

Full Length Research Paper

Effect of saponosides crude extract isolated from *Citrullus Colocynthis* (L.) seeds on blood glucose level in normal and streptozotocin induced diabetic rats

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The effect of saponosides crude extract isolated from the seeds of *Citrullus Colocynthis* L. Schrad (Cucurbitaceae) on blood glucose level were investigated in normal and diabetic rats (streptozotocin induced). A single and non toxic dose (20 mg/kg bw) of saponosides crude extract were administrated to animals by intraperitoneal route. Lethal doses of saponosides crude extract in healthy rats (DL₅₀ and DL₁₀₀) were found to be 200 and 250 mg/kg bw, respectively. The results obtained from this study showed no change of glycaemia in normal rats. Whereas, in STZ-treated diabetic animals, the blood glucose level decrease significantly ($p < 0.05$) to reach its normal value 48 h after administration of saponosides crude extract. This antihyperglycaemic effect was maintained during twenty six days after a unique intraperitoneal injection. This effect can be reproduced after return to the diabetic state. Saponosides instigate an antihyperglycaemic and sustainable action in STZ-diabetic rats.

Key words: *Citrullus colocynthis* L., antidiabetic, saponosides, streptozotocin, rats.

INTRODUCTION

Searching for new antidiabetic drugs from medicinal plants is still attractive because a wide array of plant derived active principles have demonstrated antidiabetic activity. This main active constituents include alkaloids; flavonoids and related compounds; glycosides; steroids; terpenoids; polysaccharides; amino acids; inorganic ions and miscellaneous compounds (Lamba, 2000; Pranav, 2008; (Kumar S. et al., 2008 and Sawaya et al, 1986). During the last decades, *Citrullus colocynthis* L. Schrad (Cucurbitaceae) a medicinal plant reputed for their toxicity but also for many medicinal properties as reviewed from various literatures (Diwan, 2000; Elawad, 1984; Wasfi, 1994). Several works were undertaken to evaluate its antidiabetic properties in diabetic animal models as described subsequently.

Abdelhassen et al. (2000) reviewed the effects of saponin extract of *C. colocynthis*'s rind on the fasting plasma glucose levels in alloxan induced diabetic rabbits. The saponin significantly lowered the fasting glucose levels after 1 and 2 h and highly significant ($P < 0.001$) after 3 and 6 h. Graded doses (10, 15 and 20 mg/kg) of saponin extract, when given orally to alloxan diabetic rabbits, produced a significant reduction of plasma glucose concentration. Indeed, Nmila et al. (2000) reported that the basic subfraction of free amino acids of seeds are able to stimulate the release of insulin, from perfused rat pancreas.

It was reported also, that aqueous extracts of *C. colocynthis* seeds ameliorate some of toxic effects of streptozotocin (Al-Ghaithi et al., 2004). Therefore, Benariba et al. (2009) showed that *C. colocynthis* seeds extracts had a beneficial long-term effect on glucose homeostasis and body weight maintenance in streptozotocin-induced diabetic rats. Furthermore, Al-Khateeb et al. (2009) described the physiological effects

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of the ethanol extract of the pulp portion of *C. colocynthis*. The extract exhibits hypoglycemic effect on the steady state normoglycemic levels, as well as antihyperglycemic effect on steady state hyperglycaemic levels in diabetic rats. These physiological actions were mediated, at least in part, via an increase in insulin secretion.

In North Africa folk medicine, various parts especially fruits (seeds and pulp) of *C. colocynthis* are highly valued for the treatment of diabetes disease (Ziyyat et al., 1997). Our ethnopharmacological survey revealed that *C. colocynthis* L. is used by diabetic patients under two forms: (i) as a foot bath: slices of fresh fruit are soaked in warm water, patient immerses his/her feet until the bitter taste is felt (ii) Oral ingestion of seeds: generally one to three seeds per day are swallowed. Nevertheless, to date, the scientific scrutiny of *C. colocynthis*, is insufficiently documented and warrants systematic analysis. In particular, antidiabetic activity of saponosides fraction remains untested for a long time period.

The objectives of this investigation were: 1) to observe the influence of saponosides crude extracts from *C. colocynthis* seeds on fasting blood glucose of normal and streptozotocin diabetic rats during a prolonged period (three months nearly); 2) to determine its acute toxicity (LD₁₀₀) and (LD₅₀) in healthy rats.

