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Physiological Influence of Licorice Extract on Some Hormonal and Biochemical Parameters Alterations Induced by Glucocorticoid in Male Rats

Safinaz Badie[1] and Sohier Fathey[2*]

[1]Department of Physiology, Al-Dayer, Jazan University, Jazan 82912, Kingdom of Saudi Arabia.

[2]Department of Microbiology and Immunology, National Research Centre, Dokki, Egypt.

***Corresponding author: Sohier Fathey** **Email:** sohir_syam@yahoo.com

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Abstract

The current study investigates the influence of licorice extract versus adrenal incapability induced by glucocorticoids in male rats. Forty male rats were sectioned randomly into 4 groups. The first was negative control group (G1): normal saline was given orally to rats. The second was positive control group (G2): intra peritoneal treatment with Hydrocortisone Sodium (50 mg/kg) for seven days. The third was therapeutic group (G3): intra peritoneal treatment with Hydrocortisone Sodium (50 mg/kg) for seven days and then licorice extract (100 mg /kg) given orally for 14 days. The fourth was licorice extract Group (G 4): Rats were given 100 mg/kg of licorice extract orally for 21 days. At the end of the experiment, hormonal measurement; adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), serum cortisol, as well as malondialdehyde (MDA), 11 β -hydroxysteroid dehydrogenase enzyme (11β-HSD), in addition to serum sodium and potassium were measured. The results demonstrated treatment with licorice extract improved significantly ($P<0.05$) in ACTH, CRH and serum cortisol hormones with non-significant reduction in serum MDA level in the therapeutic group compared with the positive control group. Our results concluded that licorice extract improves the alteration induced by hydrocortisone hormones and reduces the free radicals.

Keywords | Adrenal incapability, Licorice extract, 11β-HSD, Rats

Introduction

Adrenal incapability (AI) is a clinical disturbance that leads to failure of the adrenal cortex to secrete or output cortisol. There are three pathological types of adrenal incapability may present; primary, secondary and tertiary. Particularly, primary adrenal incapability (PAI) which originated from 2ry pathology of the adrenal gland which induces a defect at the adrenal

level is also accompanied by mineralocorticoid (aldosterone) deficiency (Fredrick et al., 2024; Lewis et al., 2023;).

Synthetic glucocorticoids (GCs) are able to mitigate inflammation and suppress the immune system, so they are frequently used as therapeutic agents. Their likely side effect is prohibition of the hypothalamus pituitary adrenal axis leading to adrenal incapability (Díaz-Castro et al., 2020).

Various factors are elevating the prevalence of this type of adrenal incapability such as the dose, route of administration, the duration of therapy, and potency of glucocorticoid, in addition to individual sensitivity and synchronized medicines that conflict with glucocorticoid metabolism. When the therapy of the exogenous glucocorticoid medication is minimized, patients may suffer signs of Cushing's as well as glucocorticoid withdrawal syndromes. So, prior to the return of adrenal function, the employ of glucocorticoids shouldn't be entirely cutout (Nachawi et al., 2024). While extended utilization of exogenous glucocorticoids can induce atrophy of adrenocortical layers and pituitary corticotroph cells. Whereas, mineralocorticoids are regulated by the renin-angiotensin system secreted to sustain aldosterone production, which (Borresen et al., 2022).

Natural extract from the roots of licorice plants (*Glycyrrhiza glabra*), which is thought to contain active components as glycyrrhizic acid which itself is hardly absorbed from the alimentary tract. Prior to absorption, glycyrrhizin acid is hydrolyzed to obtain glycyrrhetic acid, which is the definitive biologically active metabolite (Yaw et al., 2015). Researchers have discussed the beneficial uses of licorice extract at low accurate doses; anti-diabetic, anti-hyperlipidemia, antioxidant, anti-inflammatory, antiviral, antimicrobial, and anti-tumor merits. Moreover, it also has hepato-protective, neuro-protective effects, renal protective, as well as thrombin inhibitory and estrogenic activity (Sharifi-Rad et al. 2021).

This study was designated to demonstrate the antioxidant effect as well as therapeutic impact of licorice roots extract on pituitary adrenal axis hormones in AI induced by glucocorticoids administration in female rats.

Materials and methods

Ethical Approval

This study was performed in the animal house at National Research Centre (NRC), under the ethical approval number 13070105-1 obtained by NRC Ethics Committee.

Animals

For this experiment, forty male rats (2-3 months) aged weighing 180-210 grams. The animal was housed in ventilated cages (10 rats /cages) beneath optimum circumstances in the animal

house comprising unlimited water and free use of a commercial diet. The animals were let ten days to acclimatize to the lab environment.

