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RESEARCH ARTICLE

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EVALUATION OF ADAPTOGENIC POTENTIALS OF HYDROETHANOLIC EXTRACT OF *GREWIA HIRSUTA* VAHL. IN EXPERIMENTAL ANIMALS

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Abstract

Objectives: To assess the adaptogenic activity of hydroethanolic extract of *grewia hirsuta* vahl. in experimentally induced stress.

Methods: Hydroethanolic extract of *Grewia hirsuta* (HEEGH) was tested at 250 and 500 mg/kg. The behavioral and biochemical studies comprises of glucose, cholesterol, triglycerides, total protein, SGPT, SGOT, BUN, and cortisol were estimated.

Results: Adrenocorticotropic hormone causes tropisms of adrenal glands and spleen at the expense of liver functions as a result of the stress induced response. The extract at two different level doses reduces the secretion of corticosterone from adrenal cortex and other biochemical (see table 1&5).

Conclusion: G*rewia hirsuta* Vahl. was used in folk & tribal medicine from the times immemorial fornervine, brain tonic, antipyretic, diuretic, carminative, aphrodisiac, cardiac tonic. The pharmacological activity of the extract was not as per the literature survey. Hence, our study which is known to reduce the cortisol secretion may be the cause for the stress.

Keywords: *Grewia hirsuta;* adaptogenic, immobilization stress, anoxia stress tolerance, forced swimming endurance

BACKGROUND

Negative stress affects quality of life badly and is an epidemic scale due to complex lifestyles and habits. Although, synthetic drugs are available such as CNS depressants like barbiturates and benzodiazepines are proved to be effective in animal and clinical studies. The major limitations of

synthetic molecules are physical dependence, weight gain, and rebound anxiety on withdrawal of therapy. For long term use synthetic drugs are known to inflict psychosocial changes such as aggression, insomnia, sadness, memory problems, metabolic disorders and suicidal tendency. There is a requirement for safe and effective drugs a replacement of synthetic drugs. There is a worldwide search for natural products which are non habit forming, safe, effective and economical.[1-3]

MATERIALSAND METHODS

Plant collection and authentication

The leaves of *Grewia hirsuta* were collected from the Chittoor district, Andhra Pradesh, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Taxonomist (IAAT 357) with assigned a voucher number 072.

Animal and Ethical clearance

Adult Wistar Albino rats of either sex (aged 8-12 weeks, and body weight 150 ± 10 g), and Swiss Albino mice of either sex (aged 10-12 weeks, body weight 20±5g,) were used to evaluate the adaptogenic activity. Ethical clearance from the Institutional Animal Ethical Committee, with the registration number 112/PO/Re/S/99/CPCSEA and dated on 21/02/2019.

Preparation of Extract

Grewia hirsuta dried leaves were extracted by Soxhlet apparatus with hydroethanol. The extract was dried using rotary evaporator and stored in airtight container .[4]

Experimental Protocol

1. Immobilization Stress in rats

Wistar Albino rats weighing 150±10 g, were divided into five groups of six animals each.
Group I - Control (Saline, 10 ml/kg, p.o.)
Group II - Stress control (Saline, 10 ml/kg, p.o) subjected to stress
Group III - Standard (*Withania somnifera*, 100 mg/kg, p.o.)
Group IV - Lower dose HEEGH (250 mg/kg, p.o.)
Group V - Higher dose HEEGH (500 mg/kg, p.o.)

Duration of treatment was ten days. Stress was induced by immobilization stress model as described Pawar VS *et al* 2011[5] & Sudheer A *et al* 2015.[6]

2. Anoxia Stress Tolerance Test:

Swiss Albino mice weighing 20-30 g were selected and divided into five groups of six each.
Group I - Stress control (Saline, 10 ml/kg, p.o) subjected to stress
Group II - Standard (*Withania somnifera*, 100 mg/kg, p.o.)
Group III- Lower dose - HEEGH (250 mg/kg, p.o.)
Group IV- Median dose -HEEGH (333.33 mg/kg, p.o.)
Group V - Higher dose - HEEGH (500 mg/kg, p.o.)

Animals were treated for 21 days and the induced stress was measured as described Singh S *et al* 2021 [7] & Jahagirdar AQF *et al* 2020.[8]

3. Swimming Endurance test in mice:

Swiss Albino mice weighing 20-30 g divided into five groups of six animals each
Group I - Control (Saline, 10 ml/kg, p.o.)
Group II - Stress control, (Saline, 10 ml/kg, p.o) subjected to stress
Group III - Standard (Diazepam, 2 mg/kg, i.p)
Group IV - Lower dose - HEEGH (250 mg/kg, p.o.)