MATERIALS AND METHODS

Collection of plant material

C. colocynthis L. fruits were collected in autumn from the desertic region of Bechar in south Algeria. The plant was botanically identified in the Department of Biology at Tlemcen University (Algeria). A voucher specimen C1461 has been kept in the department herbarium. The ripe fruits were shade dried at room temperature.

Preparation of saponosides extracts

The dry seeds (200 g) were pulverized in a grinder. Powdered seeds were submitted to saponosides extraction according to protocol described by Bruneton (1999). A mixture of powdered seed (105 g), distilled water (280 ml) and ethanol 96% (120 ml) was refluxed for 8 h. After filtration of the marc, the filtrate was extracted three times with 50 ml of n-butanol. After evaporation of ethanol and water, the title extract was obtained by precipitation with diethyl ether (0.94 g, Rdt = 0.89%, mp = 210°C). The residue was dissolved in ethanol and kept in brine.

Preliminary phytochemical screening

Preliminary phytochemical screening test revealed that our extract corresponded well to saponosides components (Kapoor et al., 1969).

Experimental animals

Eighty six healthy Wistar rats weighing between 150 to 200 g were selected for the present study. They were raised and housed in an

air-conditioned animal room at 24 ± 2°C and subjected to 12 h light/dark cycle. They were allowed free access to feed pellet diet and tap water *ad libitum*.

Acute toxicity study

Healthy rats fasted for 18 h were used. Thirty six rats were randomly assigned into six groups of six animals and housed in uniform conditions. Group I was treated with NaCl solution (0.9%) as control; Groups II to VI were treated with saponosides extract of *C. colocynthis* administered intraperitoneally in graded doses of 50, 100, 150, 200, 250 mg/kg, respectively. The latency for death and the number of deaths observed in each group during 48 h following saponosides extract administration were recorded. The rats were observed for gross behavioural, neurologic, autonomic and toxic effect at regular intervals.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (65 mg/kg bw) dissolved in a 0.9% NaCl solution to overnight fasted rats. The animals were considered diabetic if fasting blood glucose level values exceed 2 g/L and selected for experimentation.

Experimental design and chronic animal treatment

Healthy and diabetic rats fasted for 16 h were used. They were divided into four groups with five animals each. Group I: normal control rats received NaCl solution (0.9%) (NDC: non diabetic control); Group II: diabetic control rats untreated given saline solution, served as positive control (DC: diabetic control); Group III: normal rats treated with saponosides crude extract (20 mg/kg bw), served as normal treated (NDT: non diabetic treated), and Group IV: diabetic rats received saponosides crude extract (20 mg/kg bw) and served as treated diabetic rats (DT: diabetic treated). Treatment duration was maintained for 84 days. During the first week period, animals were allowed to acclimatize to the laboratory environment prior to commencement of streptozotocin (STZ) administration at day 7 to Groups II and IV. Animals of Groups III and IV received the first and second IP injections of saponosides crude extract (S.C.E) (20 mg/kg bw) at days 28 and 63, respectively. Fasting blood glucose level, glycosuria and body weight were measured weekly.

Analytical techniques

Blood samples were collected from tail vein: a drop of blood were analysed for its glucose content by Accu-Chek Active strips (Roche) or withdrawn from retro-orbital plexus under light anesthesia. Blood was collected in heparinized capillaries and centrifuged (3000 rpm, 10 min). The plasma obtained after centrifugation was used for the determination of glucose levels. Glucose was estimated by glucose oxydase-peroxydase method using commercially available Diagnostic kit (Trinder, 1969). Urine sugar was detected by Keto-Diastix reagent strips (Bayer). The values of glycaemia corresponded well to plasmatic glucose level.

Statistical analysis

Results are expressed as mean ± standard error (SEM). Statistical comparison was performed by Student's test. The results were considered statistically significant if the values were 0.05 or less (Snedecor, 1967).

