Study of protective effects of glycyrrhizin in lithium chloride induced Nephrogenic Diabetes Insipidus in rats

Shivalinge Gowda KP* and Manjunath Prasad MK
Dept. of Pharmacology, PES College of Pharmacy, Hanumanthanagara, Bangalore-50

Abstract
The nephrogenic diabetes insipidus activity of isolated glycyrrhizinic acid was evaluated in lithium chloride induced animal models. NDI was induced by lithium chloride (60mmol/kg of food). Glycyrrhizinic acid at different doses (100mg/kg B.w, 200mg/kg B.w) were administered orally to experimental animals. Biochemical serum urea, serum creatinine, serum sodium, urine sodium, creatinine and urea were estimated, the histopathological examination of kidney sections. The results of study reveal that there are increased levels of these serum and urea biomarkers, when compared with the normal rats and marked protective changes in histopathological sections of the kidneys were observed in rats and mice treated with different doses of Glycyrrhizinic acid with NDI inducing agents. The NDI was induced in rats with the administration of Lithium chloride (60mmol/kg of food) for 30 days. From the results of this study it is concluded that the different doses of Glycyrrhizinic acid possesses protection effects.

Key words: Glycyrrhizinic acid, lithium chloride, Nephrogenic diabetes insipidus

Introduction:
Diabetes Insipidus: Excessive urination and extreme thirst as a result of inadequate output of the pituitary hormone ADH (antidiuretic hormone, also called vasopressin) or the lack of the normal response by the kidney to ADH. There are four types of Diabetes Insipidus (DI). NDI stands for Nephrogenic Diabetes Insipidus. It is a medical condition in which the kidney is unable to conserve water. The main symptoms are excreting large amounts of dilute urine (“polyuria”), and drinking large amounts of water (“polydipsia”) to make up for the water lost in the urine. NDI is a form of diabetes insipidus due primarily to pathology of the kidney. Kidneys are the primary organs of the urinary system, which purifies the blood by removing wastes from it and excreting them from the body in urine. Every day, the kidneys filter about 45 gal (180 L) of blood, about four times as much as the amount that passes through any other organ. Because of this high volume, the kidneys are more often exposed to toxic substances in the blood and are very vulnerable to injury from those sources in

*Corresponding Author
Shivalinge Gowda KP
contrast to central/ neurogenic diabetes insipidus, which is caused by insufficient levels of anti diuretic hormone (ADH)/ Arginine Vasopressin (AVP). Nephrogenic diabetes insipidus is caused by an improper response of the kidney to ADH, leading to a decrease in the ability of the kidney to concentrate the urine by removing free water there are two categories of nephrogenic diabetes insipidus: inherited and acquired. Drugs to Use for Nephrogenic Diabetes Insipidus Nephrogenic diabetes insipidus are a disease of the kidney that impairs water conservation. The kidney is unable to respond to the anti diuretic hormone arginine vasopressin. According to the Nephrogenic Diabetes Insipidus Foundation NDI is a well-established complication of long-term lithium therapy. Lithium-induced NDI is a serious condition that can result from taking the medication lithium for treatment of bipolar disorder or other psychiatric conditions.

Material & Methods: Animal was used are male Wister rats weighing around 150-200g the Number of animal in each group: 06 Wister male rats (150-200 g) were purchased from Raghavendra Enterprises, Bangalore. Which were maintained in the animal house of PES College of Pharmacy, Bangalore. Which were maintained in the animal house of PES College of Pharmacy, Bangalore for experimental purpose. All the animals were acclimatized for seven days under standard husbandry conditions, i.e. room temperature of 25 ± 1°C; relative humidity 45-55% and 12:12h light/ dark cycle. The animals were given free access to standard rat pellet (Amruth Animal Feeds Pvt. Ltd, Bangalore, India), with water ad libitum under hygienic conditions. Each group had separate set of animals and care was taken to ensure that animals used in an experiment were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize non-specific stress if any. The approval of the Institutional Animal Ethical Committee (IAEC) of P.E.S College of Pharmacy Bangalore (Karnataka) was taken prior to the experimentation. All the protocols and the experiments were conducted in compliance to ethical principles and guide bar lines as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Govt. of India, New Delhi. A dried root of licorice powder was procured from Amruth Kesari pharma depot Pvt Ltd, Bangalore, Karnataka. And then it is subjected for the isolation of glycyrrhizinic acid from licorice powder by acid precipitating method

