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Stachyose extract from *Rehmannia glutinosa* Libosch. to lower plasma glucose in normal and diabetic rats by oral administration

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The hypoglycemic effects of water extract and stachyose extract (Part III) from *Rehmannia glutinosa* Libosch. were investigated in this paper by oral administration to normal, glucose- and adrenaline-induced hyperglycemic and alloxan-induced diabetic rats. The results showed that Part III had the effect of lowering fasted plasma glucose level and partially preventing hyperglycemia induced by glucose ($2.5\ g \cdot kg^{-1}$, i.p.) and adrenaline ($300\ \mu g \cdot kg^{-1}$, i.p.), respectively, but no obvious dose-dependent effect was found when it was administered at the doses of 100, 200 and 400 $mg \cdot kg^{-1}$ for 6 days, i.g. In alloxan-induced diabetic rats, Part III ($200\ mg \cdot kg^{-1}$ for 15 days, i.g.) gave a significant decrease in blood glucose level. The results suggested that Part III, which is mainly composed of stachyose from *Rehmannia glutinosa* Libosch., had a significant hypoglycemic effect in glucose- and adrenaline-induced hyperglycemic and alloxan-induced diabetic rats.

1. Introduction

Rehmannia glutinosa Libosch. is a traditional Chinese medical herb and is noted in traditional Chinese medicine (TCM) as a drug with a nourishing effect on the body, especially for the growth, development and well-being of the body. It has been used for thousands of years in many TCM preparations for the treatment of diabetes mellitus. Modern experimental studies of *Rehmannia glutinosa* Libosch. in the treatment of diabetes mellitus started as early as 1935 when it was found that it induced a hypoglycemic effect in normal rabbits (Jin and Shi, 1935); since then, this effect has been confirmed by other investigators (Luo et al. 1957; Yin and Guo 1993). Two components, catalpol (Kitagawa et al. 1971) and a pectin-like polysaccharide (RG-WP) (Kiho et al. 1992) from *Rehmannia glutinosa* Libosch. have been shown to exert the hypoglycemic effect. Chemical studies showed that stachyose is the main component with a content of about 48.3% reported by Japanese researchers (Tomada et al. 1971) and 30% by Chinese researchers (Bian et al. 1995) in *Rehmannia glutinosa* Libosch., but there has been no report about the effect of this component on glucose metabolism. The aim of this study was to investigate the hypoglycemic effect of a stachyose extract from *Rehmannia glutinosa* Libosch. by oral administration to normal, glucose- and adrenaline-induced hyperglycemic and alloxan-induced diabetic rats.

2. Investigations and results

2.1. Preparation of stachyose extract

A stachyose extract from *Rehmannia glutinosa* Libosch. was prepared according to Tomada et al. (1971), slightly modified. A water extract obtained from the fresh roots of *Rehmannia glutinosa* Libosch. with yields of about 40% of raw materials, was further separated by charcoal column chromatography into four parts and identified by HPLC. Part I was composed mainly of glucose and bisaccharides, Part II mainly trisaccharides, Part III mainly stachyose and Part IV of other oligosaccharides. Chromatograms of each part are shown in the Fig. and the yields and contents of stachyose of each part are shown in Table 1.
Fig. and Table 1 show that stachyose is the dominant component of Part III separated from a water extract of *Rehmannia glutinosa* Libosch., the content of stachyose being 60.51%. Water extract and stachyose extract (Part III) were used in our pharmacological experiments.

2.2. Comparison of hypoglycemic activity of stachyose extract (Part III) with water extract from Rehmannia glutinosa Libosch. in glucose-induced hyperglycemic rats

After pretreatment of normal rats with water extract and stachyose extract (Part III) at a dose of $200\ mg \cdot kg^{-1}$ for 6 days, i.g., hyperglycemia was induced by glucose ($2.5\ g \cdot kg^{-1}$, i.p.) 6 h after the last administration. Fasted

Table 1: Yields of different parts from 1000 g water extract of *Rehmannia glutinosa* Libosch. separated by charcoal column chromatography

Parts	Eluates	Yield (g)	Percent of stachyose (%)
I	water	111.50	0
II	5% ethanol	150.10	20.99
III	10% + 15% ethanol	168.93	60.51
IV	20% ethanol	15.88	5.99