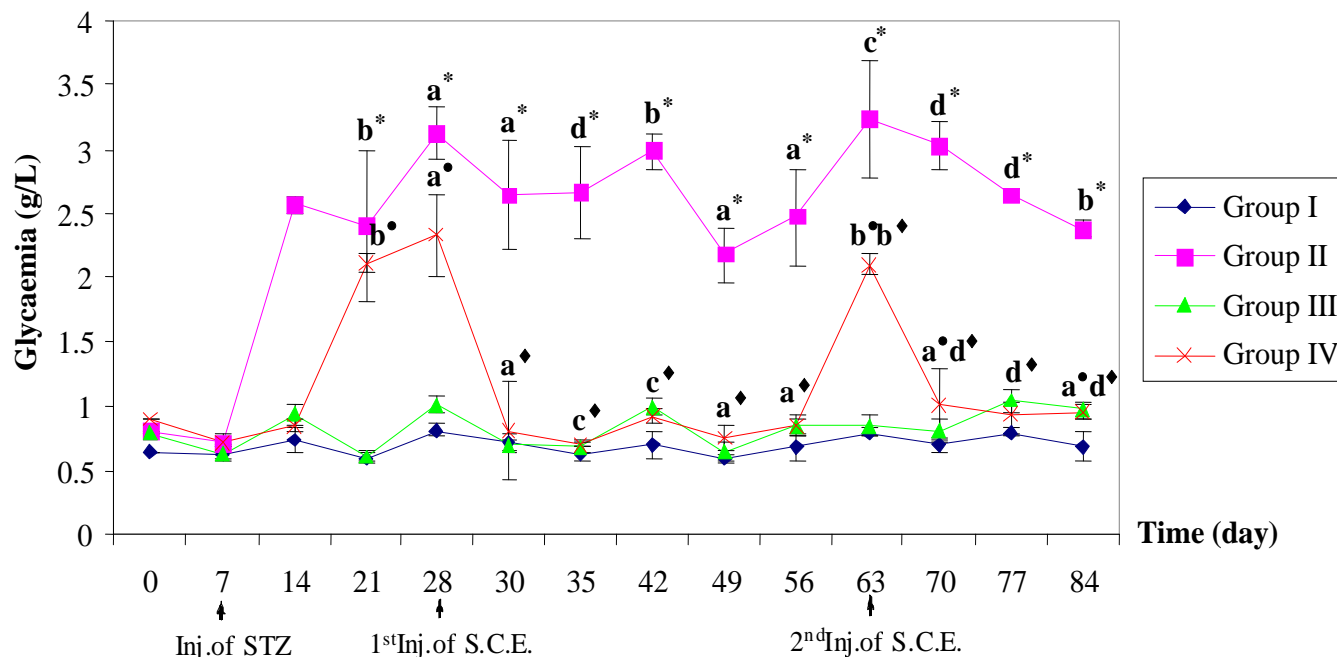


Figure 1. Long term effect of *C. colocynthis* saponosides crude extract (20 mg/kg bw, IP injection) on blood glucose level of normal and STZ diabetic rats. Values are expressed as mean \pm standard deviation ($n = 5$). a*, a*, a*: slightly significant ($P < 0.05$); b*, b*, b*: significant ($P < 0.01$); c*, c*, c*: very significant ($P < 0.001$); d*, d*, d*: high significant ($P < 0.0005$); S.C.E.: Saponosides crude extract *: significance of diabetic control rats compared to normal control. •: Significance of treated diabetic rats with saponosides extract compared to normal control. ♦: Significance of treated diabetic rats with saponosides extract compared to diabetic control.

RESULTS

Acute toxicity study (LD₅₀ and LD₁₀₀)

Administration dose of 50 mg/kg b.w induce no change in the behaviour of the treated rats and any obvious toxic effect was observed. In contrast, higher doses of 100 and 150 mg/kg produced the following signs: lose of locomotion activity, ataxia, convulsion, diarrhoea one hour after the injection, at the highest doses (200 and 250 mg/kg): same signs as described previously, lethargy, coma and death were observed. The intensity of these toxic effects was dose-dependant. The intraperitoneal median lethal dose (LD₅₀) value was found to be 200 mg/kg. Although, the lethal dose (LD₁₀₀) is estimated to 250 mg/kg.

Effect of saponosides crude extract on blood glucose level in healthy rats

Figure 1 shows that basal glycaemia stays without significant variations in non diabetic control group (NDC). Fasting blood glucose level in normal rats is ranged between 0.63 to 0.83 g/L. Administration of S.C.E produced no change of basal glycaemia in normal treated group (NT).

Effect of saponosides crude extract on blood glucose level in diabetic rats

Streptozotocin treatment produced a significant increase in plasma glucose level with a moderate diabetic state (2.4 g/L) average. The result from this study indicated that the S.C.E exhibit significant hypoglycaemic activity in STZ-diabetic rats ($p < 0.01$), while no significant effect was observed on normoglycemic animals (NT group). After the first injection, hyperglycaemia was reduced to reach its basal value. This normoglycemic state was maintained about 26 days. Then, glycaemia reflected back to diabetic values (2.09 g/L) (Figure 1). This diabetic state was characterized also by glycosurea symptom. As shown in Figure 1, after the second injection of saponosides crude extract at day 63, a significant diminution of blood glucose level was observed again, glycaemia returned to its normal value and glycosurea disappeared completely.

