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Progesterone-Induced Hyperphagia is Attenuated by *Myrica nagi* through Dopaminergic and Serotonergic Modulation in Female Mice

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Abstract

Regulation of feeding behavior to control obesity is an alternative line of research for the treatment of obesity and diabetes. Compared to other models of obesity, the progesterone-induced obesity model is more specific to the female population; it focuses on the eating behavior and behavioral and emotional changes associated with progesterone. We aimed to study the changes in feeding behavior upon progesterone administration and the effects of *Myrica nagi* Thunb (Myricaceae) extracts on these changes. Further, this study aimed to provide insights into the progesterone-induced hyperphagia modulated by serotonergic and dopaminergic systems. In this study, experimental obesity was induced in female mice by treating with a high dose of progesterone for 28 days (sub-chronic study) followed by the assessment of parameters such as food consumption behavior, behavioral parameters including ambulatory movements, rearing, and grooming, and biochemical parameters such as lipid profile (total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein), liver parameters (alkaline phosphatase and serum glutamic pyruvic transaminase), serotonin and dopamine levels, and liver histopathology. The sub-chronic administration of progesterone, via the subcutaneous route, induced significant hyperphagia and promoted fat accumulation. Even though we did not find significant differences in food intake between mice in the control group and progesterone-treated groups, the final weight in the progesterone-administered groups increased, signifying the impact of this progesterone-induced obesity model. Treatment with MEMN extract reversed the progesterone-induced effects suggesting that herbal extracts can be exploited as serotonergic and dopaminergic agents for the treatment of progesterone-induced eating disorders, especially in the female population.

Keywords: Anti-Obesity Agent, Dopamine, Feeding Behavior, Hyperphagia, Herbal Extract, Progesterone-Induced Obesity, Serotonin

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1. Introduction

Steroid hormones play a major role in the development of obesity[1]. Obesity is mainly influenced by energy intake and energy expenditure. The latter comprises the metabolic rate at rest, energy consumption related to activity, and thermogenesis related to diet, whereas the former is governed by various hormones, such as leptin, insulin, and ghrelin[2]. The metabolism of an individual at rest and during activity is decided by their endocrine profiles[3].

Progesterone stimulates hyperphagia through progestin receptors present on the serotonergic neurons[4]. Orlistat, a pancreatic lipase inhibitor, suppresses overeating behavior by promoting a change in the reuptake of serotonin in the hypothalamus[5]. Additionally, a reduction in ghrelin hormone level causes a decrease in appetite[6]. Overall, these indicate an interaction between progesterone and serotonin receptors in regulating eating behavior.

Furthermore, studies have reported that a disturbance in the progesterone level may lead to binge eating in females due to alterations in the serotonin level (serotonergic function)[7]. Reports suggest that progesterone, as a hormone, causes fattening; additionally, the use of depo-medroxyprogesterone acetate has been shown to cause weight gain. Moreover, progesterone treatment in mice resulted in a high body mass index[8,9].

Some reports have suggested that progesterone changes the pathophysiology and behavior of an organism and promotes excessive fat deposition in the body. Therefore, in the present study, it was used as a control drug to induce obesity in mice. Body weight, rearing, grooming, and ambulatory movements, food consumption behavior, and biochemical parameters were evaluated in all the groups.

Serotonin is an important neurotransmitter produced by raphe nuclei of the brainstem and plays a major role in homeostasis, and regulates food consumption via extensions to multiple regions of the brain[10]. Studies indicate that controlling serotonin levels promotes a change in eating habit. Decreased serotonin signaling in the hypothalamus contributes to adiposity by hampering the negative feedback from food intake, thus promoting overconsumption[11,12]. Evidence suggests that an increase in serotonergic signaling decreases food intake, while a decrease in serotonergic signaling leads to weight gain[13]. Serotonin activates α-melanocyte stimulating hormone leading to a loss of appetite, and it inhibits the appetite-promoting peptide Agouti and neuropeptide Y neurons present in the arcuate nucleus of the hypothalamus[14,15]. These facts prompted us to study and determine the importance of serotonin in progesterone-induced obesity.