Group V - Higher dose - HEEGH (500 mg/kg, p.o.)
The duration of treatment for 7 days and swimming endurance was assessed as described by Darbar S *et al* 2020 [9] & Kanase V *et al* 2019.[10]

Statistical Analysis
Results were expressed as mean ± SEM (n=6) and one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test at * p< 0.05, ** p< 0.01 *** p< 0.001 as compared to stress control.

RESULTS

1. Immobilization stress model
Induced stress significantly increased the levels of serum glucose, cholesterol, triglycerides, blood urea nitrogen, total protein and plasma cortisol levels in stressed rats. HEEGH treated animals showed statistically significant decrease in the biochemical parameters. (See table 1).

Table 1. Effect of HEEGH on biochemical parameters of immobilization induced stress in Albino Wistar rats

Group	Glucose (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (mg/dL)	Plasma Cortisol (µg/dL)	BUN (mg/dL)	SGPT (U/L)	SGOT (U/L)
Control (Saline)	82.29± 5.87	58.03± 5.39	63.26± 4.61	3.79± 0.16	6.79± 0.44	26.91± 1.13	37.99± 3.88	37.03± 3.71
Stress control (Saline)	154.6± 5.84	141.8± 6.07	107.4± 5.44	4.87± 0.15	17.21± 0.50	46.30± 2.16	82.39± 4.19	89.39± 4.80
Standard (*Withania somnifera)*	110.4± 5.30***	65.02± 5.52***	74.18± 4.35***	3.92± 0.13***	9.79± 0.43***	30.55± 1.58***	52.99± 4.70***	52.72± 4.09***
Lower Dose (HEEGH)	132.8± 4.10**	122.8± 5.59**	88.85± 5.50**	4.29± 0.19*	15.00± 0.64*	38.34± 1.41**	80.13± 5.31	79.71± 5.89
Higher Dose (HEEGH)	115.7± 5.28***	73.19± 5.65***	77.31± 4.70***	4.08± 0.13**	12.52± 0.71***	34.45± 1.69***	59.70± 4.01**	66.28± 4.50**

The values are expressed as mean ± SEM, (n=6), Where,* p< 0.05, ** p< 0.01 *** p< 0.001 as compared to stress control. One-way ANOVA followed by Dunnet's multiple comparison tests.

1.1 Effect on organ weight in immobilized stressed rats
Increase in the weight of the liver and adrenal glands, whereas decrease in the weight of spleen was observed. HEEGH reversed the organ weight and the results were found to be statistically significant. (See table 2).

Table 2. Effect of HEEGH on relative organs weight of immobilization stressed in Albino Wistar rats

Group	Organs weight		
	Liver (g/100g b.w.)	Spleen (g/100g b.w.)	Adrenal gland (mg/100g b.w)
Control (Saline)	3.69±0.17	0.61±0.02	17.34±0.002
Stress control (Saline)	5.80±0.23	0.24±0.01	96.12±0.004
Standard (*Withania somnifera)*	3.96±0.25***	0.61±0.01***	25.45±0.002***
Lower Dose (HEEGH)	4.84±0.24*	0.39±0.02**	76.24±0.006**
Higher Dose (HEEGH)	4.09±0.25***	0.67±0.03***	28.86±0.003***

The values are expressed as mean ± SEM, (n=6), Where, p< 0.001, *** p<0.01,** p< 0.05,* p< 0.001 as compared to stress control.