Experimental design

The Forty male rats were sectioned randomly into four groups. The first was negative control group (G1); normal saline was administered orally to Rats. The second was positive control group (G2); a dose of 50 mg/kg of Hydrocortisone Sodium was administered intra-peritoneal to rats for 7 days. The third therapeutic group (G3); the same of group 2 and then followed by oral administration of licorice roots extract by100 mg /kg for14 days. The last fourth group (G4); rats were orally given 100 mg/kg of licorice roots extract for 21 days. Blood samples were taken from cardiac punctures at the end of the experiment and placed into gel tubes for the analyses.

Hormonal and biochemical assay

The serum ACTH, cortisol, CRH hormones, 11beta-HSD enzyme, and malondialdehyde (MDA) were measurement by using commercial kits (RayBiotech / USA)**.** Serum sodium and potassium levels were measured by colorimetric method according to (Frezzotti et al., 1996).

Statistical analysis

The data were statistically analyzed using the ANOVA in the computerized SPSS program version 24.0.

Results

Influence of licorice roots extracts on ACTH, Cortisol, and CRH hormones in adrenal incapability female Rats.

The current study demonstrated a significant reduction ($P<0.05$) in serum concentration of ACTH, cortisol, and CRH hormones in the G2 group administered hydrocortisone paralleled to the control G1 (Table 1). Licorice roots extract treatment induced a significant elevation ($P<0.05$) in G3 (therapeutic) group compared with the G2 group. While, the ACTH and the cortisol values in G3 group have significant variations ($P<0.05$) compared to the control G1. On the other hand, no significant differences between G4 and the control group in all three hormones, as shown in (Figure. 1).

Table 1: influence of licorice roots extract on serum ACTH, cortisol, and CRH concentration in adrenal incapability in male rats.

Group	Treatment	ACTH (Pg/ml)	Cortisol (nmol/L)	CRH (Pg/ml)
(G1)	Control Negative	93.76 ±7.34	62.52 ±4.18	7.42 ±0.25
(G2)	Control Positive	46.57 ±2.64	30.11 ±4.88	4.82 ±0.4
(G3)	Therapeutic	70.21 ±3.05	41.35 ±9.50	6.2 ±1.25
(G4)	Licorice roots extract	100.23 ±4.56	67.41 ±7.9	6.67 ±0.55

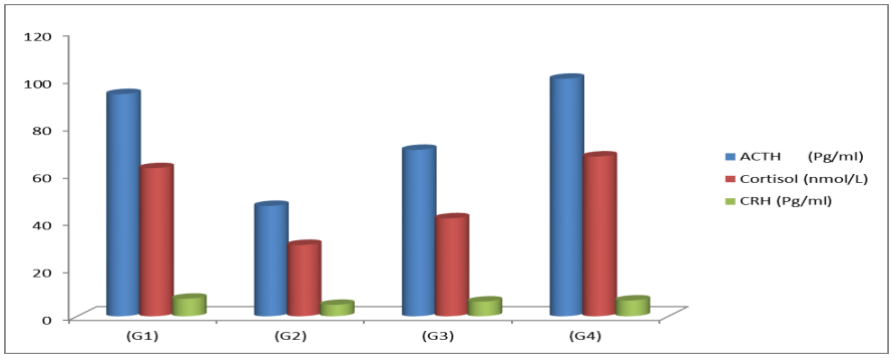

Figure 1: Impact of licorice roots extract on serum ACTH, cortisol, and CRH concentration in adrenal incapability in male rats. G1: received normal saline, G2: received hydrocortisone, G3: received hydrocortisone then licorice roots extract, G4: licorice roots extract only.

Influence of licorice roots extracts on 11beta-HSD enzyme and MDA in adrenal incapability in male rats

The obtained data displayed that the mean value of 11 β -HSD enzyme has diminished significantly ($p<0.05$) in G2 compared with the control. The effect of daily administration of licorice roots extract indicates a significant decrease ($p<0.05$) in the G3 and G4 groups compared with the control group G1 as shown in (Table 2) and (Figure 2).

Table 2: Influence of licorice roots extract on serum 11beta-HSD enzyme and MDA concentration in adrenal incapability in male rats.

Group	Treatment	11beta-HSD	MDA

		ng/dl	nmol/dl
(G1)	Control Negative	0.413 ±0.05	0.179 ±0 .03
(G2)	Control Positive	0.135 ±0.03	3.54±0.688
(G3)	Therapeutic	0.140 ±0.15	0.65 ± 0.18
(G4)	Licorice roots extract	0.110 ±0.26	0.182 ±0.03

On the other hand, the results in Table (2) revealed that a significant elevation (P≤0.05) in serum MDA in the adrenal incapability (G2) compared to the control G1, G3 and G4 groups. Hence, there were no significant variations G3 and G4 groups after being treated with licorice roots extract compared to the control G1(Figure 2).