Moreover, a decrease in dopaminergic signaling results in excessive eating and suppresses reward sensation, whereas normal dopaminergic signaling increases food-reward-associated brain activity[16]. A tendency to seek food and for adiposity is observed striatal D2 receptor knockdown animals with decreased dopamine signals[17,18]. In general, this information is in accordance with the hypothesis that a decrease in dopamine signaling (striatal pathway) may motivate individuals to over eat[17].

Several studies on hyperphagia linked to depression have reported that natural flavonoids produce antidepressant and hypophagic effects. This occurs mainly due to the activation of serotonergic and dopaminergic systems; however, activation of noradrenergic and γ-Aminobutyric Acid (GABA)-ergic neurotransmitter systems and an increase in production of Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF) are also involved[19].

Myrica nagi Thunb. (Myricaceae) is widely known as Kaiphal, Box Berry, and Katphala. Its medicinal properties include antimicrobial, anthelmintic, anti-inflammatory, anxiolytic, and mast cell-stabilizing activities, whereas the fruit of *M. nagi* possesses antimicrobial and antioxidant properties. Studies have supported that the whole plant exerts a chemoprotective effect on the skin[20]. Based on its reported properties and phytoconstituents, this plant was further selected for evaluating its anti-hyperphagic effect.

Liquid chromatography-mass spectrometry studies of the *M. nagi* leaf extract showed the presence of approximately 21 compounds. Another study showed the presence of myricanol (diarylheptanoid) that, according to molecular docking analysis, was found to be an AMP-Activated Protein Kinase (AMPK) activator with high affinity for the γ subunit of AMPK, a pathway of immense importance in obesity. *M. nagi* leaf extracts were found to contain alkaloids, tannins, flavonoids, sterols, carbohydrates, triterpenoids, and saponins[21].

The fruit and bark of *M. nagi* contain phytocompounds, such as myricetin, quercetin, and caffeic and gallic acid, which exhibit the potential to regulate metabolic disorders. *M. nagi* is known for the beneficial effects of its fruit; however, studies on its role in modulating appetite and causing behavioral alterations are limited[20].

This study was undertaken based on previous research on *M. nagi* fruit, including its phytochemical analysis

and anti-hyperlipidaemic effects[22]. The objective of this study was to establish a model for progesterone-induced obesity and understand the role of *M. nagi* in controlling hyperphagic behavior.

2. Material and Methods

2.1 Chemicals

Analytical-grade chemicals were used for all experiments. Cholesterol and triglyceride kits and o-phthaldialdehyde were purchased from Span Diagnostics, Surat, India. The insulin assay kit was obtained from CLIA Kit INC, while leptin measurements were conducted using the RayBio Rat Leptin ELISA kit (Norcross, GA, USA). Resistin levels were determined using kit from Ray Biotech, while Blood glucose measurements were carried out using the Accu-chek® glucometer (Roche Corporation, Germany), and blood glucose test strips (Roche Pvt Ltd, Mumbai, India). Tumour Necrosis Factor-alpha (TNF-α), Interleukin 6 (IL-6), Lipopolysaccharide (LPS), and C-Reactive Protein (CRP) levels were quantified using reagents from Proteintech China, Wuhan.

2.2 Plant Extraction

M. nagi was collected from the forests in Solan in the months of June and July, 2017. The study has been authenticated by the National Institute of Science Communication and Information Resources (NISCAIR) (Ref. No. NISCAIR/RHMD/Consult//2017/3102-51-4). Powdered fruit (500 g) was subjected to methanol extraction (55–65 °C) and exhalation for 4h. The extract was evaporated by a rotary evaporator (Heidolph, D-91126, Germany).

2.3 Phytochemical Testing

Complete phytochemical screening was performed as described previously[23-25].

2.4 Generation of the Mouse Model for Progesterone-Induced Obesity

Female Swiss albino mice weighing 20-25 g were housed in polypropylene cages under a 12/12 h light/dark cycle with the temperature and humidity maintained at 24 ± 5 °C and 60%, respectively. The mice were provided food pellets and water *ad libitum*. Prior approval was obtained from Institutional Animal Ethical Committee (IAEC) (Committee for the Purpose of Control and Supervision on Experiments on Animals approval numbers: RIP/IAEC/2016-2017/09 and RIP/IAEC/2018-19/01 Dated:05.05.18).

