Therapeutic Potential of Asparagus racemosus Root in Managing Hyperglycemia, Dyslipidemia, and Platelet Aggregation in Neonatal Streptozotocin-Induced Type 2 Diabetic Rats

Abstract

Lipid abnormalities frequently accompany hyperglycemia and thus a primary goal in diabetes therapy is the management of dyslipidemia. *Asparagus racemosus* root has previously been shown to reduce postprandial blood glucose in diabetic rats by delaying carbohydrate absorption and enhancing insulin secretion. In the present study, the chronic effects of *A racemosus* root on serum glucose, fructosamine, lipids, and platelet aggregation were assessed in rats with type 2 diabetes induced by neonatal streptozotocin injection. The type 2 diabetes model was created by injecting 48-hour-old pups with a single intraperitoneal dose of streptozotocin (STZ). Platelet aggregation was measured by optical aggregometry. Daily oral administration of ethanol extract of *A racemosus* to diabetic rats (n = 10) lowered serum glucose by 21% ($p<0.01$) and fructosamine by 11% ($p<0.05$) after 28 days. Total cholesterol ($p<0.05$), triglyceride ($p<0.05$), and NEFA ($p<0.01$) levels were also lowered by 9%, 16% and 38% respectively. No difference in HDL cholesterol or body weights was observed compared to control rats but platelet aggregation was significantly reduced by 18% ($p<0.05$). Food and water intake, stool formation, water content of stools, and urine formation were unchanged in extract-treated rats in a 24-hour acute observational study in Nalgene Metabolic Cages. In conclusion, this study reveals that ethanol extract of *A racemosus* root lowers circulating glucose, and atherogenic blood lipids and decreases platelet aggregation. Thus, *A racemosus* is the source of glucose-lowering bioactive agents and a useful dietary adjunct in the management of diabetes, dyslipidemia, and related complications.

Keywords: *Asparagus racemosus*, Diabetes mellitus, Serum glucose, cholesterol, triglyceride, HDL, NEFA, Platelet aggregation.

Introduction

"The increasing prevalence of diabetes mellitus represents a significant public health concern, currently impacting around 400 million individuals globally" (Khursheed, et al., 2019), and "with numbers estimated to reach more than 640 million by 2040" (Giovannini, et al., 2016). It is a long-standing, complex, and non-transmissible endocrine disease that poses significant clinical challenges worldwide due to treatment costs and the threat of life-limiting diabetic complications. Type 2 diabetes (T2DM) is the most common form characterized by chronic hyperglycaemia due to deficits in insulin secretion and action (Smushkin & Vella, 2010).

Although recent years have seen a significant upturn in the number of drug classes available to treat T2DM (Bailey & Day, 2018), medicinal plants have a vast potential for the treatment of this disease and various other ailments due to their content of therapeutically active phytochemicals (Jacob & Narendhirakannan, 2019). Indeed, the World Health Organization (WHO) has reported that about 80% of the population in developing countries depends on traditional medicinal plant treatments for their primary health care (WHO 2023). Conventional antidiabetic drugs improve blood glucose control, but they are expensive and often associated with unavoidable side effects plus the inability to totally protect against these diabetic complications. Thus, medicinal plants represent a useful alternative therapeutic strategy in many developing countries, and they may additionally serve as an alternative source for the discovery of antidiabetic agents (Salehi, et al., 2019).

"The World Health Organization has recorded 21,000 plants utilized for medicinal purposes worldwide. More than 400 plant species are used for the treatment of diabetes".(Kumar, et al., 2021). "Asparagus racemosus, also referred to as Shatavari or Satavar, is a member of the Liliaceae family. It is cultivated in the tropical regions of India, the Himalayas, Sri Lanka, Australia, and Africa". (Selvaraj, et al., 2019). Indeed, *A*

racemosus is one of the most valuable medicinal plants, regarded as a "Queen of herbs" in Ayurvedic health system, and has been used in many parts of the world to treat various diseases (Kohli, et al, 2023). For example, extracts of *A racemosus* prepared from the tubers, leaves or fruits have been used for various ailments such as gonorrhoea, piles, rheumatism, coughs, diarrhoea, gastric troubles and headache as well as diabetes (Goyal, et al., 2003). "The roots of *A racemosus* are utilized globally as a traditional herbal medicine because it contains a great number of bioactive compounds, including polyphenols, flavonoids, saponins, and minerals" (Guo, et al 2023).