1.2 Histopathology of Adrenal gland in immobilization induced stress

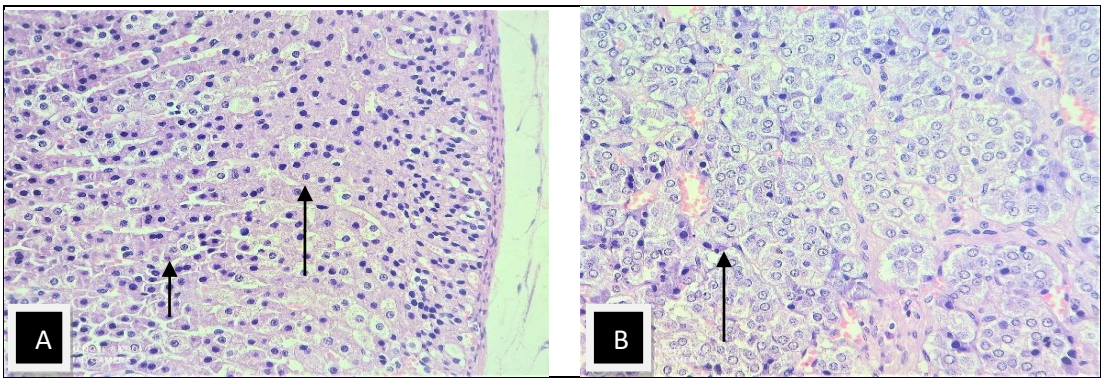

Figure 1. Photomicrograph sections of the adrenal cortex of the control group shows intact architecture (A) The zona glomerulosa [Fig.1 A, short-arrow] consists of secretory cells arranged in irregular ovoid clusters surrounded by trabeculae containing capillaries. The zona fasciculata [Fig.1 A, Long-arrow] consists of cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.1 B, Arrow] contains basophilic polyhedral chromaffin cells having granular cytoplasm, ganglion cells and intact blood vessels. Photomicrographs were at 400 x magnification using light microscope, and 5 µm thick paraffin sections, Hematoxylene and Eosin stain.

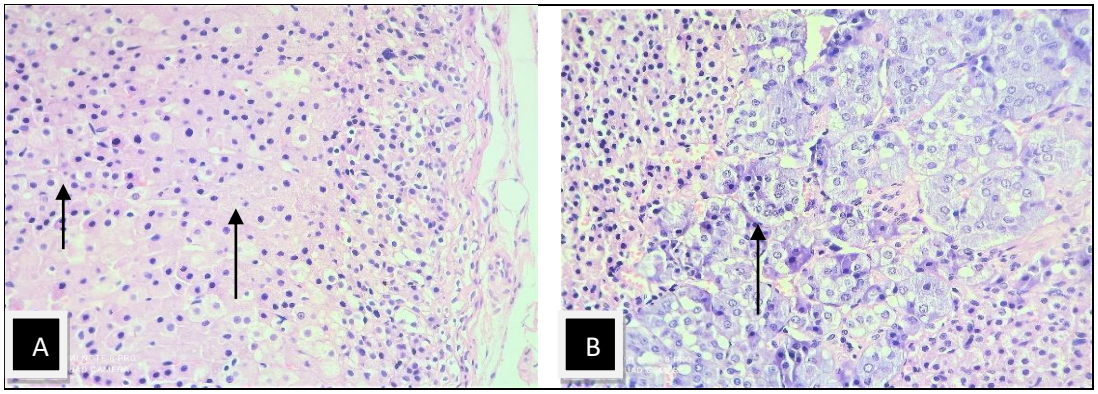

Figure 1.1 Photomicrograph sections of the adrenal cortex of the stress control group shows partially loss of architecture. (A) The zona glomerulosa [Fig.1.1 A, short-arrow] appears atrophic with moderate mononuclear inflammatory infiltration. The zona fasciculata [Fig.1.1B, Long-arrow] consists of diffuse hypertrophic secretory cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.1.1 B, Arrow] contains hypertrophic basophilic polyhedral chromaffin cells with intact ganglion cells and blood vessels.