The animals were divided into six groups of 8 individuals each as described below. Group I: normal mice administered with 2% Tween 80 per oral (p.o.); Group II: progesterone (10mg/kg, s.c.) in arachis oil; Group III: progesterone and orlistat (50mg/kg,p.o.); Group IV: progesterone and methanolic extract of *M. nagi* (MEMN) (100mg/kg,p.o.); Group V: progesterone and MEMN (200mg/kg,p.o.); Group VI: progesterone and MEMN (400mg/kg,p.o.). The study duration was set as 28 days.

2.5 Progesterone-Induced Hyperphagia

Progesterone (10mg/kg) dissolved in arachis oil was subcutaneously (s.c.) administered for 28 days in the neck region (dorsal). The test drug (0.4mL/100 g body wt) was administered 30 min before progesterone administration[26].

2.6 Generation of Progesterone-Induced Obesity Model

Group I: normal mice administered 2% Tween 80 per oral (p.o.); Group II: progesterone (10 mg/kg, s.c.) in arachis oil; Group III: progesterone and orlistat (50mg/kg,p.o.); Group IV: progesterone and methanolic extract of *M. nagi* (MEMN) (100mg/kg,p.o.); GroupV: progesterone and MEMN (200mg/kg,p.o.); Group VI: progesterone and MEMN (400mg/kg,p.o.). Six groups containing eight animals in each group were finalized for the study to be conducted for 28 days.

2.7 Acute Toxicity Study (Oral)

The study was performed according to the Organization for Economic Co-operation and Development guideline no. 423 (December 17, 2001). Stepwise doses from 50 to 2000 mg/kg were administered. Effective doses of 1/5th, 1/10th, 1/20th were selected for further study[27].

2.8 Parameters Evaluated

The animals were evaluated for body weight, feeding, and behavioral parameters such as motor activity, rearing, and grooming, and Lee index was calculated. Biochemical parameters including lipid profile (Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL)) and liver enzymes (Alkaline Phosphatase (ALP) and Serum Glutamic Pyruvic Transaminase (SGPT)) were analysed using kits. Blood

glucose (glucometer), leptin, insulin (ELISA kits), serotonin, and dopamine levels were also documented. Organ weight and fat pad weight were estimated. Histopathology of the liver and mesenteric fat was analyzed. ELISA was performed using the kits as per the manufacturer's instructions. Serotonin and dopamine levels in the mouse brains were estimated through spectrofluorimetric analysis following standard methods[28,29].

3. Results

3.1 Food Consumption Behavior

Food consumption behavior was evaluated every half an hour on days 1, 14, and 28 (Table 1). The progesterone group showed a significantly (**p<0.01) higher food consumption than the control group. All drug-treated groups showed significantly (**p<0.01) lower levels of hyperphagia than Group II. On the 14th day, the intake of food in Group II was higher at 1 h and 2 h intervals than that in the control group. Groups IV and VI showed lower food consumption than the inducer group. On the 28th day, Group II showed an increase in food intake at 1 h and 2 h intervals compared with the control group at the end of 2 h. Groups IV, V, and VI showed decreased food consumption compared with the inducer group (Table 1).

3.2 Effect of MEMN on Behavioral Parameters

Behavioral parameters were evaluated on the basis of three parameters, namely, ambulatory movements, rearing, and grooming. The inducer group showed a significant decrease (**p<0.01) in ambulatory, rearing, and grooming movements compared with the control. The test groups showed significantly higher levels (**p<0.01) of ambulatory, rearing, and grooming movements than the disease control group (Table 2).

3.3 Effect of MEMN on Body Weight

Initially, body weight was monitored on the 1st day and subsequently on the 28th day of treatment. After 28 days, all mice showed weight gain compared to the control (Table 3). Groups IV, V, and VI showed a significant (**p<0.01) decrease in weight compared with Group II.

3.4 Effect of MEMN on the Lee Index

Table 4 resents the Lee index in mice; this analysis was performed at the end of the experiment.

3.5 Effect on Insulin Level

Insulin levels were significantly(**p<0.01) increased in the progesterone-treated groups, indicating diabetes and insulin resistance. The test groups showed significantly (**p<0.01) lower insulin levels, maintained within the normal range, compared to the control group (Table 5).