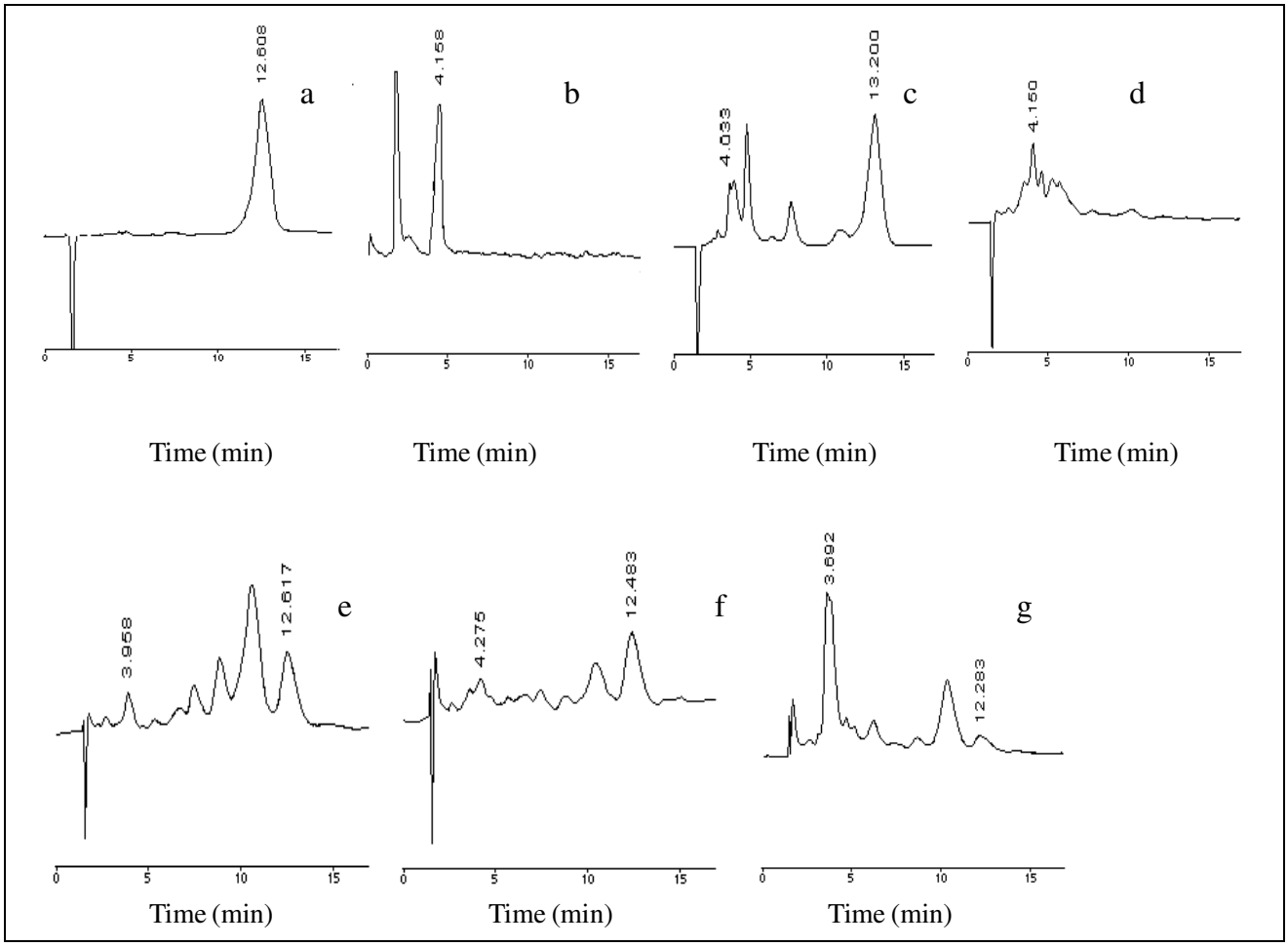

Fig.: Chromatograms of different parts of water extract from *Rehmannia glutinosa* Libosch. a. stachyose standard; b. glucose standard; c. water extract; d. Part I; e. Part II; f. Part III. g. Part IV. HPLC conditions: column: Spherisorb NH_2; flow rate: 1.2 ml/min; wave: 195 nm; mobile phase: acetonitrile: water 70 : 30, (v/v); column temperature: 25 °C

plasma glucose level was determined before glucose loading (0 min) and at 30 min, 60 min, 90 min and 120 min after glucose loading. The results obtained showed that the peak plasma glucose level (30 min) was lowered ($p < 0.05$) in the water extract and Part III groups compared with the glucose group (Table 2).