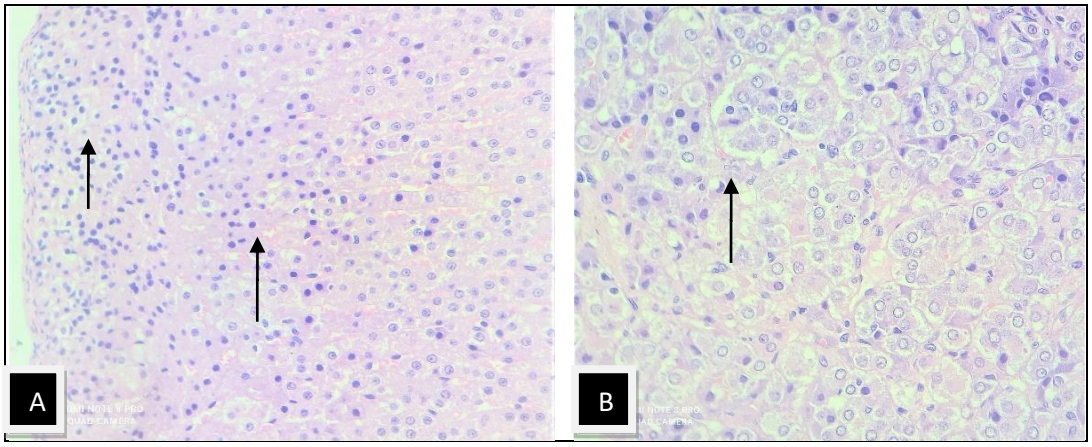

Figure 1.2 Photomicrograph sections of the adrenal cortex of the standard group shows partially loss of architecture. (A) The zona glomerulosa [Fig.1.2 A, short-arrow] exhibits focal atrophic cells with mild mononuclear inflammatory infiltration. The zona fasciculata [Fig.1.2 A, Long-arrow] consists of focal hypertrophic secretory cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.1.2 B, Arrow] contains focal hypertrophic basophilic polyhedral chromaffin cells with intact ganglion cells and blood vessels.

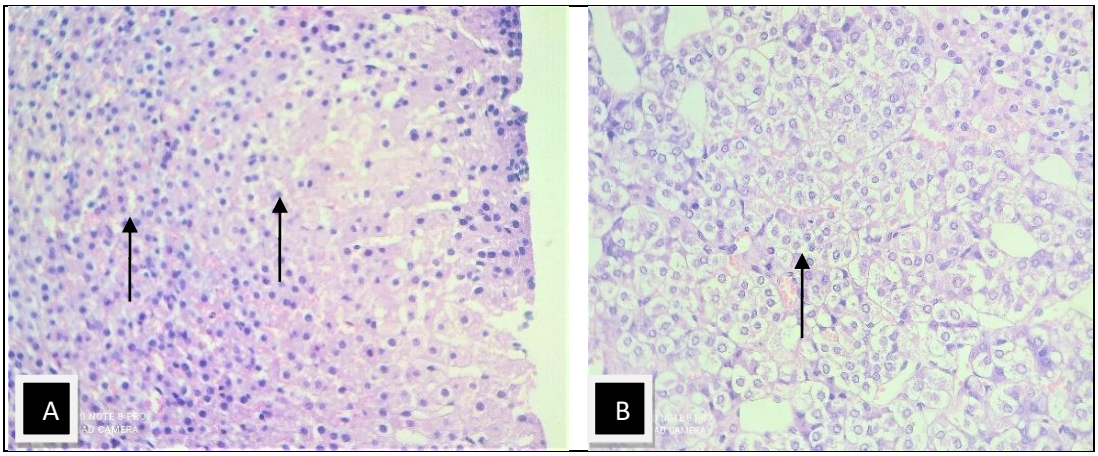

Figure 1.3. Photomicrograph section of the adrenal cortex of lower dose group shows partially loss of architecture. (A) The zona glomerulosa [Fig.1.3 A, short-arrow] consists of secretory cells arranged in irregular ovoid clusters surrounded by trabeculae containing capillaries. The zona fasciculata [Fig.1.3 A, Long-arrow] consists of cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.1.3 B, Arrow] contains basophilic polyhedral chromaffin cells having granular cytoplasm, ganglion cells and intact blood vessels.

