Remittances Review
June, 2023
Volume: 8, No: 4, pp. 961-971
ISSN: 2059-6588 (Print) | ISSN 2059-6596 (Online)
remittancesreview.com

Received: 11 November 2022 Accepted: 28 March, 2023
DOI: https://doi.org/10.33182/rr.v8i4.67

Evaluation of Hydroethanolic Extract of *Grewia Hirsuta* Vahl for In-Vivo Adaptogenic Potentials

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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 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 biochemicals (see table 1&5).* ***Conclusion:*** *Grewia hirsuta was used in folk & tribal medicine from the times immemorial for nervine, 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 hirsute, adaptogenic, immobilization stress, anoxia stress tolerance, forced swimming endurance*

Introduction

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

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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]

Materials and Methods

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 healthy Wistar Albino rats of either sex (aged 8-12 weeks, and body weight 150 ± 50 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 hydro ethanol. 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 (Distilled water)

Group II - Stress control (Distilled water, 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 10 days. Stress was induced by immobilization model as described Pawar VS et al & Sudheer A et al. [5,6]

2. Anoxia Stress Tolerance Test

Swiss Albino mice weighing 20-30 g were selected and divided into five groups of six each.

Group I - Control (Distilled water)

Group II - Stress control, (Distilled water, subjected to stress)

Group III - Standard (*Withania somnifera*, 100 mg/kg, p.o.)

Group III - Lower dose - HEEGH (250 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 & Jahagirdar AQF et al. [7,8]

3. Swimming Endurance test in mice

Swiss Albino mice weighing 20-30 g divided into five groups of six animals each

Group I - Control (Distilled water)

Group II - Stress control, (Distilled water, 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 Darbar S et al & Kanase V et al. [9,10]

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

Immobilization stress model

Induced stress significantly increased the levels of serum glucose, cholesterol, triglycerides, blood urea nitrogen (BUN), total protein and plasma cortisol levels in stressed rats. HEEGH treated animals showed statist 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

Groups	Dose	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 Distilled water	5ml/kg	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	5ml/k	154.6±	141.8±	107.4±	4.87±	17.21±	46.30±	82.39±4.1	89.39

control Distilled water	g	5.84	6.07	5.44	0.15	0.50	2.16	9	± 4.80
Standard (*Withania somnifera)*	100 mg/kg	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	250 mg/kg	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	500 mg/kg	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.

Effect on organ weight in immobilized stressed rats

Increase in the weight of the liver, adrenal glands and whereas decrease in the spleen were 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

Groups	**Dose**	**Organs weight**		
		Liver (g/100g b.w.)	**Spleen (g/100g b.w.)**	**Adrenal gland (mg/100g b.w)**
Control Distilled water	5ml/kg	3.69±0.17	0.61±0.02	17.34±0.002
Stress control Distilled water	5ml/kg	5.80±0.23	0.24±0.01	96.12±0.004
Standard *Withania somnifera*	100 mg/kg	3.96±0.25***	0.61±0.01***	25.45±0.002***
Lower Dose HEEGH	250 mg/kg	4.84±0.24*	0.39±0.02**	76.24±0.006**
Higher Dose HEEGH	500 mg/kg	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.

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 on mice

Groups	Dose	Duration of anoxia stress tolerance time (min)		
		7th Day	14th Day	21th Day
Control Distilled Water	5 ml/kg	37.33± 2.02	41.17± 2.24	44.11± 2.335
Standard (*Withania somnifera*)	100 mg/kg	60.69± 2.69***	64.32± 2.31***	70.65± 2.16***
Lower Dose HEEGH	250 mg/kg	41.30± 3.11***	46.68 ±2.315***	49.32± 2.54***
Median Dose HEEGH	333.33 mg/kg	45.29± 3.43***	51.58 ±2.276***	55.82± 2.22***
Higher Dose HEEGH	500 mg/kg	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.