The major active constituents of *A racemosus* are steroidal saponins (Shatavarins I-IV) and sarsasapogenin which are present in the roots, leaves, and fruits (Kohli, et al, 2023). Indeed, the root extracts have been shown to possess pharmacological efficacies such as antioxidant potential, antimicrobial properties, anti-tumor activity, hepatoprotective effects, and antidiabetic actions (Guo, et al., 2023; Selvaraj, et al., 2019; Kamat, et al., 2000). Root extracts of *A racemosus* also possess phytoestrogenic properties, and may be beneficial for neurodegenerative disorders, as well as antidiarrhoeal, antidyspepsia, adaptogenic, cardioprotective, immunoadjuvant, antibacterial, antifungal and antitussive effects (Bopana & Saxena, 2007). "These effects may be due to constituent alkaloids, flavonoids, tannins, saponins, phenols, terpenes, polysaccharides and steroids. Indeed, *A racemosus* root extract which contained the greatest amount of flavonoids, polyphenols and vitamin C also exhibited the best antioxidant activity" (Guo, et al., 2023). Further, the magnitude of inhibitory effects on α-amylase and α-glucosidase were closely related to the content of phytochemical constituents, particularly flavonoids and triterpenoids.

Although *A racemosus* may be used in the management of T2DM with few or no side effects, evaluation of the effects of extracts of *A racemosus* need to be performed using *in vitro* and *in vivo* models. These can be used to elucidate its mechanism of action to aid

development of medicinal preparations, nutraceuticals or functional foods for diabetes (Vadivelan, et al., 2019). The aim of this study therefore was to build on this previous research to further verify the beneficial antidiabetic actions of ethanolic root extracts of *A racemosus* and to evaluate its effects in type 2 diabetic rats on serum lipids, NEFA, and platelet aggregation which play a key role in the pathogenesis of diabetic complications.

Materials and Methods

Plant material and extract preparation:

Dried *A racemosus* roots were purchased from Ramkrishna Mission, Kolkata, India and botanically authenticated with voucher specimens deposited at the National Herbarium, Bangladesh. Roots were grounded to powder (200 mesh) by using a cyclotec-grinding machine and stored in a well-stopper plastic container. The powder (2 kg) of *A racemosus* root was extracted with 80% ethanol (10 litter x 3; 24 hours, at 22°C) as described previously (Hannan, et al., 2012). The extract was then freeze-dried (330g) and the dry sample was stored in a reagent bottle at 4°C.

Animals

Adult male Long-Evans rats weighing 180 - 220g from Bangladesh Institution of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) Animal House (Dhaka, Bangladesh) were used throughout the study. The animals were maintained on 12-hour light-dark cycle at 21±2°C. A standard pellet diet and water were supplied ad libitum unless otherwise indicated. The overall nutrient composition of the diet was 36.2% carbohydrate, 20.9% protein, 4.4% fat, and 38.5% fibre with the metabolisable energy content of 1.18 MJ/100 g (282 Kcal/100 g). Animals described as fasted were deprived of food for at least twelve hours but were allowed free access to drinking water.

Induction of type 2 diabetes:

"Single intraperitoneal injection of streptozotocin (STZ) to 48-hour-old pups was used to produce a type 2 diabetes rat model in later life. STZ was dissolved in fresh in 0.5M citrate buffer (p H 4.5) and injected immediately at a dose of 90 mg/kg body weight. Experiments were carried out 3 months after injection when the rats weighed approximately 175 - 180 g. Prior to experiments the diabetic status of the rats was checked by blood glucose estimation. The method followed has been described previously" (Bonner-Weir, et al. 1998; Portha , et al., 1989).

Chronic studies

For the chronic study, type 2 diabetic rats were administered plant extract orally (1.25 g/kg body weight) by a metallic tube twice daily for 28 days. The dose of the extract, 1.25 g/kg bw was selected for this study as it showed a significant hypoglycemic effect in the previous acute study. Control rats were administered water (10ml/kg body wt.). All the groups were maintained under similar environmental conditions and were provided with food and water ad libitum throughout the experiment. The effects of ethanol extract of *A. racemosus* on basic metabolic features were tested. After a period of 3 days of adjustment, rats were placed in Nalgene Metabolic Cages (Model 650-0100, USA) and over the next 24 hours, food and water intake, as well as urination and defecation, were measured at intervals of one hour for a total period of 24 hours. The body weight of each rat was recorded every 7th day. Blood samples were collected at the beginning of the experimental period from the tail tips under mild ether anesthesia and the end of the experiment from the abdominal aorta under pentobarbital anesthesia. The serum was separated by centrifugation for the analysis of glucose, fructosamine, total cholesterol, HDL-cholesterol, triglyceride, and non-esterified fatty acids (NEFA). Serum was preserved immediately at –70° C and stored until analyzed.