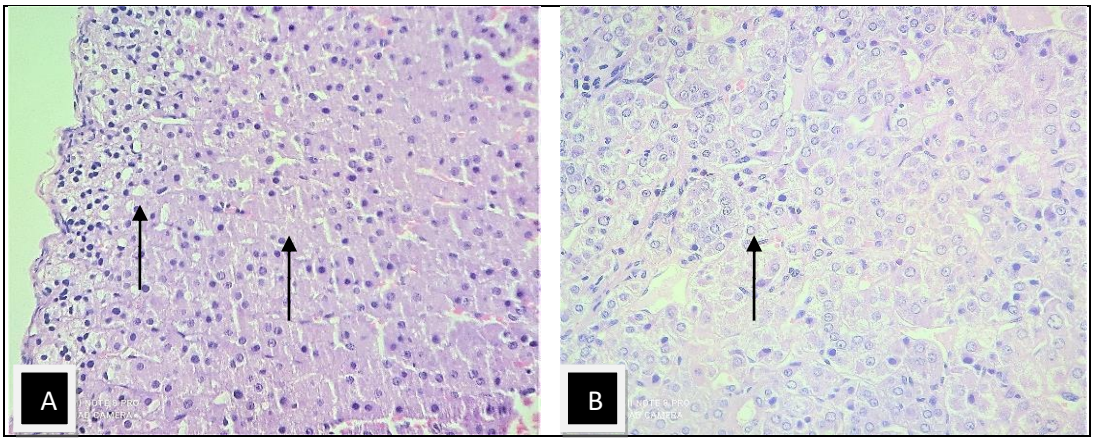

Figure 1.4 Photomicrograph section studied from the adrenal cortex of higher dose group shows partially loss of architecture. (A) The zona glomerulosa [Fig.1.4 A, short-arrow] appears focally atrophic with mild mononuclear inflammatory infiltration. The zona fasciculata [Fig.1.4 A Long-arrow] consists of focally hypertrophic secretory cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.1.4 B, Arrow] contains focal hypertrophic basophilic polyhedral chromaffin cells with intact ganglion cells and blood vessels.

2. Anoxia stress tolerance test

There is delay in anoxia time when compared to stress control group and the results were statistically significant (see table 3).

Table 3. Effect of HEEGH on anoxia stress tolerance time in Swiss albino mice

Group	Duration of anoxia stress tolerance time (min)		
	7th Day	14th Day	21st Day
Control (Saline)	37.33±2.02	41.17±2.24	44.11±2.335
Standard (*Withania somnifera*)	60.69±2.69***	64.32±2.31***	70.65±2.16***
Lower Dose (HEEGH)	41.30±3.11***	46.68±2.315***	49.32±2.54***
Median Dose (HEEGH)	45.29±3.43***	51.58±2.276***	55.82±2.22***
Higher Dose (HEEGH)	53.37±2.38***	55.97±1.865***	60.50±2.22***

The values are expressed as mean ± SEM, (n=6), Where, p< 0.001, *** p<0.01,** p< 0.05,* p< 0.001 as compared to stress control.

3. Forced swimming endurance test

The changes in behavioral, biochemical and histopathological were given in table 4,5, 6 and figure 2. Observed biochemical parameters showed decreased levels when compared to stress control group.

Table 4. Effect of HEEGH on Immobility time, mobility time, locomotor activity and muscle coordination in forced swimming edurance test

Groups	Immobility time (sec)	Mobility time (sec)	No. of counts/ 10 min	Fall off time (sec)
Control (Saline)	- -	- -	342.2±17.26	118.9±4.52
Stress Control (Saline)	132.60± 2.22	107.40± 2.44	517.2± 32.13	54.55±7.40
Standard (Diazepam)	78.24±2.39***	161.76±2.07***	320.1±19.34***	138.4±5.05***
Lower Dose (HEEGH)	97.33±2.21***	142.67±2.28***	416.9±28.41*	80.63±6.86*
Higher Dose (HEEGH)	87.29±1.92***	152.71±2.10***	374.4±21.31**	94.09±5.75**

The values are expressed as mean ± SEM, (n=6), Where, p< 0.001, *** p<0.01,** p< 0.05,* p< 0.001 as compared to stress control.

Table 5. Effect of HEEGH on biochemical parameters in forced swimming endurance test

Groups	Glucose (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (mg/dL)	Plasma Cortisol (ηg/ml)	BUN (mg/dL)	SGPT (U/L)	SGOT (U/L)
Control (saline)	90.73± 3.08	66.40± 1.74	82.70± 2.52	3.94 ±0.15	88.04± 2.71	33.77± 1.41	38.0± 1.86	38.67± 1.77
Stress Control (Saline)	150.04± 5.04	136.3± 2.40	154.8± 2.18	6.06± 0.20	179.4± 3.05	61.88± 1.88	86.5± 2.00	89.96± 2.10
Standard (Diazepam)	99.96± 3.77***	84.35± 2.41***	114.2± 2.31***	4.22± 0.14***	101.9± 2.94***	40.37± 1.38***	49.2± 1.79***	42.27± 1.62***
Lower Dose (HEEGH)	128.2± 4.91*	117.0± 2.46**	139.5± 2.56**	5.075± 0.14**	143.3± 3.34***	54.30± 1.89**	70.57± 1.98**	73.72± 1.85**
Higher Dose (HEEGH)	116.3± 4.87***	97.52± 2.53***	124.0± 2.36***	4.77± 0.13***	119.4± 3.37***	49.55± 1.36***	59.88± 1.87***	58.34± 1.74***

The values are expressed as mean ± SEM, (n=6), Where, p< 0.001, *** p<0.01,** p< 0.05,* p< 0.001 as compared to stress control.

