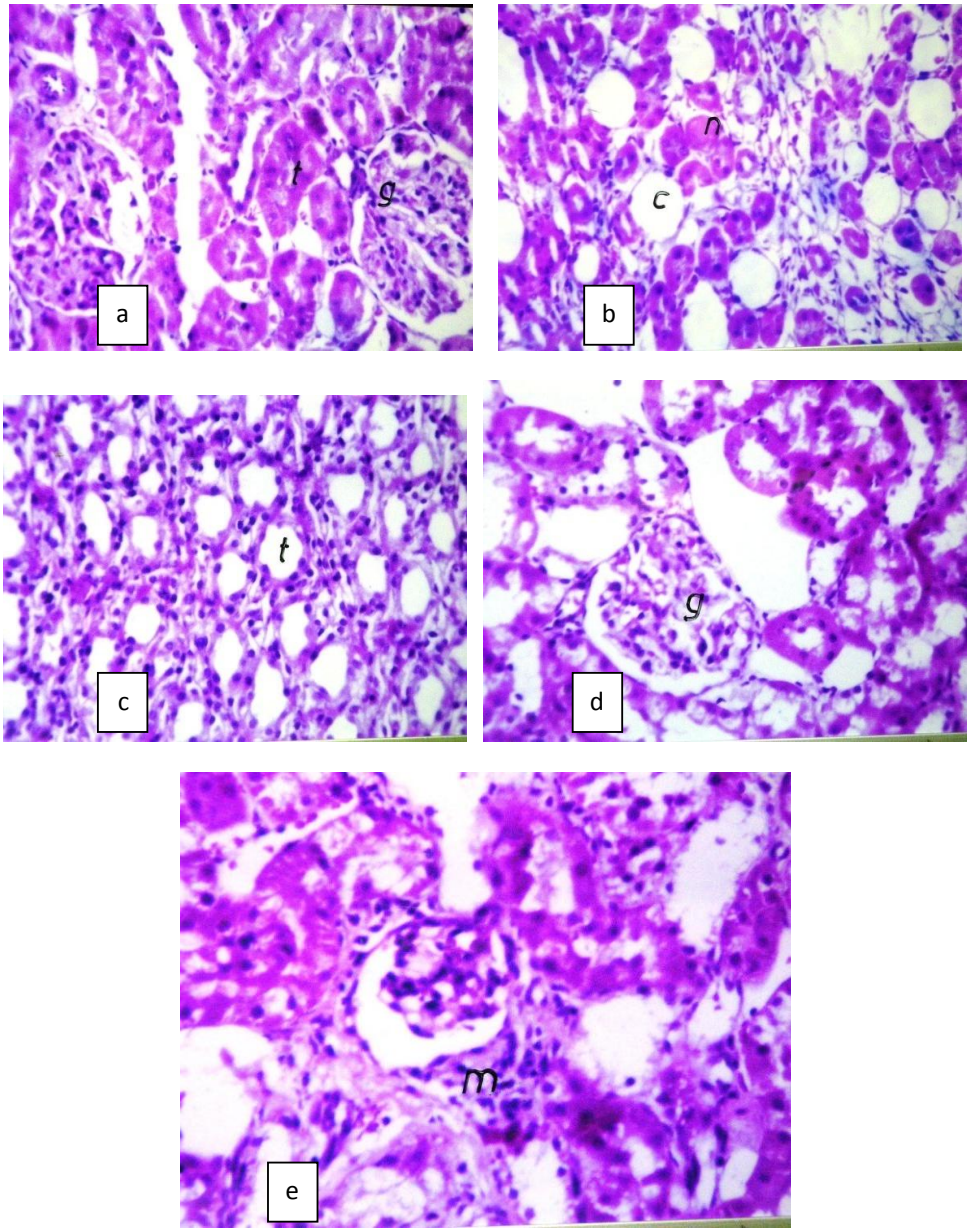

Figure 1. Histopathological changes in kidney of control and experimental rats. (a) Group I: Normal rat kidney showed glomeruli (g) and proximal convoluted tubules (t); (b) Group II: Diabetic kidney shows coagulative necrosis of the tubules (n) in the corticomedullary junction with cystic dilatation and hemorrhage; (c) Group III: Plant extract: tubules showed normal histological structure (t); (d) Group IV: Protective kidney shows vacuolization in the endothelial cells lining the tuft of the glomeruli (g); (e) Group V: Treated kidney showed periglomerular inflammatory cells infiltration (m).

before STZ injection produced minimal congestion in the intertubular blood vessels and there was minimal vacuolization of the lining endothelium of the tuft. Rats treated with the extract, 3 weeks after STZ induction had cystic dilation in the tubular lumen of some tubules. Administration of extract to control rats did not alter the structure of kidney, when compared with control group (Figure 1).