Estimation of platelet aggregation:

Platelet aggregation analysis was performed on fresh plasma taken from the abdominal aorta under pentobarbital anesthesia by using Chrono Log Lumi aggregometer (Chronolog Corp. Havertown, PA, USA) linked to a potentiometric recorder. The anticoagulant used was sodium citrate (38 g/l, 1 vol. anticoagulant for 9 vol. of blood). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation. Platelet aggregation kinetics in 200 ul of PRP was measured by optical aggregometry using two different doses of ADP (12 and 14μM) as inducers. Platelet aggregation was expressed as a percentage of the PPP transmission value.

Biochemical Analyses

Serum glucose was determined by glucose oxidase (GOD-PAP) based enzymatic colorimetric method, and serum fructosamine by a colorimetric method using kits from Boehringer Mannheim GmbH (Germany). Serum total cholesterol, triglyceride, HDL-cholesterol, and NEFA were estimated by enzymatic colorimetric method using commercial kits from SERA PAK (USA).

Statistical analysis

Results are presented as mean ± SD. Groups of data were compared using unpaired Student's unpaired *t*-test and Mann-Whitney U test where appropriate. Where data were collected over several time points, they were analysed using repeated measures ANOVA, with Bonferroni adjustment to ensure an overall error rate of 5%. One-way ANOVA was performed and pair-wise comparisons to the control group performed using Dunnett's test to preserve an overall error rate of 5%. Differences were considered significant if $p<0.05$.

Results

Chronic effects of A racemosus on serum glucose, fructosamine, lipids, and platelet aggregation

Ethanol extract of *A racemosus* significantly lowered serum glucose ($p<0.01$) and fructosamine levels ($p<0.05$) by 21% and 11% respectively after 28 days (Fig 1). Total cholesterol ($p<0.05$), triglyceride ($p<0.05$), and NEFA ($p<0.01$) levels were also significantly decreased by 9%, 16% and 38% respectively (Fig 2, Fig 3) but HDL cholesterol was unchanged (Fig 2). *A racemosus* also lowered platelet aggregation significantly ($p<0.05$) by 18%, (Fig 3).

Effects of A racemosus on body function and weight

In the 24-hour study, no significant effects of the ***A racemosus*** extract were found on food and water intake, stool formation, water content of stools, and urine formation compared to the control group (Fig 4a, Fig 4b). Neither did the ethanol extract have any effect on body weight or body weight gain during the 28-day study. Indeed, the body weight increased in all rats from the initial to the final study day (Fig 5).

Discussion

Previous studies with an ethanolic extract of *A racemosus* at doses of 100 and 250 mg/kg daily for 4 weeks demonstrated significantly decreased plasma glucose, creatinine, urea nitrogen, total cholesterol and triglyceride levels of severely diabetic rats made insulin-deficient by a single large dose of streptozotocin (Somania, et al., 2012). Other research has demonstrated that administration of *A racemosus* to type 2 diabetic rats for 28 days decreased serum glucose, increased pancreatic insulin, plasma insulin, liver glycogen, and total oxidant status. These effects were associated with significant inhibition of glucose absorption during in situ gut perfusion. Further, in support of positive effect on insulin

sensitivity, the extract was also shown to enhance insulin-mediated glucose transport cultured 3T3-L1 adipocytes (Hannan, et al., 2012) (Hannan, et al., 2007).

Lipid abnormalities are secondary to hyperglycemia and thus one of the major objectives in the management of diabetes is the correction of dyslipidaemia (R. Kenneth & MD Feingold, 2023). The present study investigated the chronic effects of *A racemosus* on the serum lipids and glucose as well as platelet aggregation status in type 2 diabetic rats. Thus, as well as confirming previous observations on the antihyperglyaemic action of the ethanol extract, the present 28-day experiment with *A racemosus* revealed a clear ability to lower total cholesterol and triglyceride. Since previous investigations showed that the extract increased insulin production, this suggests that raised insulin levels might be responsible for the observed decrease of triglycerides mediated by activation of enzyme lipoprotein lipase (Hannan, et al, 2012).

This observation of a hypolipidaemic action of the *A racemosus* root extract is important because an abnormal serum lipid profile, particularly elevated triglycerides and low high-density lipoprotein cholesterol (HDL-C), is commonly seen in patients with type 2 diabetes at risk of cardiovascular disease (Boden W E, 2000). In such subjects, increased plasma free fatty acid (FFA) levels induce peripheral insulin resistance and promote hyperglycaemia. Indeed, it is possible that NEFA may influence platelet aggregation and vascular changes by accelerating the rate of prostacyclin in plasma. Thus, by lowering NEFA levels the root extract might have prompted the decrease in platelet aggregation (Sobczak, et al, 2019).