DISCUSSION

From our results, it appears that administration of 65 mg/kg of STZ induced diabetes in normal rats (3.09 g/L). Our observations are in agreement with the reports by several workers that STZ-induced diabetes mellitus and insulin deficiency leads to increased blood glucose (Chaude et al., 2001).

The findings of this study indicate that a single IP injection of this extract reduced fasting blood glucose in STZ-diabetic rats. In addition, this antihyperglycaemic effect via blockage of the serum glucose level was maintained and persisted for a relatively long period of time (26 days). The hyperglycaemia decreased significantly and reached its normal value 48 h after extract administration; the glycosuria disappeared simultaneously in treated diabetic rats.

As indicated in Figure 1, a high pick was observed after 63 days, so it is logical, because the extract during the time are catabolised and the positive effect is terminated. The antihyperglycaemic action can be reproduced again by a second injection of extract when animals returned to diabetic state.

This antihyperglycaemic effect was attributed to saponosides (or saponin), a natural constituent of *C. colocynthis* and other plant species, that may interact with several metabolic pathways or insulin metabolism and influence directly and indirectly on glucose homeostasis (Pranav et al., 2008). It is well documented in the literature that they are glycosides of steroids or triterpenoids found in plants. Charantin, a steroidal saponin, isolated from *Momordica charantia* L. (Cucurbitaceae) is reported to possess an insulin-like activity by enhancing the release of insulin and slowing down the glucogenesis (Ng et al., 1986). Gymnemic acid, a saponin isolated from *Gymnema sylvestre* R. (Asclepiadaceae), exhibit potent hypoglycaemic activity in animals models, including stimulation of insulin secretion and release, regeneration of β cells of Langerhans islets, activation of enzymes responsible for glucose utilization and reduction of glucose and fatty acid assimilation in the small intestine (Sugihara, 2000; Spasov, 2008).

On the basis of literature and from our experimental data, we assumed that saponosides may be active constituents of *C. Colocynthis*, which regulate efficiently blood glucose in diabetic rats. They may exert their effect by potentializing the insulin-secretion of residual insulin cells or increase the ability of cells to keep glucose (NDF, 2008; Patrick, 2008; Ashok, 2011).

From this study, we had successfully provided that our results are in agreement and supplementary with the previous studies that reported hypoglycaemic effect of saponosides extract isolated from *C. colocynthis* in normal and alloxan-diabetic rabbits at short period (0 to 24 h) (Abdel-Hassan et al., 2000) and *in vitro* stimulation potency of the same extract on insulin release from isolated islets of Langerhans (Nmila et al., 2000).

Conclusion

This study support that *C. colocynthis* possesses antidiabetic properties and saponosides are one of the active constituent. The seeds of this plant seem to have a promising value for the development of potent phytomedicine for diabetes. Further pharmacological and

biochemical investigations are underway to elucidate mechanisms of the antidiabetic effect of *C. colocynthis* and understanding its folkloric use in the management of diabetes mellitus.

