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DECREASED AMNIOTIC FLUID GLUCOSE LEVEL AND WEIGHT GAIN OF CHICK EMBRYO BY ALCOHOLIC EXTRACT OF *GYNURA PROCUMBENS*

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ABSTRACT

Patients suffering from diabetes mellitus are gradually increasing throughout India. The burden was 31 million in the year of 2000 and within 2030 it is expected to rise more than 100 per cent to reach 79.4 million whereas the global load is expected to reach 360 million or more by that time. This disease is associated with major complications like ischemic heart disease, retinopathy, nephropathy, neuropathy etc. So there is a scope to search newer drugs to control the disease effectively. *Gynura procumbens* which belongs to the family Asteraceae has been claimed to have anti-hyperglycaemic, anti-cancer and anti-inflammatory activities. In this experiment amniotic glucose lowering activity of *Gynura procumbence* leaf extract was tested in alloxan treated chick embryo. The results of this experiment revealed that the leaf extract is not only capable of reducing the amniotic fluid glucose level, but it has got also capability of increasing body weight of the chick embryo. Thus this plant extract may prove to be an ideal medicine for underweight diabetes mellitus patients in future days to come.

KEYWORD: *Gynura procumbens*, chick embryo, glucose lowering activity, alloxan, weight gain.

INTRODUCTION

Gynura procumbens also known as" longevity spinach" is habitat in China, South East Asia and Africa. The plant is 3.5-8cm long and 3-3.5cm in width.[1] In many parts of Bangladesh,

India, and Thailand it is commonly known as "diabetes plant" due to its use in reducing blood glucose level. Leaf extract of *G. procumbens* have shown remarkable efficacy against hyperglycaemia by improving sensitivity towards insulin and by interfering with the pathway of gluconeogenesis in liver.[2-4] There are also other names of this plant- thus in Malay it is known as "SamburgNyawa" which means "prolongation of life" where as in China it is known as "Bai Bing Cao" which means "100 ailments".[5] Though it is commonly known as "diabetes Plant" but it is systematically and topically used in various other diseases.[6] In Indonesia it is commonly used in various kidney diseases and in Vietnam it is used in pyrexia, while in Thailand, it is used as anti-viral and anti-inflammatory agent.[7] The beneficial effect of *G. procumbens* is due to its variety of bioactive compounds present in the plant. Taxonomically the plant *G. procumbens* (Fig.1) belongs to angiosperms, order – Asterales, Family – Asteraceae, Tribe- Senecioneae, Genus – *Gynura*.

Fig 1: *Gprocumbens* plant

Active compounds

Several experiment have shown its activity due to presence of many bioactive compounds (Fig. 2) like flavonoids, saponins, tanins, sterol glycosides etc.[8-10] Studies has also shown the presence of two highly potential anti-oxidant compound in leaves extracts of *G. procumbens*, they are kaempferol-3-O rutinoside and astragalin.[11] Many of these compounds were also found to have some other beneficial activities like anti-inflammatory[12,13], anti allergic, anti cancerous[14] etc.