It is important to note that in the 28-day chronic study, there was no significant change in the body weight of the *A racemosus* group compared to the control group. Also, in the acute metabolic experiment, the extract neither changed food and water intake, stool formation, water content of the stools nor urine volume as compared to control rats. These findings suggest that the extract of *A racemosus* root is well tolerated and lacks adverse effects which

would otherwise alter these various parameters as well as the behaviour of the rats (Hannan, et al., 2012).

Conclusion

we have shown that ethanol extract of *A racemosus* root counters hyperglycaemia and exerts beneficial effects to correct dyslipidemia and platelet aggregation in T2DM rats. *A racemosus* may therefore be a useful therapeutic adjunct and identification of active principle(s) may provide an opportunity to develop a new agent for the treatment of diabetes complications.

Ethical Approval

All authors hereby declare that 'Principles of laboratory animal care' (NIH publication No. 85-23, revised 1985) were followed, as well as the UK Animals (Scientific Procedures0 Act 1986 and EU Directive 2010/63 EU for animal experiments. All experiments were examined and approved by the appropriate ethics committee.

Disclaimer (Artificial intelligence):

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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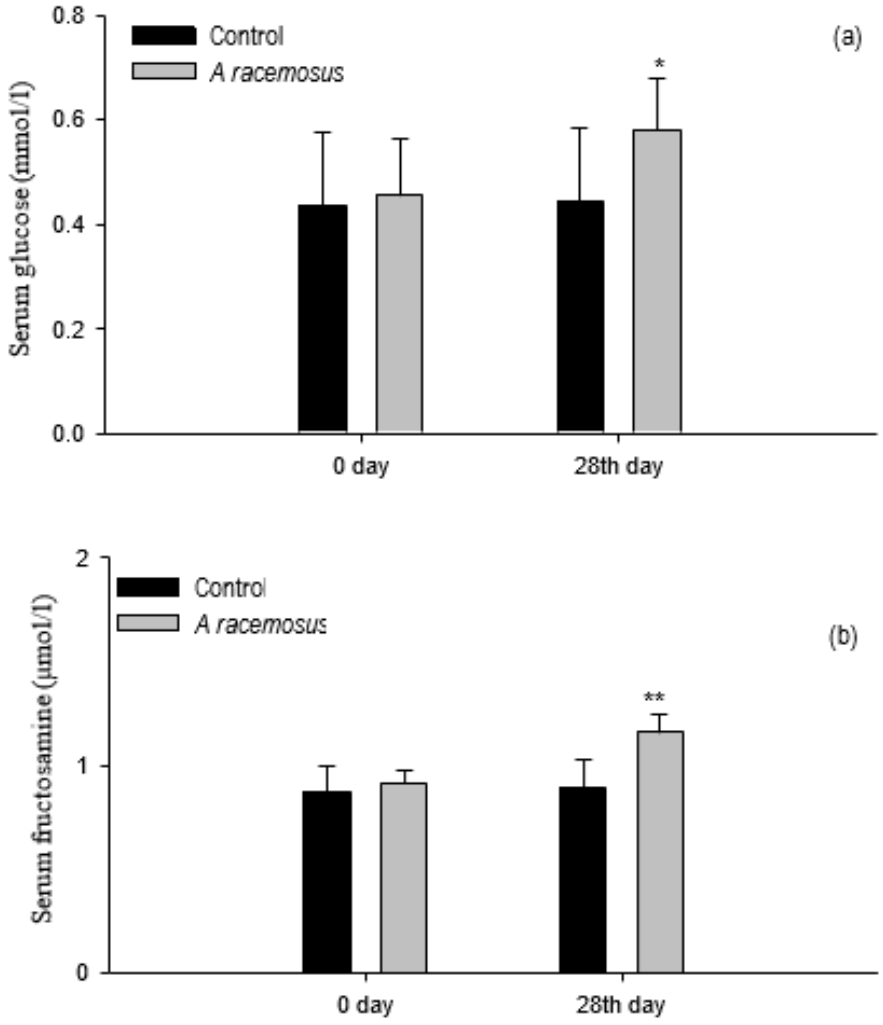

Fig 1: Effects of ethanol extract of *A racemosus* on serum levels of glucose (a) and fructosamine (b) in type 2 rats during 28 days of feeding.

