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Effects of *Inula racemosa* root and *Gymnema sylvestre* leaf extracts in the regulation of corticosteroid induced diabetes mellitus: involvement of thyroid hormones

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The efficacy of *Inula racemosa* (root) and *Gymnema sylvestre* (leaf) extracts either alone or in combination was evaluated in the amelioration of corticosteroid-induced hyperglycaemia in mice. Simultaneously thyroid hormone levels were estimated by radio-immunoassay (RIA) in order to ascertain whether the effects are mediated through thyroid hormones or not. While the corticosteroid (dexamethasone) administration increased the serum glucose concentration, it decreased serum concentrations of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). Administration of the two plant extracts either alone or in combination decreased the serum glucose concentration in dexamethasone induced hyperglycaemic animals. However, the administration of *Inula racemosa* and *Gymnema sylvestre* extracts in combination proved to be more effective than the individual extracts. These effects were comparable to a standard corticosteroid-inhibiting drug, ketoconazole. As no marked changes in thyroid hormone concentrations were observed by the administration of any of the plant extracts in dexamethasone treated animals, it is further suggested that these plant extracts may not prove to be effective in thyroid hormone mediated type II diabetes, but for steroid induced diabetes.

1. Introduction

There are two types of diabetes mellitus, insulin dependent (IDDM, type-I) and non-insulin dependent (NIDDM, type-II). These two forms account for about 95% of total diabetes cases reported worldwide. However, four additional types of diabetes were recognized [1]. These include malnutrition-related diabetes mellitus (MRDM), gestational diabetes (GD), impaired glucose tolerance diabetes (IGT) and diabetes associated with other conditions such as pancreatic disease, diseases of hormonal etiology, drug or chemical induced or those as a result of abnormalities of insulin or its receptors or certain genetic syndromes. Corticosteroid diabetes is hormone related diabetes.

While ample work has been done on the herbal regulation of two main types of diabetes viz. IDDM and NIDDM, almost no report is available on the herbal regulation of diabetes associated with hormonal disorders, mainly steroid diabetes, which results from an excess increase in the level of glucocorticoids within the body [2].

The leaves of *Gymnema sylvestre* (Asclepidaceae) and the roots of *Inula racemosa* (Compositae) commonly known as Gurmar and Pushkarmool in India are known for their anti-inflammatory, expectorant, cardio tonic, digestive and diuretic properties [3]. However, no information is available on their regulatory effects on corticosteroid-induced diabetes mellitus. Therefore, the primary aim of the present investigation was to reveal the possible regulation of corticosteroid-induced hyperglycaemia by these two plant extracts. Investigations have also been made in order to find out whether a combination of the two extracts can be more effective or not.

2. Investigations, results and discussion

In the present investigation the effects of *Inula racemosa* root and *Gymnema sylvestre* leaf extracts either alone or in combination have been studied in corticosteroid induced hyperglycaemic adult mice of either sex. Simultaneously changes in total serum thyroid hormone concentrations and in hepatic lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities were also investigated following methods described earlier [4, 5].

Administration of dexamethasone significantly increased serum glucose concentration (Table), as observed earlier [6], with a concomitant decrease in the level of both the thyroid hormones, T_3 and T_4. Interestingly, administration of the plant extracts either alone or in combination to dexamethasone induced hyperglycaemic animals reversed the effect produced by dexamethasone.

Although previous studies on these two plant extracts indicated their hypoglycaemic effects [7, 8] their importance in regulating steroid diabetes was not studied. The present findings for the first time indicated that *Gymnema sylvestre* and *Inula racemosa* are also capable of reducing corticosteroid-induced hyperglycaemia. The inhibition was

Table: Effects of *Inula racemosa* (IR) root and/or *Gymnema sylvestre* (GS) leaf extracts on serum concentrations of glucose (mg/dl), T_3 (ng/ml), T_4 (ng/ml), hepatic LPO, (nM MDA formed/hr/mg protein), SOD (units/mg protein) and CAT (µM of H_2O_2 decomposed/min/mg protein) activities in normal and dexamethasone induced hyperglycaemic mice: a comparison with ketoconazole

Groups	Glucose	T_3	T_4	LPO	SOD	CAT
Control	62.47 ± 3.30	0.71 ± 0.06	29.71 ± 2.04	0.82 ± 0.04	4.75 ± 0.32	54.31 ± 3.04
IR	$48.80^{**} \pm 3.01$	$0.52^{*} \pm 0.05$	$23.42^{*} \pm 1.32$	$0.60^{***} \pm 0.03$	$5.90^{*} \pm 0.42$	61.77 ± 4.14
GS	$43.78^{**} \pm 3.30$	$0.40^{***} \pm 0.04$	$21.14^{**} \pm 0.86$	$0.58^{***} \pm 0.03$	$6.73^{***} \pm 0.35$	59.19 ± 3.65
IR + GS	$46.70^{**} \pm 3.30$	0.54 ± 0.08	$21.92^{**} \pm 0.98$	$0.60^{***} \pm 0.03$	$6.18^{*} \pm 0.46$	61.17 ± 3.48
Ketoconazole (Keto)	$50.60^{*} \pm 3.17$	0.63 ± 0.07	25.61 ± 1.07	0.79 ± 0.01	4.86 ± 0.20	55.61 ± 2.87
Dexamethasone (Dexa)	$88.22^{**} \pm 5.70$	$0.48^{*} \pm 0.07$	$24.71^{*} \pm 0.75$	$0.68^{*} \pm 0.04$	$6.05^{*} \pm 0.45$	59.53 ± 3.93
Dexa + IR	$67.30^{\$\$} \pm 2.47$	0.59 ± 0.09	25.57 ± 0.89	$0.54^{\$} \pm 0.04$	6.38 ± 0.43	61.70 ± 3.98
Dexa + GS	$72.08^{\$} \pm 2.39$	0.88 ± 0.19	28.42 ± 2.39	0.59 ± 0.05	6.55 ± 0.30	70.47 ± 3.31
Dexa + IR + GS	$59.14^{\$\$\$} \pm 3.69$	0.70 ± 0.09	28.71 ± 1.94	0.60 ± 0.05	6.73 ± 0.40	66.47 ± 4.70
Dexa + Keto	80.22 ± 4.07	0.50 ± 0.06	26.05 ± 1.32	0.81 ± 0.09	5.64 ± 0.43	58.75 ± 2.25