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 mobility, immobility time, locomotor activity and muscle coordination of forced swimming edurance test

Groups	Dose	Immobility time (sec)	Mobility time (sec)	No. of counts/ 10 min	Fall off time (sec)
Control Distilled Water	5 ml/kg	--	--	342.2± 17.26	118.9± 4.52
Stress Control Distilled Water	5 ml/kg	132.60± 2.22	107.40± 2.44	517.2± 32.13	54.55± 7.40
Standard Diazepam	2 mg/kg	78.24± 2.39***	161.76± 2.07***	320.1± 19.34***	138.4± 5.05***
Lower Dose HEEGH	250 mg/kg	97.33± 2.21***	142.67± 2.28***	416.9± 28.41*	80.63± 6.86*
Higher Dose HEEGH	500 mg/kg	87.29± 1.92***	152.71± 2.10***	374.4± 21.31**	94.09± 5.75**

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

Groups	Dose	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 Distilled water	5 ml/kg	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 Distilled water	5 ml/kg	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	2 mg/kg,	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	250 mg/kg	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	500 mg/kg	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***

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

Hematological parameters	Group I (Control)	Group II (Stress control)	Group III (Diazepam, 2 mg/kg)	Group IV HEEGH (250 mg/kg p.o.)	Group V HEEGH (500mg/kg p.o.)
RBC (x10^6/µL)	4.28 ±0.17	4.02 ±0.26	4.21 ±0.17	4.12 ±0.20 ns	4.16 ±0.19ns
Total WBC (x10^3/µL)	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 ±2.9ns	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***

Histopathology of Adrenal gland in immobilization induced stress

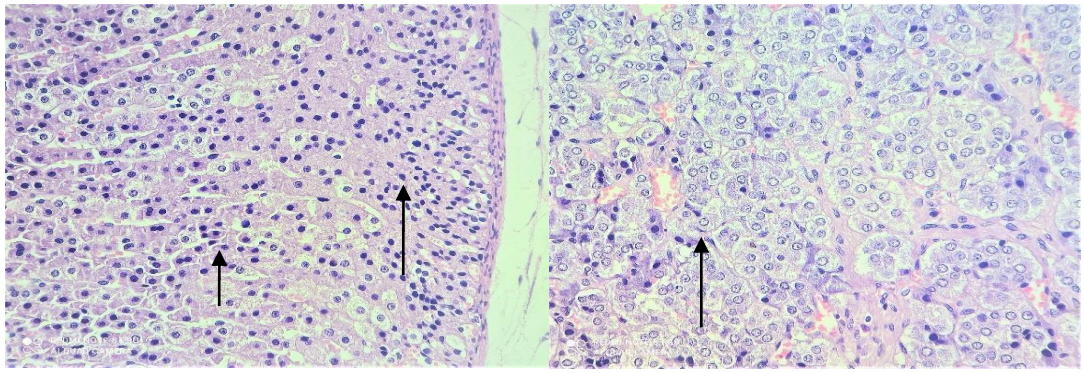

Figure 1: A Photomicrograph section of the control group at 400 x magnification Hematoxylene and Eosin stain [H&E] shows the intact architecture.

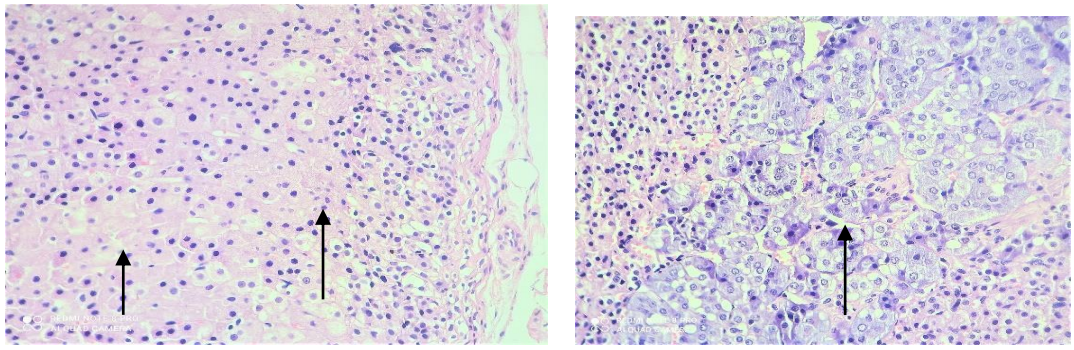

Figure 1.1: A Photomicrograph section of the stress control group shows partial loss of architecture with moderate mononuclear inflammatory infiltration.