Table 6. Effect of HEEGH on hematological parameters in forced swimming endurance test

Hematological parameters	Group I (Control, Saline)	Group II (Stress control, Saline)	Group III (Diazepam, 2 mg/kg)	Group IV HEEGH (250 mg/kg p.o.)	Group V HEEGH (500mg/kg p.o.)
RBC ($x10^6/\mu L$)	4.28±0.17	4.02±0.26	4.21±0.17	4.12±0.20 ^{ns}	4.16±0.19 ^{ns}
Total WBC ($x10^3/\mu L$)	8.21±0.33	6.14±0.32	8.24±0.28***	7.55±0.33*	7.83±0.32**
Neutrophils (%)	28.17±1.55	46.12±2.6	31.15±2.2***	37.53±2.9^{ns}	34.18±2.6***
Lymphocytes (%)	72.13±2.23	107.2±4.05	73.73±3.12***	91.84±3.60*	87.17±3.35***
Monocytes (%)	1.81±0.12	3.88±0.23	1.88±0.16***	2.82±0.21**	2.28±0.18***
Eosinophils (%)	2.02±0.17	3.702±0.22	2.015 ±0.18***	2.715±0.21**	2.405±0.20***

3.1 Histopathology of Adrenal gland in forced swimming endurance test

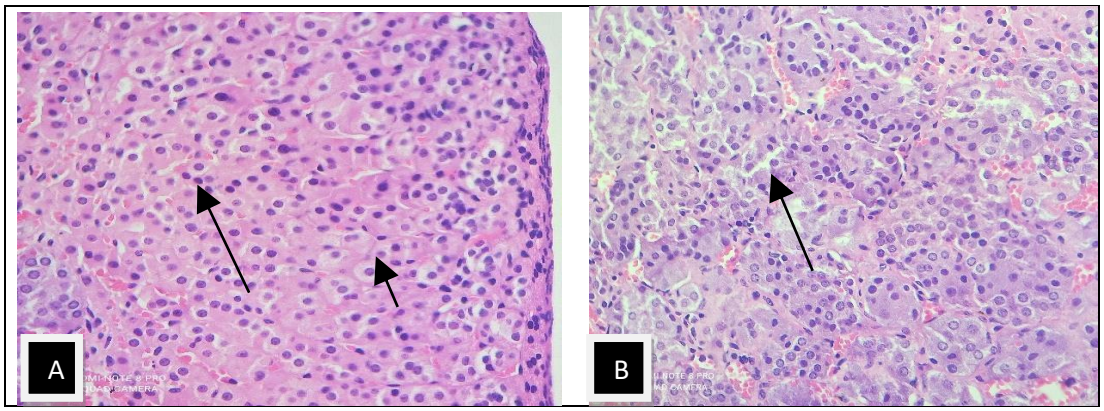

Figure 2. Photomicrograph section of the adrenal cortex of control group shows intact architecture with three layers. (A) The zona glomerulosa [Fig.2A, short-arrow] consists of secretory cells arranged in irregular ovoid clusters surrounded by trabeculae containing capillaries. The zona fasciculata [Fig.2A, Long-arrow] consists of cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.2B, Arrow] contains basophilic polyhedral chromaffin cells having granular cytoplasm, ganglion cells and intact blood vessels. Photomicrographs were at 400 x magnification using light microscope, and 5 μm thick paraffin sections, Hematoxylene and Eosin stain.