3.6 Effect of MEMN on Fasting Glucose

The progesterone-treated group showed an elevated blood level of glucose. In comparison, the MEMN-treated groups showed significantly (**p<0.01) lower blood glucose levels (Table 5).

3.7 Effects on Leptin

The effect of progesterone on plasma leptin levels was monitored (Table 5). The mice in Group II exhibited

Table 1. Average food consumption (g) behavior in mice on day 1, day 14, and day 28

Group	Day 1	Day 14	Day 28
Control	0.98 ± 0.1	0.97 ± 0.1	1.02 ± 0.1
Progesterone	1.01 ± 1.14	1.87 ± 0.6	1.91 ± 0.13###
Orlistat	1.17 ± 0.13	1.11 ± 0.12	1.21 ± 0.09***
MEMN (100mg/kg)	1.11 ± 0.15	1.17 ± 0.14	1.13 ± 0.13***
MEMN (200mg/kg)	1.01 ± 0.13	1.08 ± 0.13	1.09 ± 0.13***
MEMN (400mg/kg)	0.98 ± 0.13	1.11 ± 0.13	1.03 ± 0.1***

The food consumed by animals at different time points post treatment was measured. The MEMN, methanolic extract of *M. nagi*; p.o., per oral, The data is represented as mean ± SEM; n=8. One-way ANOVA is used for data analysis, following multiple comparisons through Tukey-Kramer test; *p<.05; **p<.0l; ***p<.00l; ***comparison with inducer group, ###comparison with control, ns-non-significant

Table 2. Effect of MEMN on behavioral parameters in mice (Progesterone-Induced Obesity)

Group	Ambulatory Movements	Rearing	Grooming
I-Normal control	124.18 ± 1.25	2.95 ± 0.19	15.06 ± 0.13
II-Progesterone	45.77 ± 1.12$^{###}$	0.66 ± 0.19$^{###}$	8.27 ± 0.17$^{###}$
III-Orlistat10 mg/kg.	108.94 ± 1.31**	2.62 ± 0.13**	12.64 ± 0.14**
IV-MEMN100 mg/kg	70.57 ± 1.28**	1.96 ± 0.16**	10.05 ± 0.18**
V-MEMN 200 mg/kg.	98.56 ± 1.20**	2.12 ± 0.18**	11.72 ± 0.14**
VI-MEMN 400 mg/kg	90.56 ± 1.25**	2.12 ± 0.19**	11.82 ± 0.19**

MEMN, methanolic extract of *M. nagi*; p.o., per oral. The above-listed data is represented as mean ± SEM; where the value of n = 8. One-way ANOVA is used for data analysis, following multiple comparisons through the Tukey-Kramer test; ns-non-significant, *p-value <.05; **p-value<0.01; ***p-value<.001; ***comparison to inducer group, ###comparison with control group.

Table 3. Effect of MEMN on body weight (in g)

Groups	Treatment	Weight on Day 1	Weight on Day 28
I	Control	26.68 ± 0.59 g	27.68 ± 0.57 g
II	Progesterone	27.11 ± 0.44	38.43 ± 0.69$^{###}$
III	Orlistat (10 mg)	26.61 ± 0.67	30.09 ± 0.77**
IV	MEMN (100 mg)	26.65 ± 0.53	34.16 ± 0.74**
V	MEMN (200 mg)	26.85 ± 0.79	32.13 ± 0.90**
VI	MEMN (400 mg)	25.92 ± 1.32	30.58 ± 1.90**

MEMN, methanolic extract of *M. nagi*. The above listed data is represented as mean ± SEM; where the value of n = 8. One-way ANOVA is used for data analysis, following multiple comparisons through the Tukey-Kramer test; *p<.05; **p<.0l; ***p<.00l; ***comparison with inducer group, ###comparison with control, ns-non-significant.