Results are mean±SD (n=12). *p<0.05 compared to type 2 diabetic control rats. Rats were given twice orally for 28 days with ethanol extract of *A racemosus* at a dose of 1.25g/Kg body weight. (a) Serum glucose, (b) Serum fructosamine. Significances are derived from repeated measures ANOVA and adjusted using a Bonferroni correction.

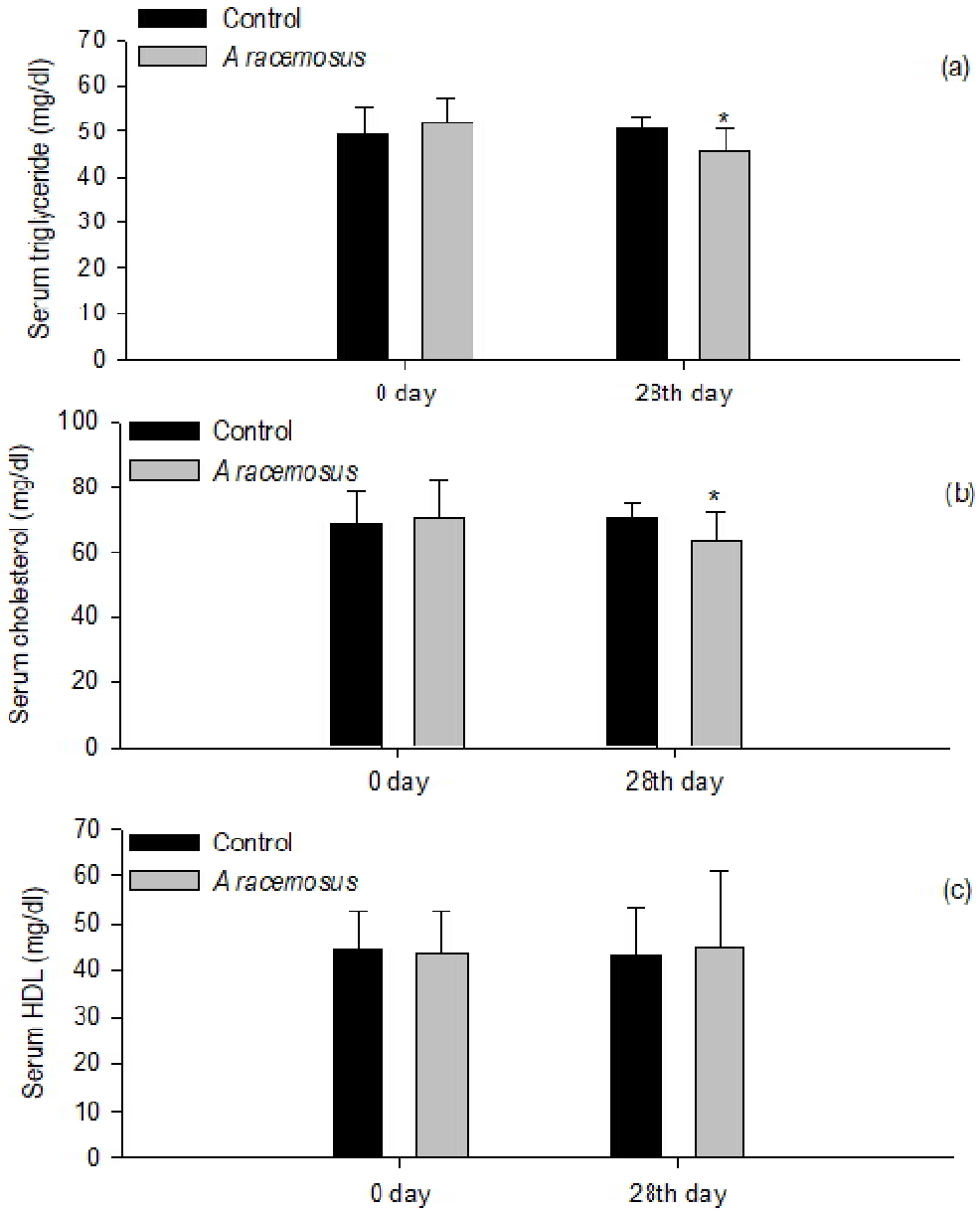

Fig 2: Effects of ethanol extract of *A racemosus* on serum levels of triglyceride (a), cholesterol (b), and HDL (c) in type 2 rats after 28 days of feeding.