2.3. Effect of stachyose extract (Part III) on glucose level in normal, glucose-induced and adrenaline-induced hyperglycemic rats

Non-fasted and fasted plasma glucose levels were determined in normal rats after treatment with Part III at doses of 100, 200 and 400 $mg \cdot kg^{-1}$ for 6 days, i.g. The results obtained showed that there was no obvious effect of Part III at each dose on non-fasted plasma glucose level but a significant decrease in fasted plasma glucose level at doses of 100, 200 and 400 $mg \cdot kg^{-1}$, i.g., in normal rats ($p < 0.05$, 0.05 and 0.01, respectively), while no significant dose-dependency was found (Table 3).

After pretreatment of normal rats with Part III at doses of 100, 200 and 400 $mg \cdot kg^{-1}$ for 6 days, i.g., hyperglycemia was induced by glucose (2.5g $\cdot kg^{-1}$, i.p.) and adrenaline (300 $\mu g \cdot kg^{-1}$, i.p.) 6 h after the last administration. Plasma glucose level was determined before glucose load (0 min) and at 30 min, 60 min, 90 min and 120 min after glucose load or before adrenaline injection (0 min) and at 30 min and 90 min after adrenaline injection. The results obtained showed that the peak of plasma glucose level (30 min) for each dose group of Part III was lowered ($p < 0.01$) relative to that of the glucose control group (Table 4). Pretreatment with Part III at doses of 200 and 400 $mg \cdot kg^{-1}$ showed a similar preventive effect against hyperglycemia induced by adrenaline ($p < 0.05$ and 0.01, respectively) (Table 5).

Table 2: Hypoglycemic activity of different parts of the water extract from *Rehmannia glutinosa* Libosch. on plasma glucose level in glucose-induced hyperglycemic rats (mean ± SD)

Group	Dose ($mg \cdot kg^{-1}$)	Plasma glucose level ($mmol \cdot L^{-1}$)			
		0 min	30 min	60 min	120 min
Saline	0	5.03±0.57(10)	4.88±1.19(10)	4.98 ±1.13(9)	5.30±1.25(9)
Glucose	0	5.26±0.87(10)	10.31±2.62(9)***	8.01±0.72(8)**	7.48±1.04(8)**
Water extract + glucose	200	5.14±0.91(9)	7.37±1.52(8)$^{\#\#}$	7.36±1.33(8)	6.24±1.62(8)
Part III + glucose	200	4.99±0.64(9)	7.01±0.67(9)$^{\#\#}$	6.89± 1.08(9)$^{\#}$	6.05±1.05(9)$^{\#\#}$

Values are obtained from the number (N) of rats in each group. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with glucose group

Table 4: Effect of Part III on plasma glucose level in rats with glucose-induced hyperglycemia (mean ± SD)

Group	Dose ($mg \cdot kg^{-1}$)	Plasma glucose level ($mmol \cdot L^{-1}$)			
		0 min	30 min	60 min	120 min
Saline	0	4.97 ± 0.86(10)	5.11 ± 0.88(10)	5.04 ± 1.07(9)	5.12 ± 1.21(9)
Glucose	0	5.03 ± 1.07(10)	11.77 ± 2.39***(9)	9.36 ± 1.45***(8)	8.16±1.48***(8)
Part III + glucose	100	5.41 ± 0.49(8)	7.97 ± 0.63$^{\#\#\#}$(8)	7.48 ± 0.85(8)	6.82 ± 0.91(8)
Part III + glucose	200	4.93 ± 0.99(9)	7.35 ± 1.56$^{\#\#\#}$(9)	7.12 ± 1.46(8)	6.30 ± 1.39(8)
Part III + glucose	400	5.12 ± 0.52(9)	6.87 ± 0.56$^{\#\#\#}$(9)	7.05 ± 0.67$^{\#}$ (9)	6.08 ± 7.08$^{\#\#}$(9)