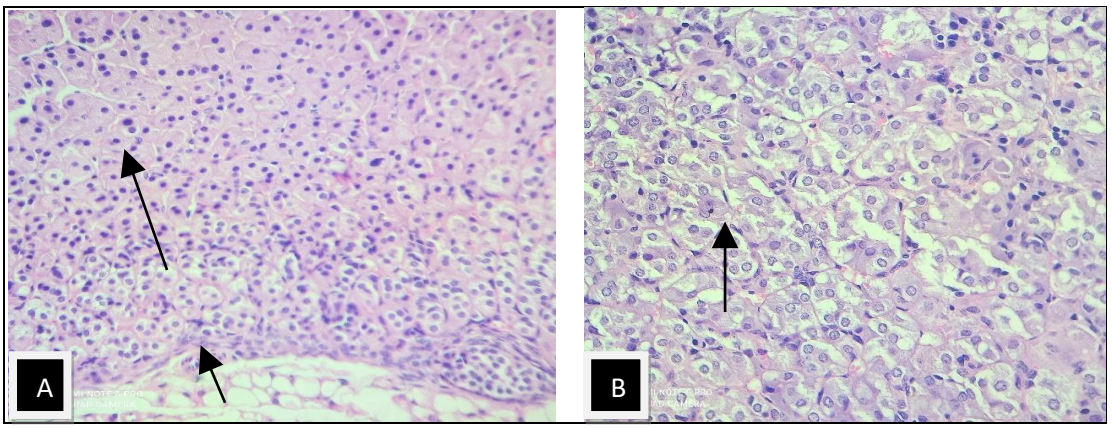

Figure 2.1 Photomicrograph section of the adrenal cortex of stress control group shows partial loss of architecture. (A) The zona glomerulosa [Fig.2.1 A, short-arrow] appears atrophic with mild to moderate mononuclear inflammatory infiltration. The zona fasciculata [Fig.2.1 A, Long-arrow] consists of diffuse hypertrophic secretory cells arranged in parallel cords. (B) The innermost layer zona reticularis consists of intact cells. The adrenal medulla [Fig.2.1 B, Arrow] contains hypertrophic basophilic polyhedral chromaffin cells with intact ganglion cells and blood vessels.

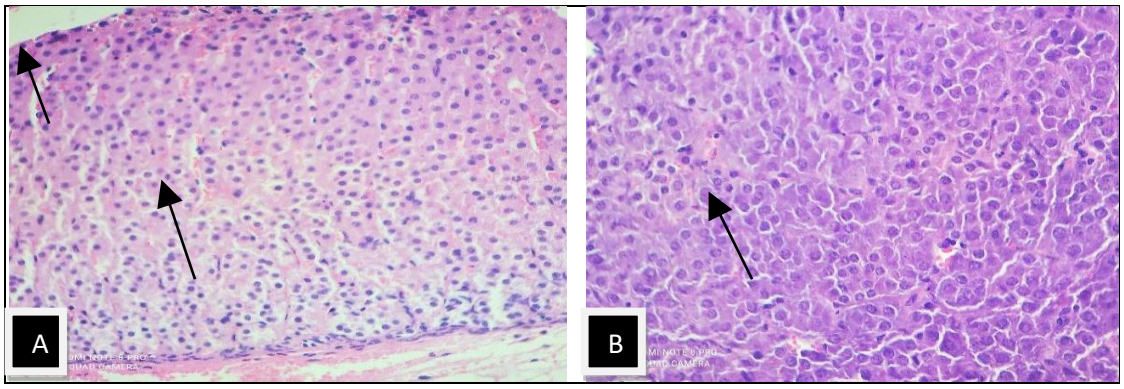

Figure 2.2 Photomicrograph section of the adrenal cortex of standard group shows intact architecture. (A) The zona glomerulosa [Fig.2.2 A, short-arrow] exhibits secretory cells with mild mononuclear inflammatory infiltration and congested capillaries. The zona fasciculata [Fig.2.2 A, Long-arrow] consists of focal hypertrophic secretory cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.2.2 B, Arrow] contains focal hypertrophic basophilic polyhedral chromaffin cells with intact ganglion cells and blood vessels.