Table 4. Effect of MEMN on the lee index

Day	Control group	Progesterone	Orlistat	MEMN (100mg/kg)	MEMN (200mg/kg)	MEMN (400mg/kg)
28	333.44 ± 0.007	365.59 ± 0.002$^{###}$	351.36 ± 0.009***	361.5 ± 0.008***	354.39 ± 0.007***	346.54 ± 0.009***

MEMN, methanolic extract of *M. nagi*. The above listed data is evaluated using mean ± SEM; where the value of n = 8. One-way ANOVA is used for data analysis, following multiple comparisons through Tukey-Kramer test; *p<.05; **p<.0l; ***p<.00l; ***comparison with inducer group, ###comparison with control, ns-non-significant

Table 5. Effects of MEMN on insulin, blood glucose, and leptin levels in mice

Groups	Insulin (ng/mL)	Blood Glucose (mg/dL)	Leptin (ng/mL)
Control	0.6 ± 1.04	78.74 ± 0.79	1.3 ± 1.04
Progesterone	3.6 ± 1.04$^{###}$	166.90 ± 0.86$^{###}$	15.01 ± 1.04$^{###}$
Orlistat 10 mg/kg	1.6 ± 1.56***	120.09 ± 0.43**	5.23 ± 1.56**
MEMN 100 mg/kg	3.1 ± 1.77***	130.23 ± 0.44**	7.0 ± 1.77**
MEMN 200 mg/kg	2.9 ± 1.19***	124.63 ± 0.35**	6.23 ± 1.19**
MEMN 400 mg/kg	2.0 ± 1.22***	128.80 ± 0.42**	6.0 ± 1.22**

MEMN, methanolic extract of *M. nagi*; p.o., per oral. The above listed data is evaluated using mean ± SEM; where the value of n = 8. One-way ANOVA is used for data analysis, following multiple comparisons through Tukey-Kramer test; *p<.05; **p<.0l; ***p<.00l; ***comparison with inducer group, ###comparison with control, ns-non-significant.

a significant increase in plasma leptin (***p<0.001) compared to those in the control group; this result demonstrates the considerable impact of progesterone on leptin levels. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited a significant decrease (**p<0.01) in leptin levels compared with Group II mice.

3.8 Effect of MEMN on Liver Enzymes

As presented in Table 6, Group II mice exhibited a significant increase (**p<0.01) in the levels of ALP and SGPT compared with the normal control mice; this indicates the considerable impact of progesterone on the liver. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited a significant decrease (**p<0.01) in the levels of these enzymes compared to the mice treated with progesterone alone.

3.9 Effect of MEMN on Lipid Profile

The following parameters were evaluated: TC, TG, HDL, and LDL. The disease control group showed a significant increase (**p<0.01) in TC, TG, and LDL compared to the control group. The mice of Groups IV, V, and VI treated with the extract at doses of 100, 200, and 400 mg/kg, respectively, exhibited a significant (**p<0.01) decrease in the levels compared to the mice of Group II (Table 7). The HDL levels were significantly (**p <0.01) increased in mice of Group VI than in mice of Group II.

3.10 Effect of MEMN on Brain Serotonin and Dopamine Levels

The mice in Group II showed a significant (**p<0.01) decrease in the brain serotonin levels compared to the mice in the control group. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited

Table 6. Effect of MEMN on liver enzymes

Groups	ALP (IU/L)	SGPT (IU/L)
Group I Normal control	220.2 ± 1.684	43.86 ± 2.395
Group II Progesterone	415.15 ± 2.019$^{\#\#\#}$	91.57 ± 2.369$^{\#\#\#}$
Group III Orlistat 10mg/kg, p.o.	370.72 ± 2.393**	56.83 ± 1.195**
Group IV MEMN 100mg/kg, p.o.	361.35 ± 1.077**	64.46 ± 1.278**
Group V MEMN 200mg/kg, p.o.	354.39 ± 1.739**	74.055 ± 1.343**
Group VI MEMN 400mg/kg, p.o.	344.65 ± 1.821**	54.46 ± 1.35**

b.w., body weight; MEMN, methanolic extract of *M. nagi*; p.o., per oral; ALP, alkaline phosphatase; SGPT, serum glutamic pyruvic transaminase. $^{\#\#\#}$p < 0.001 , **p < 0.01, ***p < 0.001, $^{\#\#\#}$when compared with the normal control group, ***when compared with the inducer group.