Values are obtained from the number (N) of rats in each group.$^{***}p < 0.001$, compared with saline control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with glucose control group

2.4. Effect of stachyose extract (Part III) on body weights and plasma glucose level in alloxan-induced diabetic rats

Diabetes was induced in Wistar rats by 150 $mg \cdot kg^{-1}$ of alloxan (i.p.). Body weights were significantly lowered and fasting plasma glucose level was elevated in alloxan-induced diabetic rats compared with that of saline-treated normal rats at d0, d3, d9 and d15. Treatment of diabetic rats with Part III at d3, d9 and d15 at a dose of 200 $mg \cdot kg^{-1}$ (i.g.) increased the body weight but the differences were not significant compared with the DM group ($p > 0.05$), while blood glucose level was decreased compared with the diabetes group ($p < 0.01$). Administration of metformin (500 $mg \cdot kg^{-1}$, i.g.) also showed a hypoglycemic effect in alloxan-induced diabetic rats ($p < 0.05$). (Table 6, Table 7)

Table 3: Effect of Part III on non-fasted and fasted plasma glucose level in normal rats (mean ± SD, n = 10)

Group	Dose ($mg \cdot kg^{-1}$)	Non-fasted plasma glucose level ($mmol \cdot L^{-1}$)	Fasted plasma glucose level ($mmol \cdot L^{-1}$)
Saline	0	6.59 ± 0.82	5.51 ± 0.52
Part III	100	6.66 ± 0.62	4.88 ±0.67*
Part III	200	7.40 ± 1.13	4.48 ± 0.73*
Part III	400	6.89 ± 0.87	4.46 ± 0.69**

Values are obtained from the number of rats in each group.$^{*}p < 0.05$, $^{**}p < 0.01$, compared with saline control group.

3. Discussion

Rehmannia glutinosa Libsoch. has been used as a drug with a nourishing effect on the body and for treatment of diabetes in China for thousands of years. Although early studies demonstrated that *Rehmannia glutinosa* Libsoch. exerted a hypoglycemic effect, only limited progress was made because of limitations of phytochemical techniques until two hypoglycemic components, catalpol (Kitagawa et al. 1971) and a polysaccharide (RG-WP) (Kiho et al. 1992), were successfully separated from the drug. Studies also revealed that polysaccharides from *Rehmannia glutinosa* Libosch. possessed immunopotentiating (Chen et al. 1993; Chen et al. 1994) and P_{53} gene expression increas-

Table 5: Effect of Part III on plasma glucose level in rats with adrenaline-induced hyperglycemia (Mean ± SD)

Group	Dose ($mg \cdot kg^{-1}$)	Plasma glucose level ($mmol \cdot L^{-1}$)		
		0 min	30 min	90 min
Saline	0	5.46 ± 0.41(10)	5.57 ± 0.84(10)	5.54 ± 0.59(9)
Adrenaline	0	5.33 ± 0.51(10)	17.44 ± 3.09(9)***	16.84 ± 3.3(9) ***
Part III + adrenaline	100	5.41 ± 0.49(8)	15.21 ± 2.86(8)	13.04 ± 3.13(8)
Part III + adrenaline	200	5.28 ± 0.52(9)	14.42 ± 2.22 (9) $^{\#\#}$	11.49 ± 2.34(9) $^{\#\#}$
Part III + adrenaline	400	5.25 ± 0.63(9)	14.27 ± 2.36(9) $^{\#}$	11.18±2.31(9) $^{\#\#}$

Values are obtained from the number (N) of rats in each group. $^{***}p < 0.01$, compared with saline control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with adrenaline control.