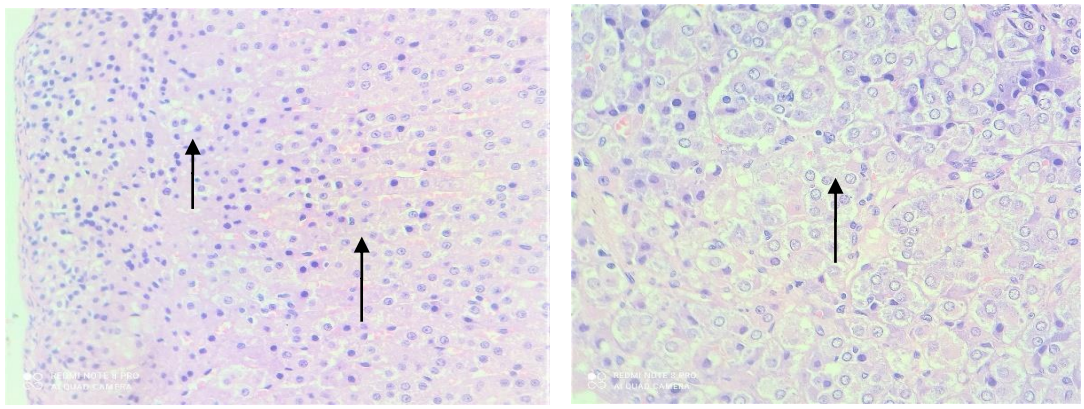

Figure 1.2: A Photomicrograph section of the standard group shows mild partial loss of architecture.

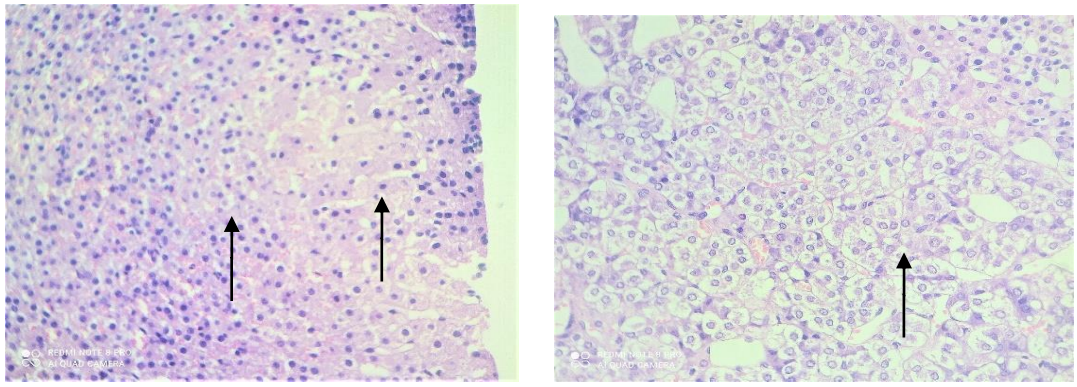

Figure 1.3: A Photomicrograph section of the lower dose HEEGH shows partial loss of architecture and consists of secretory cells arranged in irregular ovoid clusters surrounded by trabeculae containing capillaries.