Results are mean±SD (n=12). *p<0.05 compared to type 2 diabetic control rats. Rats were given twice orally for 28 days with ethanol extract of *A racemosus* at a dose of 1.25g/Kg body weight. (a) Serum triglyceride, (b) Serum cholesterol, (c) serum HDL.

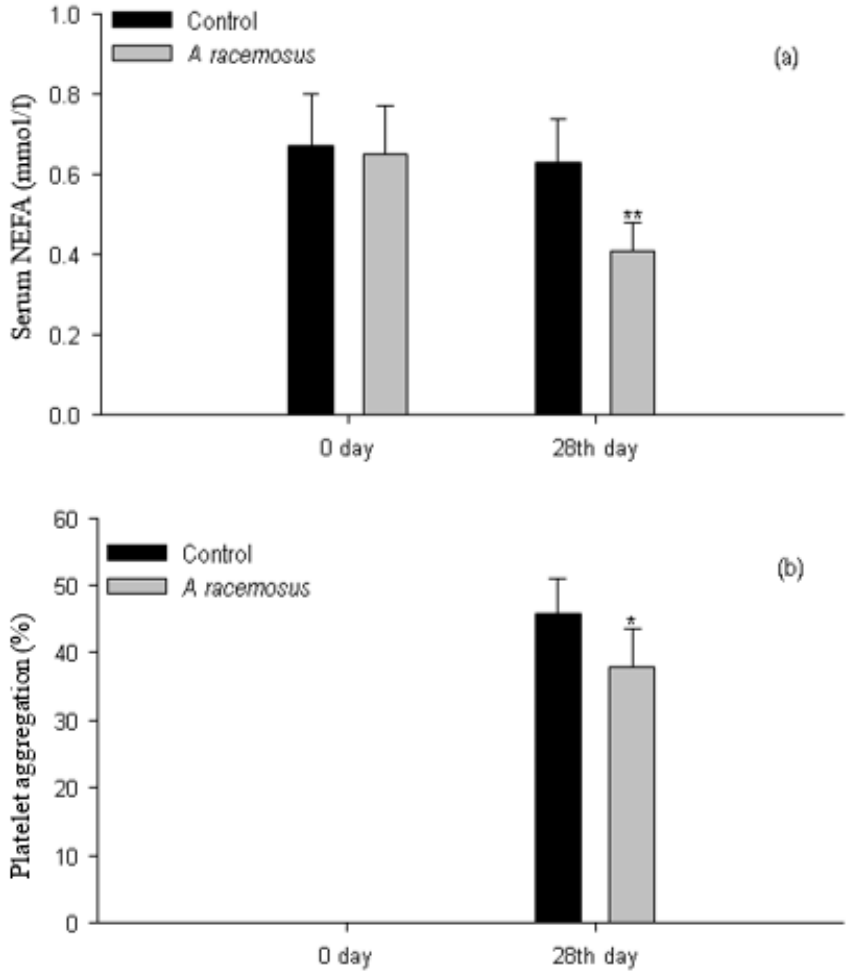

Fig 3: Chronic effects of ethanol extract of *A racemosus* on serum levels of NEFA (a) and platelet aggregation (b) in type 2 diabetic rats.

Results are mean±SD (n=12). **p<0.01, ***p<0.001 compared to type 2 diabetic control rats. Rats were given twice orally for 28 days with ethanol extract of *A racemosus* at a dose of 1.25g/Kg body weight. (a) Serum NEFA, (b) Platelet aggregation.