Data are mean ± SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared to the respective control values, $ $P < 0.05$; $$ $P < 0.01$; $$$, $P < 0.001$ as compared to the respective dexamethasone treated values

found to be more pronounced when a combination was administered (33%) as compared to the individual plant extracts (18 and 24% respectively). Interestingly, the hypoglycaemic efficacy of the two plant extracts was found to be higher than that of a standard anti-corticoid drug, ketoconazole.

Very often hyperthyroidism is related to hyperglycaemia [9]. However, in the present study when the serum concentrations of thyroid hormones were correlated, it appeared that hypoglycaemic effects of the plant extracts are not mediated through alteration in thyroid hormone concentrations, as total T_3 and T_4 levels were not significantly altered in dexamethasone treated groups by any of the plant extracts, suggesting that these plant extracts may not affect thyroid hormone induced diabetes, but the corticosteroid induced diabetes.

The results on LPO, SOD and CAT revealed no marked alteration in any of the groups indicating that the plant extracts are apparently not toxic in the administered doses. The increased level of glucocorticoids within the body, either due to prolonged therapy in certain diseases, viz. rheumatic fever, ulcerative colitis or disease of adrenal cortex including Cushing's syndrome invariably results in hyperglycaemia [10]. Therefore in the present study dexamethasone induced increase in serum glucose level is obvious. This could be due to an increase in gluconeogenesis and a decrease in tissue glucose consumption by inducing resistance, which in turn could be through the decreasing number of insulin receptors or by intracellular interference of insulin activity [2, 11]. Therefore in the present study, the antihyperglycaemic activity of the plant extracts might have been mediated by increasing the activity of enzymes responsible for glucose uptake and utilization or by decreasing the peripheral insulin resistance.

Whatever may be the mode of action of the plant extracts, present findings suggest the safe use of the two plant extracts particularly, in the amelioration of corticosteroid-induced diabetes mellitus/hyperglycaemia. However, further investigations on dose standardization are required before considering the two plant extracts for human use.

3. Experimental

3.1. Plant material

Aqueous extract of *Gymnema sylvestre* (leaf) containing 23% gymnemic acid and alcoholic extract of *Inula racemosa* (root), were obtained in powder form from Amsar Lab. Pvt. Ltd., Indore, India that normally procures the plant material, gets them authenticated by the taxonomist and then processes for extraction. The yield of *Gymnema sylvestre* and *Inula racemosa* were reported to be 10 and 33% respectively. The aqueous suspension of these plant materials at different concentrations was prepared for experimental use.

3.2. Animals

Healthy colony bred Swiss albino mice of either sex (2 months old), weighing 28 ± 2 g were maintained in a constant temperature (27 ± 1 °C) and photo schedule (14 h light and 10 h dark) controlled room. The mice were provided with feed (Golden feeds, New Delhi, India) *ad libitum* and free access to drinking water. Standard ethical guidelines were also followed.

3.3. Experimental design

Forty mice were rendered hyperglycaemic by daily intra-muscular (*i.m.*) injection of dexamethasone (a glucocorticoid) at a pre-standardized dose of 1 mg/kg body wt/day for 7 consecutive days and then divided into five groups of eight each. One group continued to receive only dexamethasone, while the other four groups were treated with equivalent dose of dexamethasone along with *Inula racemosa* extract (IRE, 400 mg/kg b. wt./d), *Gymnema sylvestre* extract (GSE, 600 mg/kg b. wt./d). Combination of IRE and GSE (400 mg and 600 mg/kg b. wt./d respectively) and ketoconazole (24 mg/kg b. wt./d, i.p.) respectively for 15 days. Simultaneously, five other groups, each with eight-normoglycaemic animals were treated with equivalent amounts of vehicle, IRE, GSE, IRE + GSE and ketoconazole respectively. Doses of the plant extracts and ketoconazole were taken from earlier studies [8, 12, 13]. Both the plant extracts and ketoconazole were administered orally between 10 and 11 a.m. to avoid any circadian variation. Treatment was continued for 15 days.

On the last day all the animals were weighed and then sacrificed by cervical dislocation after an overnight fast. Blood samples from each one were collected, allowed to clot and centrifuged to get clear serum, which was then stored at −20 °C until assayed for glucose and thyroid hormones. The liver was removed quickly, cleaned and washed twice with phosphate buffered saline (pH 7.4) and immediately processed for the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activity.

3.4. Biochemical estimations

Serum concentrations of total tri-iodothyronine (T_3) and thyroxine (T_4) were estimated by radioimmunoassay (RIA) [4, 5]. The serum glucose was estimated by the modified glucose oxidase method [14] using GOD-POD kits from Sigma diagnostic Ltd, Baroda, India and the hepatic LPO, SOD and CAT activities using routine protocols [5].

3.5. Statistical analysis

The data was analyzed for significance using analysis of variance (ANOVA) followed by Student's t-test. A P value of 5% and less was considered as significant.

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