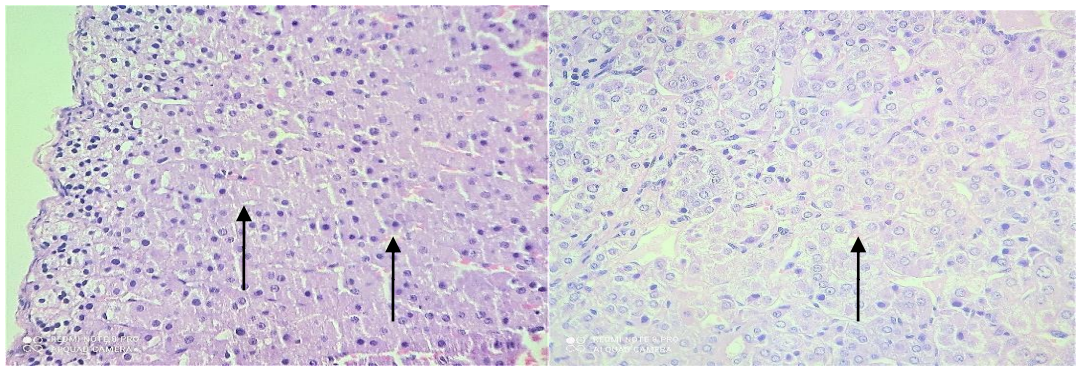

Figure 1.4: A Photomicrograph section of the higher dose HEEGH shows partial loss of architecture with mild mononuclear inflammatory infiltration.

Histopathology of Adrenal gland in forced swimming endurance test

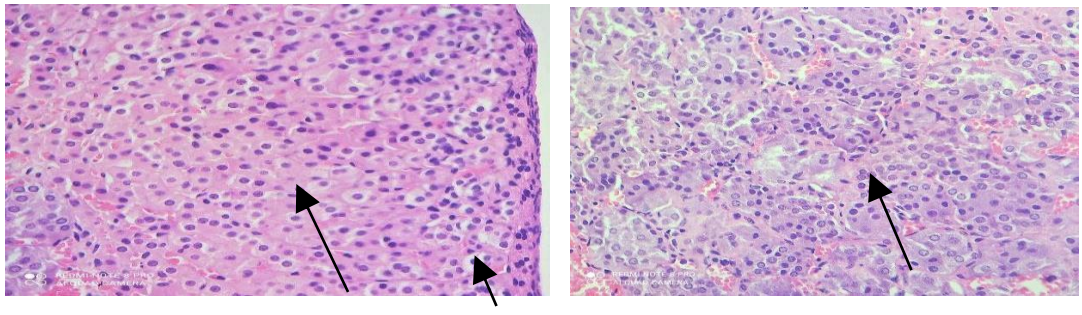

Figure 2: A Photomicrograph section of the control group at 400 x magnification Hematoxylene and Eosin stain [H&E] shows intact architecture with three layers.

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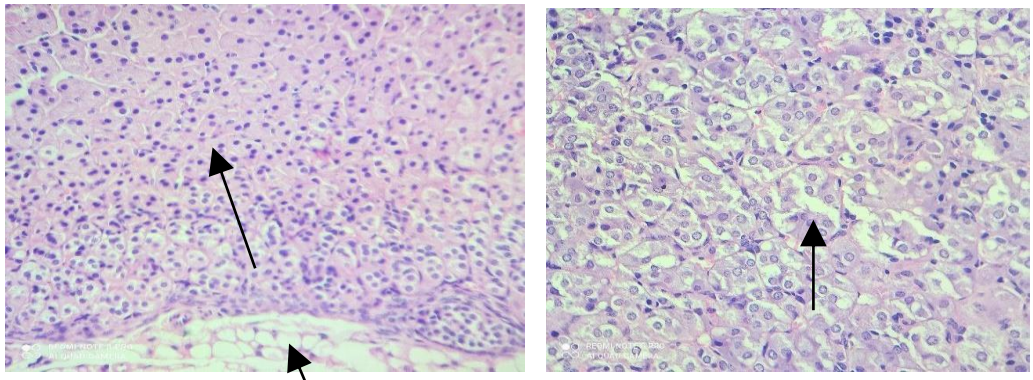

Figure 2.1: A Photomicrograph section of the stress control group shows partial loss of architecture with mild to moderate mononuclear inflammatory infiltration and congested capillaries.