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REFERENCES

- Abdel-Hassan IA, Abdel-Barry JA, Mohammeda TS (2000). The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. *J. Ethnopharmacol.*, 71(1/2): 325-330.
- Al-Ghaithi F, El-Ridi MR, Adeghate E, Amiri MH (2004). Biochemical effects of *Citrullus colocynthis* in normal and diabetic rats. *Mol. Cell. Biochem.*, 261: 143-149.
- Al-Khateeb M, Dallak M, Al-Hashem F, Bashir N, Abbas M, Elessa R, Khalil M (2009). *In vivo* acute normo-Hypoglycemic antihyperglycemic insulinotropic actions of orally administered ethanol extract of *Citrullus colocynthis* (L.) Schrab pulp. *Am. J. Biochem. Biotechnol.*, 5(3): 118-125.
- Ashok KB, Jagadish S, Tapan KM, Vaiyaburi TS, Suresh BP (2011). Ethanol extract of *Melothria Maderaspatana* inhibits glucose absorption and stimulates insulin secretion in male C57BL/6 mice. International conference on Bioscience, Biochemistry and Bioinformatics IPCBEE, 5: 429-434 IACSIT Press, Singapore.
- Benariba N, Djaziri R, Zerriouth BH, Boucherit K, Louchami K, Sener A, Willy JM (2009). Antihyperglycemic effect of *Citrullus colocynthis* seed aqueous extracts in streptozotocin-induced diabetic rats. *Met. Funct. Res. Diab.*, 2: 71-76.
- Bruneton J (1999). Pharmacognacy, Phytochemistry, Medicinal Plants. 3th Edn, Lavoisier Publishing.
- Chaudh MA, Orisakwe OE, Afonne OJ, Gamenial KS, Vongtau OH, Ob E (2001). Hypoglycemic effect of the aqueous extract of *Boerhavia diffusa* leaves. *Ind. J. Pharmacol.*, 33: 215-216.
- Diwan FH, Abdel-Hassan IA, Mohammed ST (2000). Effect of saponin on mortality and histopathological changes in mice. *East Mediterr. Health J.*, 6(2-3): 345-351.
- Elawad AA, Abdel Bari EM, Mahmoud OM, Adam SE (1984). The effect of *Citrullus colocynthis* on sheep. *J. Vet. Hum. Toxicol.*, 26: 481-485.
- Kapoor LD, Singh A, Kapoor SL, Srivastava SN (1969). Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. *Lloydia*, 32: 297-302.
- Kumar S, Kumar D, Manjusha Saroha K, Singh N, Vashishta B (2008). Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *Acta Pharm.*, 58(2): 215-20.
- Lamba SS, Buch KY, Lewis H, Lamba J (2000). Phytochemicals as potential hypoglycaemic agents. Atta-ur-Rahman (Ed.) *Studies in Natural products Chemistry*. Elsevier Sci, 21: 457-496.
- National Diabetes Fund (NDF) published as a public service (2008). Prevention and Treatment of Diabetes with Natural Therapeutics. 4th Edn, program of Project Cure, P.O. Box 96673 Washington, D.C. 20090-6673.
- Ng TB, Wong CM, Li WW, Yeung HW (1986). Insulin-like molecules in *Momordica Charantia* seeds. *J. Ethnopharmacol.*, 15: 107-117.
- Nmila R, Gross R, Rchid H, Roye M, Manteghetti M, Petit P, Tijane M, Ribes G, Sauvaire Y (2000). Insulinotropic effect of *Citrullus colocynthis* fruits extract. *Planta Med.*, 66: 418-423.
- Patrick L.O, Louis C.M, Dayna C, Pierre S.H, Teatulohi M, Timothy J (2008). Consumption of guava (*Psidium guajava* L.) and noni (*Morinda citrifolia* L) may protect betel quid-chewing Papua New

- Guineans against diabetes. Asia Pac. J. Clin. Nutr., 17(4): 635-643.
- Pranav K, Prabhakar PK, Doble M (2008). A target based therapeutic approach towards diabetes mellitus using medicinal plants. Curr. Diabetes Rev., 4: 291-308.
- Sawaya WN, Daghir NJ, Khalil JK (1986). Citrullus colocynthis seeds as a potential source of protein for food and feed. J. Agric. Food Chem., 34(2): 285-288.
- Snedecor GW (1967). Statistical Methods. Oxford and IBH Publishing Com., p. 33.
- Spasov AA, Samokhina MP, Bulanov AE (2008). Antidiabetic properties of *Gymnema Sylvestre* (a review). Pharma. Chem. J., 42(11): 626-629.
- Sugihara Y, Nojima H, Matsuda H, Murakami T, Yoshikawa M, Kinura I (2000). Antihyperglycaemic effects of gymnemic acid IV, a compound derived from *Gymnema sylvestre* leaves in streptozotocin-diabetic mice. J. Asian Nat. Prod. Res., 2: 321-327.
- Trinder P (1969). Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol., 22: 246.
- Wasfi IA (1994). Some pharmacological studies on *Citrullus colocynthis*. J. Herbs Species Med. Plants, 2: 65-79.
- Youn JY, Park HY, Cho KH (2004). Anti-hyperglycemic activity of *Commelina communis* L.: inhibition of α -glucosidase. Diabetes Res. Clin. Pract., 66S: S149-S155.
- Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W (1997). Phytotherapy of hypertension and diabetes in oriental Morocco. J. Ethanopharmacol., 58: 45-54.