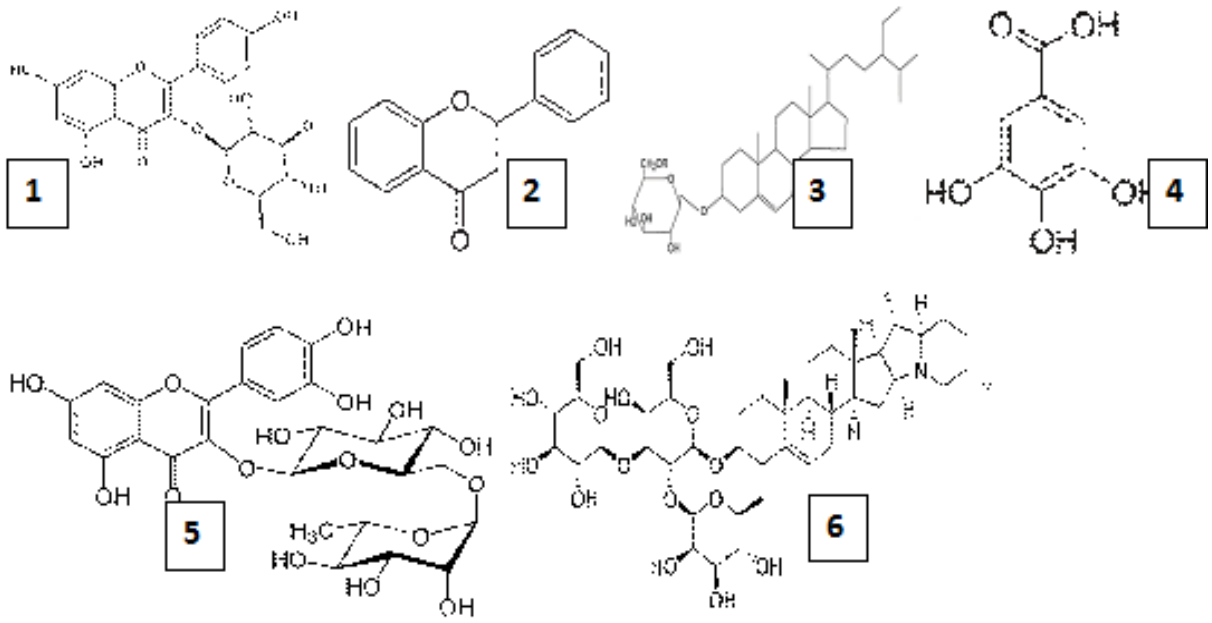


Fig2 : Showing structures of flavonoids (1), astragalin (2), sterol glucosides (3), tannin (4), rutin (5), saponin(6).

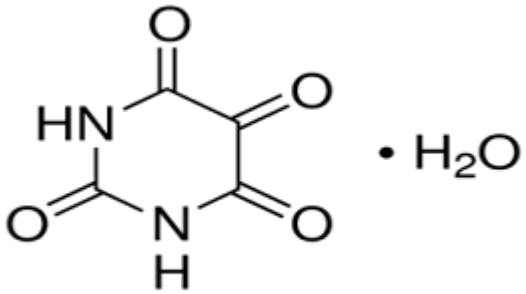


Fig 3: Showing Structure of Alloxan($C_4H_2N_2O_4$)

Hyperglycaemic effect of alloxan

Alloxan is a chemical compound having ability to induce diabetes when administered in an animal commonly used to evaluate anti-hyperglycaemic properties of different type of drugs. Alloxan is chemically known as 5,5 di hydroxyl pyrimidine-2,4,6 trione, which is an organic compound derived from Urea and a potent cytotoxic glucose analogue having chemical formula $C_4H_2N_2O_4$ and its molecular weight is 142.06.[15] Alloxan induces insulin dependent type of diabetes which occurs as a result of injection of single or multiple doses of the chemical through different routes.[16,17] The change it causes in the organism mainly by two ways selective inhibition of glucose stimulated insulin secretion and by inducing production of ROS which ultimately causes beta Cell destruction leading to develop

hyperglycaemia.[16,18] Alloxan shares a structural resemblance with glucose.[19] Basically it is a toxic beta glucose being hydrophilic in nature and it exists as alloxan monohydrate in aqueous solution. Because of structural resemblance with glucose[20], alloxan moves from plasma membrane to cytosol of pancreatic beta cell via GLUT2 receptor.[21] Peculiarity of alloxan is that it does not interfere with the activity of GLUT2 receptor which actually promotes its uptake by β-Cells causing selective bio accumulation and increased toxicity inside the cell leading to destruction.[22,23]

Glucose level in amniotic fluid of chick embryo

It was Aristotle who first started working with chick embryo in 350 BC, and dissected it for the first time. After that, several path breaking experiments performed on chick embryo. Many experiments done to estimate the change in sugar/glucose level of amniotic fluid of chick embryo. However, most of these studies estimated sugar level which contains mainly fructose not glucose. On an average the sugar.[24, 25] levels which contains mainly fructose usually remains unperturbed from 8-day to 19- day old embryo (133-148 mg/dL) and then there is usually an upsurge of the sugar level (177-195 mg/dL) till hatching. On the other hand glucose level usually varies from 10-40 mg/dL.

MATERIALS AND METHODS

G. procumbens leaf: Initially saplings of *G. procumbens* were taken from a plant supplier and then it was further confirmed by a Botanist. Then the plant was taken from the nursery to the Lab and it was placed under well airy place and nurtured properly with water for almost 15 days.