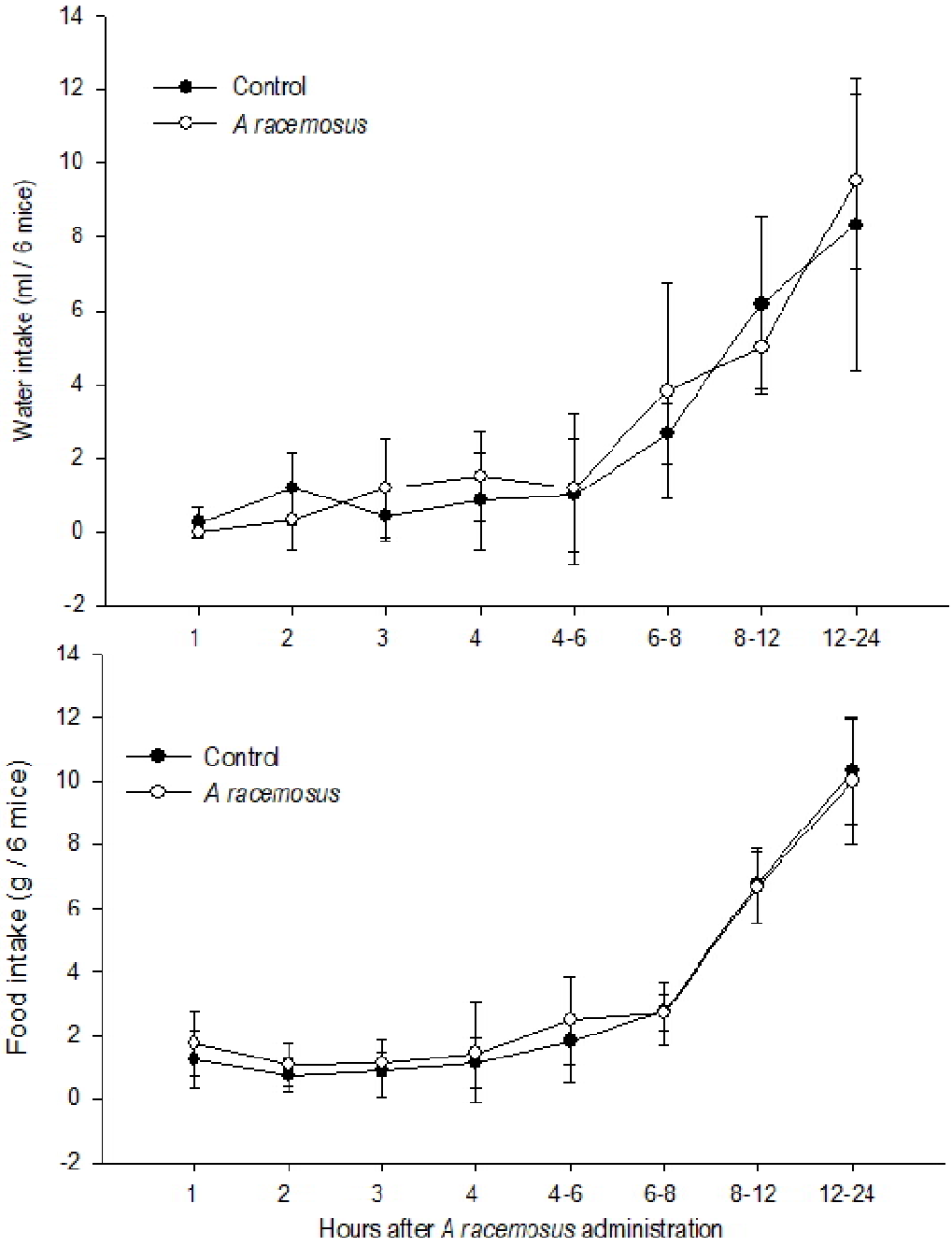

Fig 4: Acute effects of ethanol extract of *A racemosus* on food and water intake of normal mice.

Results are mean±SD (n=6). Mice were given once orally ethanol extract of *A racemosus* at a dose of 1.25g/kg body weight. Significances are derived from repeated measures ANOVA and adjusted using a Bonferroni correction.