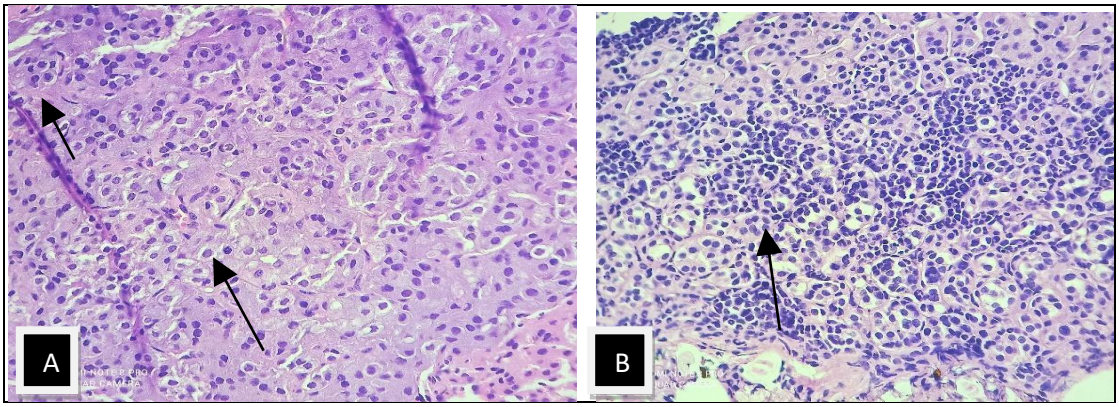

Figure 2.3 Photomicrograph section of the adrenal cortex of lower group shows partially loss of architecture. (A) The zona glomerulosa [Fig.2.3 A, short-arrow] appears focally atrophic with fibrosis and mild mononuclear inflammatory infiltration. The zona fasciculata [Fig.2.3 A, Long-arrow] consists of focal hypertrophic secretory cells arranged in parallel cords. (B) The innermost layer zone reticularis consists of intact cells. The adrenal medulla [Fig. 2.3 B, Arrow] contains dense mononuclear inflammation with focal hypertrophic basophilic polyhedral chromaffin cells and blood vessel.

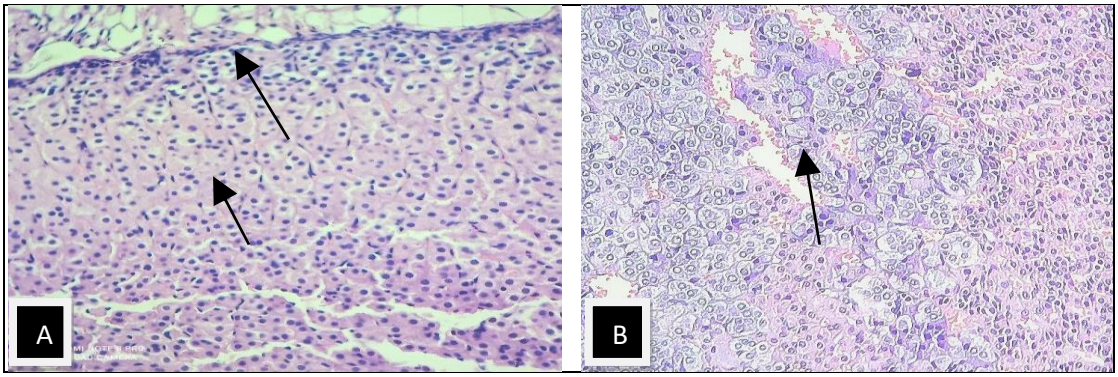

Figure 2.4 Photomicrograph section of the adrenal cortex of higher group shows intact architecture. (A) The zona glomerulosa [Fig.2.4 A, short-arrow] consists of secretory cells arranged in irregular

ovoid clusters surrounded by trabeculae containing capillaries. The zona fasciculata [Fig.2.4 A, Long-arrow] consists of cells arranged in parallel cords. The innermost layer zona reticularis consists of intact cells. (B) The adrenal medulla [Fig.2.4 B, Arrow] contains basophilic polyhedral chromaffin cells having granular cytoplasm, ganglion cells and intact blood vessels.

DISCUSSION

Secretion of cortisol maintains homeostasis through the gluconeogenesis and lipogenesis. In stress, the impairment of carbohydrate and lipid metabolism is observed by increased secretion of corticosterone. Stress elevates serum cholesterol through hypothalamic-Pituitary adrenal axis (HPA) which secretes hormones of adrenal glands (catecholamines and corticosteroids). Epinephrine (catecholamine) mobilizes lipids from the adipose tissues leading to increase in blood cholesterol.The effect of stressincrease in the release of catecholamines results in variable of serum triglycerides and BUN.[11] The elevated serum glucose, cholesterol and triglycerides were found to be decrease (statistically significant) in immobilization stress model. In our studies, we observed an increase in the weight of adrenal glands and liver in stressed animals. In treatment groups, the weight of adrenal glands and liver don't gain the weight indicating the action of the extracts on adrenal glands is preventing the stimulation caused by ACTH.[12] Anoxia induced convulsions are prevented in the treatment group, as compared to stress control group. In theforced swimming test, immobility time and mobility were observed. In the treatment group, there is a reduction in immobility time increase in mobility time when compared tostress control group.The enhancement of stamina in treatment group compare to stress control group is a strong indication for adaptogenic activity.

CONCLUSION

The extract of *Grewia hirsuta* is having abundant polyphenols, flavonoids and tannins which are established antioxidants. The plant extract is having a good adaptogenic activity due to phytochemicals of antioxidant activity.

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