DISCUSSION

Since the prevalence of diabetes is globally increasing, its management and control is an urgent priority. The

major classes of oral hyperglycemic agents currently available for the treatment of diabetes include sulphonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors (Sharma et al., 2010). Animal models of diabetes are increasingly being used for elucidating the pathophysiology and pharmacological effect on diabetes mellitus. Advantages of animal studies in the examination of alternative medicines and their efficacy include the ability to define experimental conditions more tightly and to undertake more detailed studies of the biologic effects of the agents being used (Bailey and Flatt, 1986). STZ, a β-cytotoxin is commonly used for induction of experimental diabetes. It acts through rapid depletion of pancreatic β-cells, which leads to reduction in insulin secretion. STZ-induced diabetic animals represent a good experimental diabetic state with residual or remnant insulin production by the β-cells. The diabetic state in these animals is, therefore, not the same as that obtained by total pancreatectomy, as daily administration of insulin is not required for survival of STZ-induced diabetic rats (Chauhan et al., 2008).

This study was conducted on the STZ-induced diabetic rats and describes the renal protective effect of rosemary. The intraperitoneal administration of STZ to normal rats effectively induced diabetes as reflected by hyperglycemia, hyperinsulinemia and body weight loss. Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins (Tavafi et al., 2011). Diabetic rats treated with the plant extract showed significant gain in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting (that is the reversal of gluconeogenesis and glycogenolysis), and may also be due to the improvement in insulin secretion and glycemic control.

According to the study, the water extract of rosemary produced significant reduction in blood glucose level both in normal and diabetic rats. The capacity of rosemary to decrease the elevated blood glucose level to normal glycemic level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. A number of other plant extracts have been reported to have antihyperglycemic activity through a stimulatory effect on insulin secretion (Sharma et al., 2006). The possibility also exists that plant extracts mimic or improve insulin action and/or may have extra pancreatic mechanism of action. Significant reduction of blood glucose in rats treated with rosemary confirms previous reports demonstrating the hypoglycaemic and antihyperglycemic effect of rosemary in normal and diabetic rats (Tavafi et al., 2011).

Optimal pancreatic β-cell function is essential for the regulation of glucose homeostasis in both humans and animals and its impairment leads to the development of diabetes (Fatehi-Hassanabad and Chan, 2005). Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and are secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable index of insulin secretion than insulin alone (Kunt et al., 1999). In the present study, treatment with rosemary showed significant increase in plasma insulin and C-peptide levels in diabetic rats. These results indirectly indicate that part of the antihyperglycemic activity of this plant is through release of insulin from the pancreas. Diabetics have greater insulinase activity (a proteolytic enzyme that is involved in the conversion of proinsulin to insulin) than non-diabetics (Achrekar et al., 1991). Our results also showed that treatment of diabetic rats by rosemary could significantly inhibit the increase of BUN, serum creatinine and serum uric acid in comparison to untreated diabetic animals. Since diabetes is a multifactorial disease that leads to several complications, it demands a multiple therapeutic approach. We believe that rosemary with multi-beneficial properties such as antioxidant (Bakirel et al., 2008), anti-inflammatory (Erkan et al., 2008), can be introduced to diabetic patients.

Furthermore, the kidney histopathology data of STZ-induced diabetic rats showed marked focal degenerated tubules, coagulative necrosis in the lining epithelium and focal hemorrhages in comparison with the untreated diabetic animals. The results indicate a primary effect of the diabetic state on the kidney of the rat. The primary effect, the diabetes factor, was associated with hyperglycemia and was responsible for dilatation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes (Leegwates and Kuper, 1984). Diuresis is a common feature associated with diabetes, which may be the reason for structural changes observed with glomeruli (Das et al., 1996). The excellent recovery of renal function expected with treatment of rosemary can be explained by the regenerative capability of the renal tubules. Similar results have been observed with the treatment of alloxan-induced diabetic rats with *Trigonella foenum-graecum* seed powder by Thakran et al. (2004). The role of Rosemary in reversing the diabetic state at the cellular level besides the metabolic normalization, further proves its potential as an antidiabetic assert.