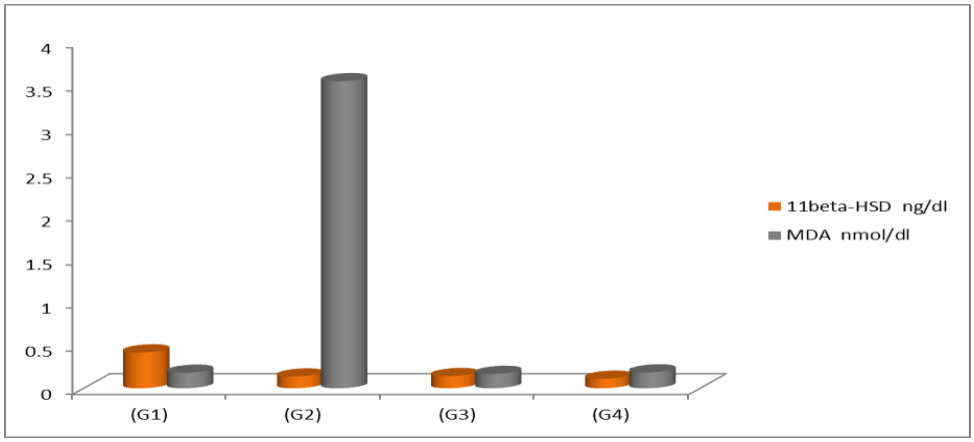

Figure 2: Impact of licorice roots extract on 11-β HSD enzyme and MDA in adrenal incapability in male rats. G1: received normal saline, G2: received hydrocortisone, G3: received hydrocortisone then licorice roots extract, G4: licorice roots extract only.

Influence of licorice roots extracts on serum sodium and potassium concentration in adrenal incapability in male rats

Table 3: Influence of licorice roots extract on serum sodium and potassium concentrations in adrenal incapability in male rats.

Group	Treatment	Serum sodium mg/dl	Serum potassium mg/dl

(G1)	Control Negative	142.23 ±0.56	5.23 ±0.56
(G2)	Control Positive	130.23 ±1.33	6.47 ±0.44
(G3)	Therapeutic	145.27 ±1.24	5.42 ±0.33
(G4)	Licorice roots extract	170.25 ±2.16	5.33 ±4.56

Our outputs in table (3) exhibited that there was a significant elevation in serum sodium accompanied with significant reduction in potassium levels in groups administered with GA compared to control group. Contrarily, the results showed a significant decreasing in serum sodium accompanied with rise in potassium levels in G2 which constituted adrenal incapability compared to control group (G1) as shown in figure (3).

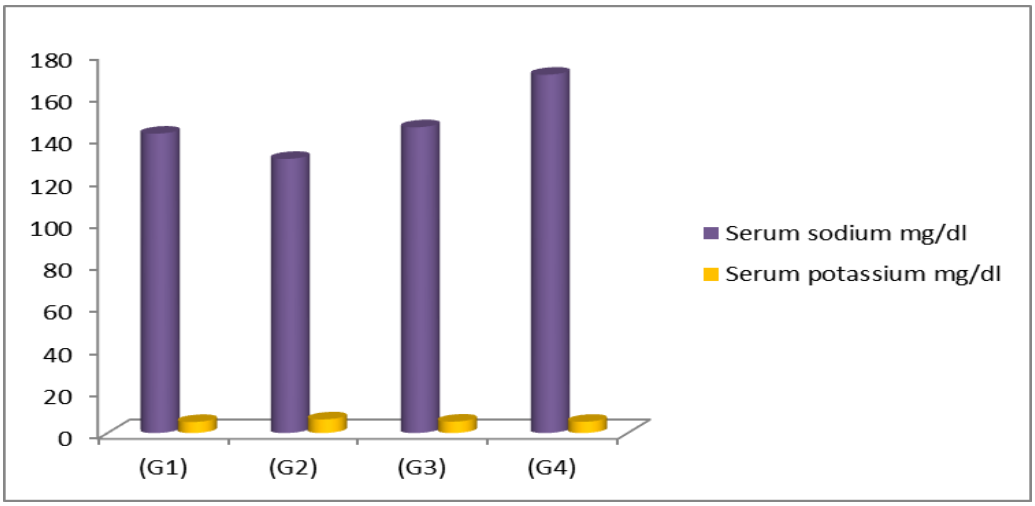

Figure 3: Impact of licorice roots extract on serum sodium and potassium concentration in adrenal incapability in male rats. G1: received normal saline, G2: received hydrocortisone, G3: received hydrocortisone then licorice roots extract, G4: licorice roots extract only.