Male wistar rats will be divided into five groups of six animals each. Group I: Normal which receives only normal diet and vehicle throughout the course. Group II Control (Glycyrrhizinic acid 200mg/kg bw through oral route ) + Normal diet for thirty days. Group III: Lithium diet feed 60mmol/kg of food. + Vehicle through oral route for thirty days. Group IV: Glycyrrhizinic acid 100mg/kg, bw + Lithium diet feed 60mmol/kg of food for thirty days. Group V: Glycyrrhizinic acid 200mg/kg,bw + Lithium diet feed 60mmol/kg of food for thirty days.
Histopathological examinations of kidney sections:

Renal tissues were collected after animal culling, fixed in 10% formalin, processed routinely, and embedded in paraffin. Paraffin-embedded tissues were sectioned at 5 µm thickness and stained with haematoxylin and eosin for histological assessment. Six coded slides from each group were examined by a pathologist in a blinded manner.

Statistical analysis:

The values were expressed as Mean ± SEM data was analyzed using one-way ANOVA followed by Tukey test.

Results and discussions: The significant increase in the urine and serum biomarkers like sodium, urea, creatinine was observed in lithium diet rats when it is compared to the normal diet rats. The lithium diet rats treated with glycyrrhizin have shown significant decrease in the urinary sodium levels when it is compared with lithium diet rats.

Figure 1: The significant increase in the urinary sodium, urea, creatinine was observed in lithium diet rats when it is compared to the normal diet rats.

Histopathological studies:

The glycyrrhizinic acid treated rats showed the tubular cell necrosis when this compared with control group where there was not such type of lesion. Treatment with different doses of Glycyrrhizinic acid (100 and 200 mg/kg b.w.p.o.) for 30 days significantly improved when compared with gentamicin alone treated rats. The treatment of glycyrrhizinic acid and higher dose was showed the protective action compared to lower dose.

Figure 2: The significant increase in the urinary sodium, urea, creatinine was observed in lithium diet rats when it is compared to the normal diet rats. The lithium diet rats treated with glycyrrhizin (100 and 200 mg/kg b.w.p.o.) have shown significant decrease in the urinary sodium levels when it is compared with lithium diet rats.
Nephrogenic diabetes insipidus is a well-established complication of long-term lithium therapy. Lithium induces irreversible reduction of urinary-concentrating capacity partly reversible reduction in the glomerular filtration rate. Patients are characterized with polydipsia, polyuria. Thus a model for lithium chloride induced nephrogenic diabetes insipidus as a side effect which could clearly reveal the loss of urine License is a plant, which has anti diuretic activity as it contains glycyrrhizinic acid according to clinical pharmacology. Since glycyrrhizin including glycyrrhizinic acid found in roots of licorice. The present study was carried out to evaluate protective effects of glycyrrhizin extracts of licorice by using suitable animal models. The effect of glycyrrhizinic acid extract on biochemical and histopathological parameters are studied and presented in the previous sections. In the lithium chloride induced, nephrogenic diabetes insipidus, the rats are administered with lithium diet have shown significant increased levels of urinary sodium, creatinine, urea, and also shows the significant increase in serum sodium, creatinine, urea when compared to normal rats administered with normal diet. The rats administered with lithium diet along with glycyrrhizinic acid have shown significant decrease in urine & serum biomarkers when compared to the lithium diet administered rats. Further the SEM studies also supports for its anti-diuretic activity in which glycyrrhizinic acid extracts shows protective effects against lithium carbonate induced NDI that may be due to its like antidiuretic activity. Thus from the present study, it can be justified that, the glycyrrhizinic acid (200mg/kg and 100mg/kg) and lower dose
of AQ extract (100mg/kg) that has shown better activity; which is indicated by improvement in the biochemical and biomechanical parameters higher dose of AQ extract (200mg/kg) has shown better anti-diuretic activity in comparison to the other treated groups. Though all the parameters were not fully justifiable, still it can be claim that glycyrrhizinic acid posses the anti-diuretic activity.

**Conclusion:**
This study demonstrates the beneficial protective effects of orally administrated AQ extract of glycyrrhizinic acid in nephrogenic diabetic insipidus induced rats. From the present study it is evident that extracts of *Glycyrrhizinic acid* could be considered as a natural alternative for the prevention and treatment of lithium induced NDI. Test parameters such as, Urine & serum Bio-markers could be much useful in evaluating the efficacy of the extracts by estimating the parameters. Further studies are required to determine the active components that are responsible for its anti-diuretic activity. Followed by, testing the isolated active principle *in vitro* and in vivo are desirable to understand and establish its mechanism of action.

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