Conclusion

The results obtained in the present study indicates that the water extract of rosemary exhibits strong antihyperglycemic activity and attenuate BUN, serum creatinine and uric acid rise in diabetic rats. Hence, the data of present study provide impetus for further molecular and mechanistic studies on the therapeutic

action of rosemary extract, before it can be considered as a possible insulin replacement or adjuvant in the management of diabetes mellitus. Further investigations on identification of the active principles and their mode of action are also needed to unravel the molecular mechanisms involved in the observed effects.

REFERENCES

Achrekar S, Kaklij GS, Pote MS, Kelkar SM (1991). Hypoglycemic activity of *Eugenia jambolana* and *Ficus bengalensis*: mechanism of action. *In vivo*. 5:143-147.

Bailey CJ, Flatt PR (1986). Animal models of diabetes. In: Nattras M.[Ed], Recent advances in Diabetes II. Churchill Livingstone, Edinburg, UK pp: 71-89.

Bakirel T, Bakirel U, Keleş OU, Ulgen SG, Yardibi H (2008). *In vivo* assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan – diabetic rabbits. J. Ethnopharmacol. 116:64-73.

Carleton H (1979). Histopathological techniques, 4th Edn., London, Oxford University Press, New York, USA, Toronto.

Chauhan S, Nath N, Tule V (2008). Antidiabetic and antioxidant effects of *Picrorhiza kurrooa* rhizome extracts in diabetic rats. Ind. J. Clin. Biochem. 23:238-242.

Das AV, Padayatti PS, Paulose CS (1996). Effect of leaf extract of Aegle marmelose (L.) Correa ex Roxb. On histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. Indian J. Exp. Biol. 34:341-345.

Erkan N, Ayranci G, Ayranci E (2008). Antioxidant activities of rosemary extract, black seed essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem. 110:76-82.

Fatehi-Hassanabad Z, Chan CB (2005). Transcriptional regulation of lipid metabolism by fatty acids: a key determinant of pancreatic β-cell function. Nutr. Metab. (Lond). 2:1.

Huang SS, Zheng RI (2006). Rosmarinic acid inhibits angiogenesis and its mechanism of action in vitro. Cancer Lett. 239:271-280.

Khalil OA, Ramadan KH, Danial EN, Alnahdi HS, Ayaz NO (2012). Antidiabetic activity of *Rosmarinus officinalis* and its relationship with the antioxidant property. Afr. J. Pharm. Pharmacol. 6(14):1031-1036.

Kunt T, Forst T, Pfutzner A, Beyer J, Wahren J (1999). The physiological impact of proinsulin C-peptide. Pathophysiol. 5:257–62.

Leegwates, DC, Kuper CF (1984). Evaluation of histological changes in the kidneys of the alloxan diabetic rat by means of factor analysis. Food Chem. Toxicol. 22(7):551 - 557.

Li MK, Crawford JM (2004). The Pathology of Cholestasis. Seminars in Liver Disease. 24(1):21–42.

Makino T, Ono T, Liu N, Nakamura T, Muso E, Honda G (2002). Suppressive effects of rosmarinic acid on Mesangio proliferative glomerulionephritis in rats. Nephron 92:898-904.

Sharma SB, Nasir A, Prabhu KM, Murthy PS (2006). Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus. J. Ethnopharmacol. 104:367-73.

Sharma SB, Tanwar R, Rini A, Singh U, Gupta S, Shukla S (2010). Protective effect of *Morus ruba* L. leaf extract on diet induced atherosclerosis in diabetic rats. Indian J. Biochem. Biophys. 47:26-31.

Siddiqui O, Sun Y, Lin JC, Chien YW (1987). Facilitated transdermal transport of insulin. J. Pharm. Sci. 76:341–45.

Tavafi M, Ahmadvand H, Khalatbari A, Tamjidipoor A (2011). Rosmarinic Acid Ameliorates Diabetic Nephropathy in Uninephrectomized Diabetic Rats. Iran. J. Basic Med. Sci. 14:275-283.

Thakran S, Siddiqui MR, Baquer NZ (2004). Trigonella foenum graecum seed powder protects against histopathological abnormalities in tissues of diabetic rats. Mol. Cell Biochem. 266(1-2):151-159.

World Health Organization (WHO) (2004). Web page http://www.who.org (accessed January 2004).

Zimmermann T, Muller A, Machnik G, Franke H, Schubert H, Dargel R (1987). Biochemical and morphological studies on production and regression of experimental liver cirrhosis induced by thioacetamide in Uje: WIST rats. Zeitschrift fur Versuchstierkunde 30:165–180.