Table 6: Effects of Part III on body weight in alloxan-induced diabetic rats (mean ± SD)

Group	Dose ($mg \cdot kg^{-1}$)	Body weights (g)			
		d 0	d 3	d 9	d 15
Saline	0	225 ± 17(10)	226 ± 17(10)	242 ± 20(10)	252 ± 20(10)
DM	0	163 ± 20***(9)	152 ± 24***(9)	158 ± 27***(9)	162 ± 30***(8)
DM + Part III	200	164 ± 10(9)	165 ± 17(9)	174 ± 17(8)	184 ± 19(8)
DM + Metformin	500	160 ± 16(9)	164 ± 19(9)	175 ± 20(9)	187 ± 16(8)

Values are obtained from the number (N) of rats in each group. $^{***}p < 0.01$, compared with saline control. DM: diabetes mellitus

Table 7: Effect of Part III on plasma glucose level in alloxan-induced diabetic rats (Mean ± SD)

Group	Dose ($mg \cdot kg^{-1}$)	Plasma glucose level ($mmol \cdot L^{-1}$)			
		d 0	d 3	d 9	d 15
Saline	0	5.28 ± 8.3(10)	5.76 ± 11.1(10)	5.34 ± 16.9(10)	7.72 ± 5.6(10)
DM	0	28.89 ± 87.6***(9)	24.19 ± 87.0***(9)	23.3 ± 68.7***(9)	21.66 ± 46.3*** (8)
DM + Part III	200	28.56 ± 70.8(9)	18.90 ± 60.3(9)	18.34 ± 69.6$^{\#}$(8)	14.59 ± 34.6$^{\#\#\#}$(8)
DM + Metformin	500	27.62 ± 67.4(9)	22.58 ± 51.4(9)	19.39 ± 61.0$^{\#}$ (9)	16.03 ± 39.5$^{\#\#\#}$(8)

Values are obtained from the number (N) of rats in each group. DM: diabeties mellitus. $^{***}p < 0.01$, compared with saline control; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$, compared with DM control

ing effects (Wei et al. 1997) and that oligosaccharides from *Rehmannia glutinosa* Libosch. had the effect of promoting proliferation of hemopoietic progenitors in SAMP8 mice (Liu et al. 1998). Hence researchers have recently paid much attention to saccharides from *Rehmannia glutinosa* Libosch.

In our present study, a water extract from *Rehmannia glutinosa* Libosch. was separated into four parts by charcoal column chromatography and HPLC analysis showed that the main component of Part III was stachyose with a content of 60.51%. The present paper reports the hypoglycemic effect of the stachyose extract (Part III) from *Rehmannia glutinosa* Libosch. given by oral administration to normal, glucose- or adrenaline-induced hyperglycemic and alloxan-induced diabetic rats. We found that Part III at doses of 100, 200 and 400 $mg \cdot kg^{-1}$ for 6 days, i.g., had the effect of lowering fasted plasma glucose level and partially preventing of hyperglycemia induced by glucose ($2.5\ g \cdot kg^{-1}$, i.p.) and adrenaline ($300\ \mu g \cdot kg^{-1}$, i.p.) in normal rats, but no obvious dose-dependent effect was observed. In alloxan-induced diabetic rats, Part III ($200\ mg \cdot kg^{-1}$ for 15 days, i.g.) significantly decreased blood glucose level. The above results indicated that the stachyose extract from *Rehmannia glutinosa* Libosch. exerted a significant hypoglycemic effect on glucose metabolism in normal, hyperglycemic and alloxan-induced diabetic rats. The results strongly support the conclusion that stachyose extract (Part III) from *Rehmannia glutinosa* Libosch. is a new active principle with a hypoglycemic effect in addition to catalpol and polysaccharides (RG-WP).

A recent study (Zheng et al. 2000) showed that absorption of orally administered stachyose in rat intestine is rapid but low with bioavailability of 3.82%. Stachyose is a non-digestible oligosaccharide with a prebiotic effect of increasing the number and/or activity of bifidobacteria and lactic acid bacteria in the body (Loo et al. 1999). The question thus raised is whether there is any relationship between the modulatory effects of stachyose extract on glucose metabolism in rat models and the increase in the number and/or activity of bifidobacteria and lactic acid bacteria in the body? The hypoglycemic mechanism of stachyose extract from *Rehmannia glutinosa* Libosch. remains to be investigated further.