Fresh (>48 h) fertilised chicken egg: Chicken eggs were collected from state poultry farm of Tollygunj, Kolkata. They were taken to the laboratory and carefully placed inside of an incubator for further use.

Alloxan: As a source of alloxan we used an homeopathic preparation of alloxan (6CH) containing approximately 1 pg/mL of alloxan.

Preparation of extract: Fresh green leaves of *G. procumbens* were washed with sterile distilled water and were allowed to dry in an incubator. The leaves were then chopped with a sterile knife on a chopper board. Chopped leaves were then smashed in a porcelain mortar with pestle. Then the pulp were taken within a linen cloth and squeezed to extract the juice

from the pulp into a sterile clean beaker. Then with the help of a micropipette the juice was transferred in a sterile brown glass vial with air tight screw. After this equal quantity of ethyl alcohol was added in the vial, and after proper labelling it was placed in a dark room for 8 days and kept undisturbed following guidelines from Homoeopathic Pharmacopoeia of India, 1971. After 8 days the fluid was taken out and placed in another small sterile vial after collecting the supernatant following centrifugation at about 1200 rpm for 3 minutes.

The final experiment and amniotic fluid collection: After incubating the eggs at 37^{O}C for 10 days the eggs were taken out of the incubator and washed with alcohol. After that the eggs were observed inside a dark room with a light source to observe the blood vessels. On the opposite side of the vascular part a small puncture was done with a sterile needle through which different materials were inoculated after numbering and dividing the eggs in three groups. Group A was with alloxan (50µL), group B was with both alloxan (50µL) and leaf extract (50µL), and group P was only with plant extract (50µL). After inoculation the eggs were then incubated for 18 h and after cutting the egg shells the amniotic fluids were collected in different vials. All the embryos were macroscopically observed and their body weights were recorded in a balance.

Amniotic fluid glucose estimation: This was done in a semiautomated biochemistry analyzer (Microlab 300) utilizing Human glucose estimation kits (Human Diagnostic company, Germany).

RESULTS

Macroscopic examination of the embryo (Fig 4) showed mostly degenerated and atrophied embryo in group A with an average body weight of 5.3 g (Mean± SD±SEM = 5.3±2.26±1.6 g) , Group B embryos were healthy with average body weight was 5.55 g (Mean±SD±SEM = 5.55±1.20±0.85 g) and embryos of group P were also of good health with an average weight of 7.70 g (Mean±SD±SEM = 7.70±0.28±0.20 g). Vascularization was also maximum in group P (Fig. 5).

Amniotic glucose levels (Graph 1) were 56.5 mg/dL (Mean±SD±SEM = 56.5±57.2±40.5 mg/dL), 0 mg/dL, 0.65 mg/dL (Mean± SD±SEM = 0.65±0.91±0.65 mg/dL) in group A, group B and group P respectively.

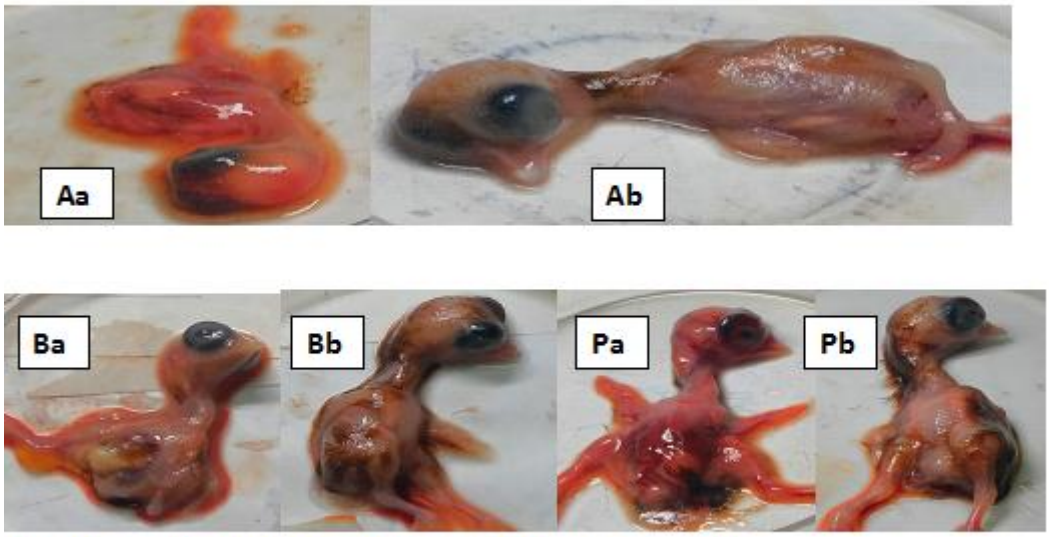
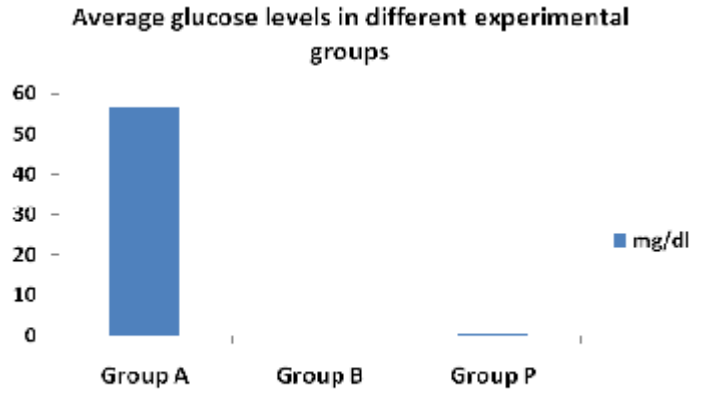