Table 7. Effect of MEMN on lipid profile in progesterone-induced obesity

Treatment	Lipid Profile				
	Cholesterol (TC)	**Triglycerides**	**HDL**	**LDL**	**VLDL**
Control	101.1 ± 4.41	78.93 ± 2.25	32.16 ± 1.73	53.15 ± 5.12	15.78 ± 5.19
Progesterone	130.98 ± 8.48$^{\#\#\#}$	141.24 ± 10.76$^{\#\#\#}$	18.86 ± 1.27$^{\#\#\#}$	83.86 ± 8.50$^{\#\#\#}$	28.24 ± 4.16$^{\#\#\#}$
Orlistat (10 mg/kg)	63.06 ± 6.76**	59.24 ± 8.12**	31.33 ± 1.80**	19.87 ± 7.18**	11.84 ± 3.11**
MEMN (100 mg/kg)	129.58 ± 8.49**	148.67 ± 5.60**	19.49 ± 1.78*	80.35 ± 8.50**	29.73 ± 6.19**
MEMN (200 mg/kg)	126.01 ± 8.70**	120.22 ± 5.98*	22.61 ± 1.64**	79.35 ± 10.92**	24.04 ± 7.19**
MEMN (400 mg/kg)	103.91 ± 8.77**	88.37 ± 5.27**	29.64 ± 1.38**	56.59 ± 9.30**	17.67 ± 4.19**

HDL, high-density lipoprotein; LDL, low-density lipoprotein; MEMN, methanolic extract of *M. nagi*; TC, total cholesterol; TG, triglycerides. The above-listed data is evaluated using mean ± SEM; where the value of n=8. One-way ANOVA is used for data analysis, following multiple comparisons through the Tukey-Kramer test; *p<.05; **p<.0l; ***p<.00l; ***comparison with inducer group, ###comparison with control, ns-non-significant

Table 8. **Effect of MEMN on brain serotonin and dopamine levels in mice and obesity**

	Treatment	Brain Serotonin (ng/mg)	Dopamine
I	Control	375 ± 10.75	851.50 ± 3.18
II	Progesterone	175 ± 5.42$^{\#\#}$	581.66 ± 4.49$^{\#\#\#}$
III	Orlistat (10 mg)	328 ± 7.45**	898.50 ± 3.59**
IV	MEMN (100 mg)	225 ± 7.85**	896 ± 1.77**
V	MEMN (200 mg)	252 ± 7.82**	776.83 ± 4.3**
VI	MEMN (400 mg)	277 ± 7.66**	810 ± 4.4**

MEMN, methanolic extract of *M. nagi*. The above-listed data is evaluated using mean ± SEM; where the value of n=8. One-way ANOVA is used for data analysis, following multiple comparisons through the Tukey-Kramer test;*p<.05;**p<.0l;***p<.00l;***comparison with inducer group, ###comparison with control, ns-non-significant

Table 9. Effect of MEMN on organ weight

Organ weight (g)	Control	Progesterone	Orlistat	MEMN (100mg/kg)	MEMN (200mg/kg)	MEMN (400mg/kg)
Liver	1.22 ± 0.10	1.701 ± 0.136$^{\#\#\#}$	1.31 ± 0.155**	1.509 ± 0.164**	1.42 ± 0.180**	1.35 ± 0.169**
Heart	0.119 ± 0.01	0.208 ± 0.058$^{\#\#\#}$	0.089 ± 0.01**	0.12 ± 0.03**	0.071 ± 0.03**	0.068 ± 0.54**
Brain	0.249 ± 0.02	0.482 ± 0.02$^{\#\#\#}$	0.271 ± 0.01**	0.308 ± 0.01**	0.29 ± 0.04**	0.276 ± 0.78**

MEMN, methanolic extract of *M. nagi*. The above-listed data is evaluated using mean ± SEM; where the value of n=8. One-way ANOVA is used for data analysis, following multiple comparisons through the Tukey-Kramer test; ns-non-significant, *p<.05; **p<.01; ***p<.001; ***comparison with inducer group, ###comparison with control group.