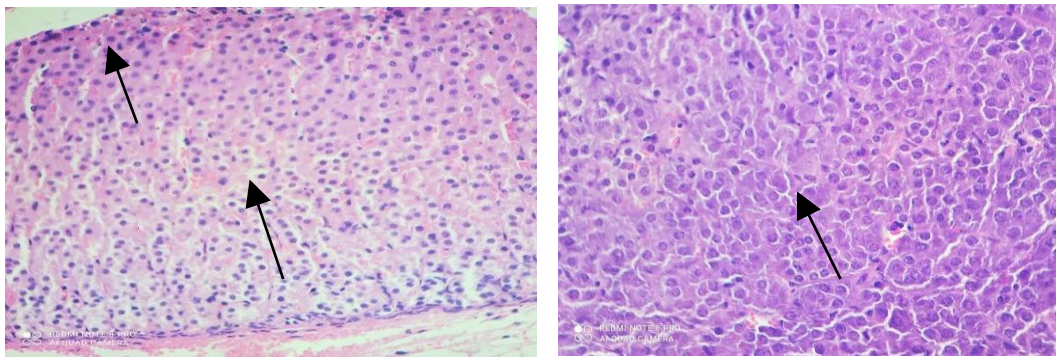

Figure 2.2: A Photomicrograph section of the standard group shows intact architecture with mild mononuclear inflammatory infiltration.

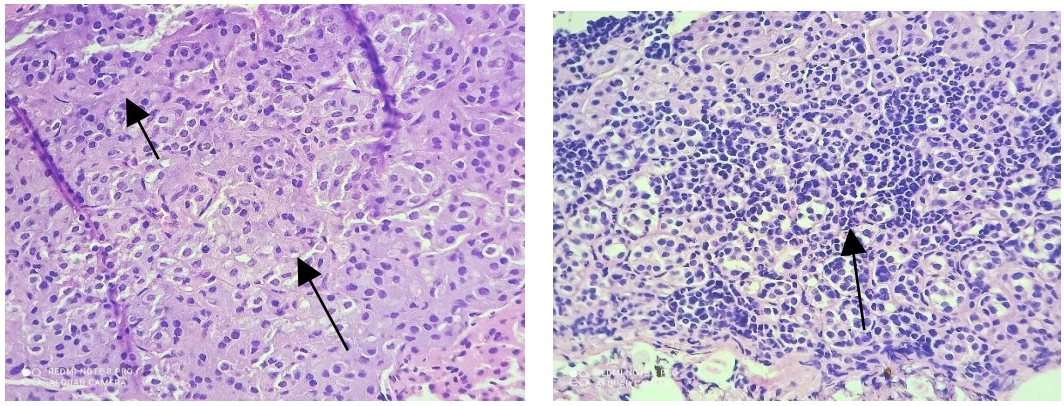

Figure 2.3: A Photomicrograph section of the lower dose HEEGH shows partial loss of architecture with moderate mononuclear inflammatory infiltration and congested capillaries.

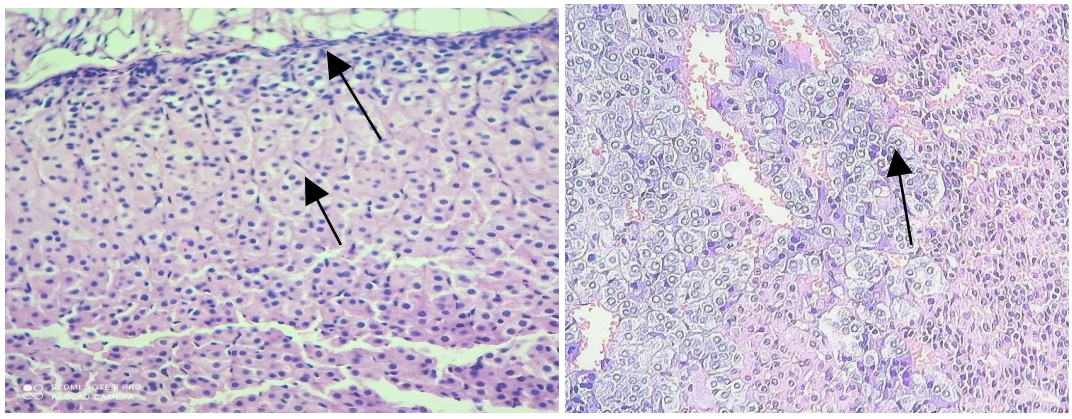

Figure 2.4: A Photomicrograph section of the higher dose HEEGH shows partial loss of architecture with mild mononuclear inflammatory infiltration and congested capillaries.

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 stress increase 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 the forced 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 to stress 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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