Discussion

Animal models are often employed to understand the pathophysiology of glucocorticoids caused adrenal incapability and to test pharmacological remedy. In our study hydrocortisone intra-peritoneal administration for 7 days induces a significant reduction in the serum concentration of ACTH, cortisol and CRH. A parallel finding from Téblick *et al*., (2022) elucidated that exogenous hydrocortisone causes a negative effect on the hypothalamus

pituitary gland axis, leading to a decreasing of CRH and ACTH, accompanied with the reduction in cortisol production. The pathophysiology of glucocorticoids is multi-reason, and it probably act via suppressed CRH, dopaminergic and central noradrenergic system, due to chronic suppression of HPA axis, and rise in cytokines, and prostaglandins (Kao et al., 2014) and (Improda et al., 2024).

The administration of licorice roots extract to groups treated with hydro- cortisone result in a significant elevation in ACTH, cortisol, and CRH levels and overcome the low levels that occurred in the adrenal incapability group (G2). Our data uphold the earlier studies (Lin et al., 2012) which administered glycyrrhetinic acid in the male rats which exposed a higher concentration of cortisol levels. The authors assumed this adrenal incapability condition attributed to the suppressive effect on 11β-HSD.

On the other side, our results revealed that the licorice roots extract treated groups were a rise in the 11β-HSD concentration than the group administered with hydrocortisone only. This enzyme is important in the conversion process of cortisol to other derivatives and is substantial for regulating the glucocorticoid and mineralocorticoid receptors. The typical suppression of 11-HSD by, bioactive constituents of licorice is endogenous steroidal substances acting as glycyrrhetinic acid-like agents that block 11-HSD and enable glucocorticoid-induced mineral-receptors and glucocorticoid-receptors stimulation, may employ as competitive substrates, while others only act as suppressors (Bailly, and Vergoten 2020, Matchanov et al., 2022).

Moreover, glycyrrhetinic acid prevents the conversion of cortisol to inactive cortisone by inhibiting 11β-HSD (Hardy et al., 2013). Certain investigations displayed the selective suppression of glycyrrhizic acid as it was mentioned that 18α- glycyrrhizic acid preferentially selectively inhibits type 1- 11β-HSD, while 18β-GA preferentially suppress type 2-11β-HSD (Sakoda et al., 2024).

Lipid peroxidation is an important concern induced by free radicals within an organism. Malondialdehyde is considered a by-product of the polyunsaturated fatty acids peroxidation in the cells. An elevation in free radicals induces excessive output of MDA. Commonly, malondialdehyde level is employed as an indicator for oxidative stress and the existence of antioxidants (Tyagi, et al., 2015, Alobaidi, 2024). Extracted from our data, by glucocorticoid injection in G2, adrenal oxidative stress was asserted by significant elevation estimating of MDA. The glucocorticoids enhance the output of free radicals as relative oxygen species (ROS), which is a reason of adrenal injury by oxidizing cell membrane lipids, DNA damage and protein denaturation (Flaherty et al., 2017). Whereas, treatment with licorice roots extract mitigated the glucocorticoid-caused oxidative damage by decreasing MDA levels. This could be explicated by the efficiency of licorice roots extract to amend certain enzymes embraced in inflammation, oxidative stress, and the inhibition of some pro-inflammatory interleukins,

safeguarding cells from destruction induced by inflammation or ROS (Ageeva et al., 2022). Our results coincided with (Feng et al., 2013; Galanis et al., 2019; Wang et al., 2022) who reported that licorice minimized serum levels of malondialdehyde (MDA) in rats. Additionally, many researches correlate the anti- oxidant merit of licorice with its anti-inflammatory ability. It has been illustrated that licorice inhibits the formation of nitric oxide and inflammatory interleukins together with other components of licorice extract (Li et al., 2011; Richard, 2021).

Our result revealed a marked elevation in serum sodium associated with significant decreasing in potassium levels in groups administered with licorice roots extract paralleled to control group and can restored the significant decreasing in serum sodium and rise in potassium levels in G2 which constituted adrenal incapability.

Hejazi et al. (2017) developed in vitro and in silico evaluation based pharmacokinetic (PBPK) model in rat to assess administration of licorice roots extract and reported increase in sodium and reduction in potassium levels. On other sight, It was demonstrated that oral taken 100 mg/kg per day licorice roots extract did not influence serum electrolyte as potassium and sodium levels, referring no onset of undesirable edema (Fernando et al. 2014).

Conclusion

Extracting from our findings, we can conclude that licorice roots extract components have a beneficial impact in the treatment and protection of hormonal, biochemical and oxidation alterations in adre- nal insufficiency rats induced by glucocorticoid.

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