4. Experimental

4.1. Animals

Female Wistar rats were obtained from the Laboratory Animal Department of Lanzhou General Hospital of PLA and housed in a house with controlled temperature ($22 \pm 2\ ^\circ C$) on a 12 : 12 light/dark cycle (lights on at 06 : 00 AM). Animals had free access to pellet food with tap water *ad libitum.*

4.2. Materials

Stachyose and Alloxan (ALX): Sigma Chemical Co.; Glucose (A. R.): Beijing Chemical Regent Co.; Glucose Assay Kits: Beijing Chemical Plant; UV-160A spectrophotometer: Shimadzu, Tokyo, Japan; HPLC system consisted of LC-6A pump (Shimadzu,Tokyo, Japan), RID-6A refractive index detector and Spherisorb NH_2 column. The chromatographic data were recorded and processed by a Chromatopac CR3A data processor.

4.3. Preparation of stachyose extracts from Rehmannia glutinosa Libosch. (Tomada et al. 1971)

The raw material was obtained from Anguo County, Hebei Province of P. R. China and authenticated as being *Rehmannia glutinosa* Libosch. by Professor Yun Liu, Institute for Drug and Instrument Control of PLA. The voucher specimen was deposited in the Department of Phytochemistry, Beijing Institute of Pharmacology and Toxicology. 1000 g of fresh roots were crushed and extracted three times with hot water (3000 ml) for 60 min. After suction filtration, all extracts were collected and concentrated to 1000 ml, then applied to a cation exchange resin column. After washing with water, the eluates were collected and concentrated, then applied to an anion resin column. Washing with water again, the eluates with positive Monish reaction were collected, concentrated and vacuum-dried. The yields of the neutral fraction were 401.9 g (about 40.19% of raw materials), which was further separated by charcoal column chromatography.

Active charcoal was treated before use with hot 15% AcOH, followed by washing with water. The neutral fraction of the water extract was applied to the top of the charcoal column (5.4×97 cm), followed by successive elution with water, 5% ethanol, 10% ethanol, 15% ethanol and 20% ethanol. Fractions were collected and carbohydrates in the eluates were measured by the phenol-sulfuric acid method. The elutes obtained from the column were divided into four groups: Part I, Part II, Part III and Part IV.

4.4. Collection of blood and plasma glucose estimation

Blood samples of the non-fasted and fasted rats were collected immediately from the retrobulbar venous plexus with capillary tubes under ether anaesthesia and with 0.1 M EDTA as anticoagulant. The glucose-oxidase-oxygen method was used for the determination of the plasma glucose level.

4.5. Glucose- and adrenaline-induced hyperglycemia in normal rats

Sterile normal saline and *Rehmannia glutinosa* Libosch. extract samples were given by i.g. at different doses to the rats for 6 days. Prior to the glucose- and adrenaline-induced hyperglycemic test on d6, rats were fasted for 6 h. Thirty minutes later, glucose ($2.5\ g \cdot kg^{-1}$, i.p.) or adrenaline ($300\ \mu g \cdot kg^{-1}$, i.p.) was administrated to the rats in each group. Blood samples were taken from the retrobulbar venous plexus at 0 min (just before glucose load), 30 min, 60 min, and 120 min (after glucose load) or at 0 min (just before adrenaline administration), 30 min and 90 min (after adrenaline administration) for the assay of plasma glucose level.

4.6. Preparation of alloxan-induced diabetic rats

Diabetes was induced in rats that had been fasted for 12 h by intraperitoneal injection of $150\ mg \cdot kg^{-1}$ body weight (bw) of alloxan, freshly dissolved in sterile normal saline immediately before use to give a concentration of $30\ g \cdot L^{-1}$. The diabetic state was assessed by measuring fasted serum glucose concentration 72 h after alloxan treatment. Rats with a serum glucose level above $19.44\ mmol \cdot L^{-1}$, as well as with polydipsia, polyuria and polyphagia, were selected for the experiment.

4.7. Effects of stachyose extract on body weight and fasting glucose levels in diabetic rats

Stachyose extract at a dose of $200\ mg \cdot kg^{-1}$ was given i.g. to diabetic rats after drawing the first blood samples. A reference drug, metformin ($500\ mg \cdot kg^{-1}$, i.g.), was given to another group of rats, while control animals received the vehicle (saline). Blood samples were drawn from these groups of rats at d 0 (before administration) and at d3, d9, d15 (after administration).

4.8 Statistical analysis

The results are expressed as mean $\pm$ SD. The significance of the differences was determinded by the one-way AVOVA test. $P < 0.05$ was considered to be a significant difference.

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