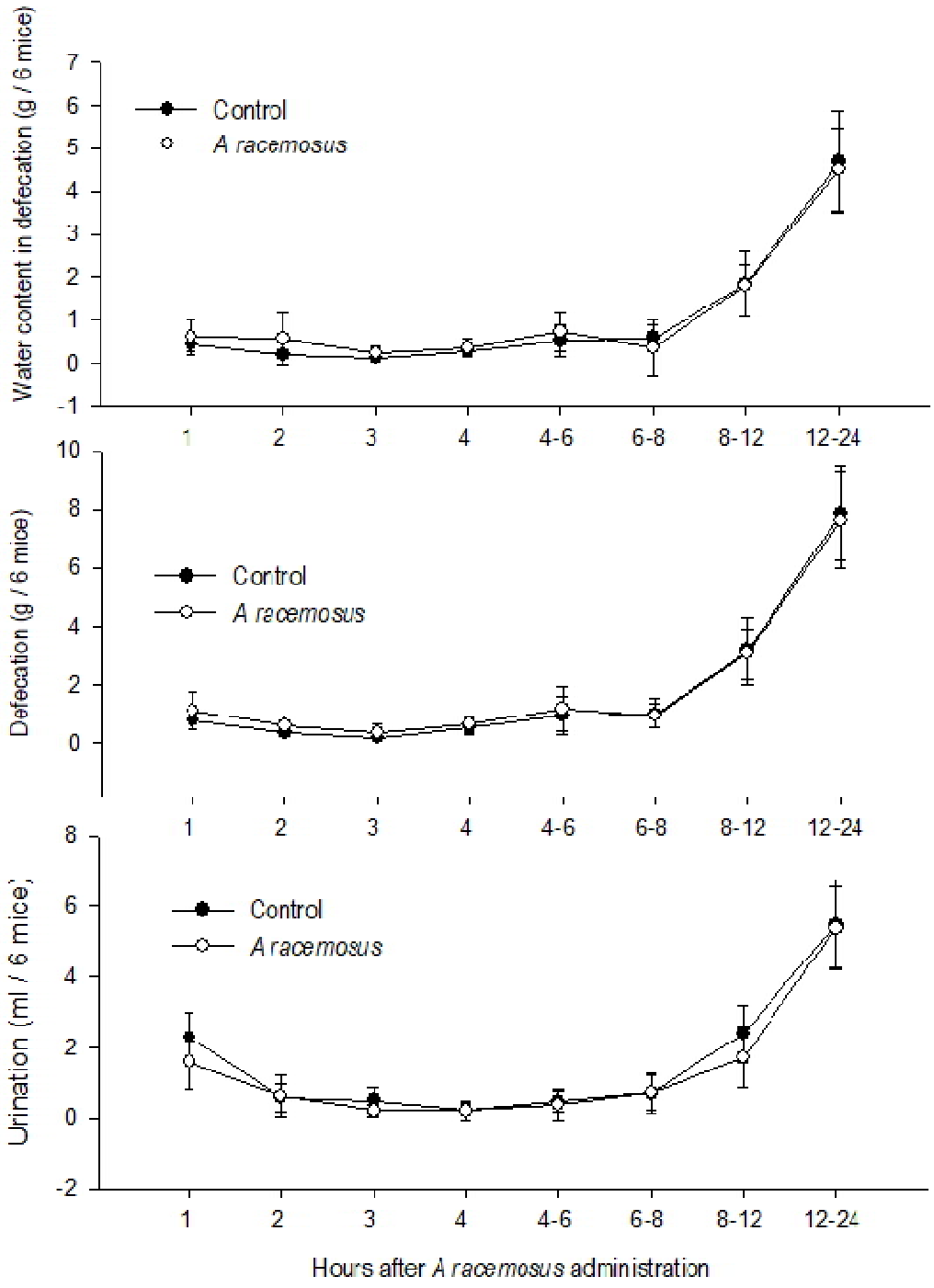

Fig 5: Acute effects of ethanol extract of *A racemosus* on urination, defecation, and water content of defecation of normal mice.

Results are mean±SD (n=6). Mice were given once orally ethanol extract of *A racemosus* at a dose of 1.25g/kg body weight. Significances are derived from repeated measures ANOVA and adjusted using a Bonferroni correction.

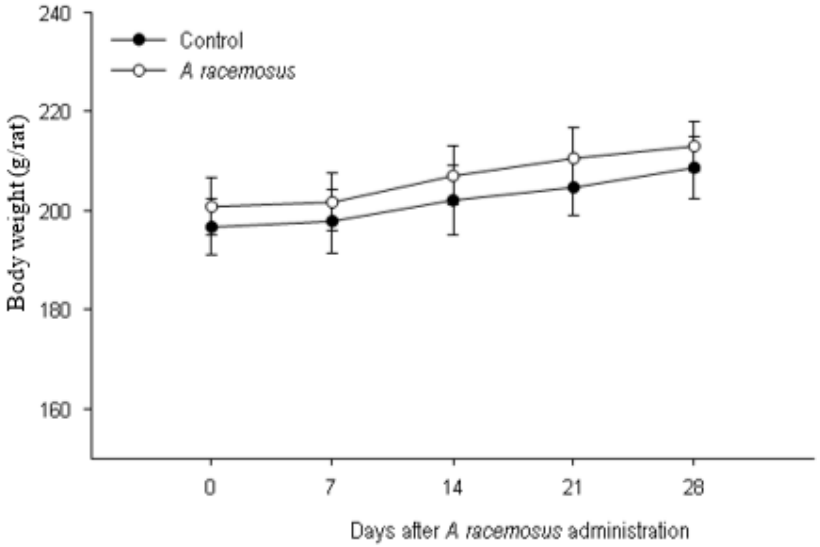

Fig 6: Chronic effects of ethanol extract of *A racemosus* on body weight of type 2 diabetic rats.

Results are mean±SD (n=12). Rats were given twice orally for 28 days with ethanol extract of *A racemosus* at a dose of 1.25g/kg body weight. Significances are derived from repeated measures ANOVA and adjusted using a Bonferroni correction.