Fig 4: Showing representative embryos of different experimental groups.

Fig. 5 : Vascularization in different groups.



Graph 1: Showing glucose levels in different groups.

DISCUSSION

The therapeutic activity of plant species are mostly due to their active constituents and bioactive chemical compounds which plays the key role.[26,27] Variety of compounds till date have found in experiments with potential therapeutic effects on diabetes mellitus like maintaining glucose homeostasis[28], promoting insulin secretion[29,30], increasing uptake of

glucose by muscles[31,32] and other antioxidant properties etc. Singh et al[33] in a simple way did their experiment on streptozoin induced diabetic rats with leaf extract of *Catharanthus roseus* and showed its therapeutic potential to combat the diabetes.

In this experiment chick embryo is used as model for the purpose of study and they were injected with alloxan 6 CH to induce increased glucose level artificially. The experiment done on the 12th day after fertilization as Liheng Shi et al[34] have shown in their experiment the day having highest and safest for study in chick embryo model. The *G. procumbens* leaf extracts are enriched with flavonoids, tannin, astragalin having potential to act against increased glucose level in the body fluid. Flavonoids are potential alpha amylase inhibitor and intermediary biosynthetic compound trans-chalone which shows the anti-hyperglycaemic property and flavonoids can also combat dyslipidaemia in type 1 diabetes as observed in rat by Najafian et al.[35] Further studies were done by Ma et al[36] where they have proposed the possible mechanism of this glycaemic control linked with the upregulation of hepatic superoxide dismutase activity, reduced hepatic malondialdehyde and increased GLUT-4 expression in skeletal muscles. Saponin[37] and tannin[38] has also exhibited their anti-diabetic property as proved in different experiments. It was reported by Hamid et al[2] the insulin secretion simulated by *G. procumbens* leaf extract whereas on the contrary when the leaf extracts are added to clonal pancreatic cells, it showed no response as shown by Hassan et al.[39] This contradiction of the above two experiments somehow gives an indication of different levels of responses in different cell lines due to various reasons. Lee et al[40] in their experiment shown no change in plasma glucose level in diabetic rat which may signify some extra pancreatic effect of *G. procumbens* extract by which it alleviates hyperglycaemia. The extract somehow interferes with hepatic glucose metabolism which was demonstrated to cause phosphorylation with inactivation of glycogen synthetase kinase 3 suggesting that its active or passive involvement in the Insulin signalling pathway.[41] So it may indicate that *G. procumbens* leaf extract reduces endogenous insulin production but increases Glucose uptake which helps to establish the body weight gaining property of the extract.

CONCLUSION

In this experiment it appears that *G. procumbence* leaf extract is capable of reducing amniotic fluid glucose level when challenged in alloxan treated chick embryo. It not only reduces glucose level in the fluid but it also increases glucose uptake by liver and adipocytes resulting weight gain of the embryo.

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