Table 10. Effect of MEMN on fat pad weight

Fat pad weight (g)	Control	Progesterone	Orlistat	MEMN (100 mg/kg)	MEMN (200 mg/kg)	MEMN (400 mg/kg)
Mesenteric Fat	0.33 ± 0.10	0.614 ± 0.12$^{\#\#\#}$	0.397 ± 0.14***	0.44 ± 0.18**	0.355 ± 0.13**	0.325 ± 0.22**
Perirenal Fat	0.261 ± 0.19	0.483 ± 0.20$^{\#\#\#}$	0.305 ± 0.40***	0.38 ± 0.30***	0.266 ± 0.11***	0.239 ± 0.17***

MEMN, methanolic extract of *M. nagi*. The above-listed data is evaluated using mean ± SEM; where the value of n = 8. One-way ANOVA is used for data analysis, following multiple comparisons through the Tukey-Kramer test; *p<.05; **p<.0l; ***p<.00l; ***comparison with inducer group, ###comparison with control, ns-non-significant.

a significant (**p<0.01) increase in the brain serotonin levels compared to Group II mice (Table 8). Group II mice showed a significant (**p <0.01) increase in dopamine levels compared to the control group mice. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited a significant (**p <0.01) decrease in dopamine levels compared with Group II mice (Table 8).

3.11 Effect of MEMN on Organ Weight

The effect of MEMN on organ weight was studied on the liver and kidneys (right and left) (Table 9). The disease group exhibited a significant (**p<0.01) increase in organ weights compared with the control group. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited a significant (**p<0.01) decrease in organ weights compared to Group II mice.

3.12 Effect of MEMN on Fat Pad Weight

Group II mice exhibited an increase in fat pad weight compared with the control mice. The fat level was high in Group II mice; however, the mesenteric and perirenal fat were significantly (***p<0.001) lower in all test groups than in Group II (Table 10).

3.13 Estimation of TNFα

The effect of progesterone on plasma TNFα levels was monitored (Figure 1). The mice in Group II exhibited

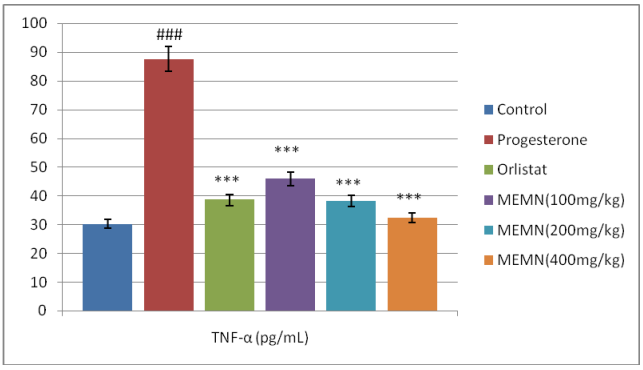


Figure 1. Effect of MEMN on TNFα.

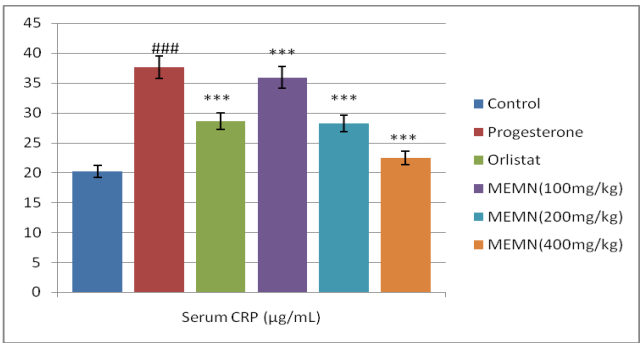


Figure 2. Effect of MEMN on CRP.

a significant increase in plasma TNFα (***p<0.001) compared to those in the control group; this result demonstrates the considerable impact of progesterone on TNFα levels. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited a significant decrease (**p<0.01) in TNFα levels compared with Group II mice.

MEMN, methanolic extract of *M. nagi*. ###p<0.001 when compared with the normal control group, ***p<0.001 when compared with the inducer group.

3.14 Estimation of CRP

The effect of progesterone on plasma CRP levels was monitored (Figure 2). The mice in Group II exhibited a significant increase in plasma CRP (***p<0.001) compared to those in the control group; this result demonstrates the considerable impact of progesterone on CRP levels. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited a significant decrease (**p<0.01) in CRP levels compared with Group II mice.

MEMN, methanolic extract of *M. nagi*. ###p < 0.001 when compared with the normal control group, ***p < 0.001 when compared with the inducer group.

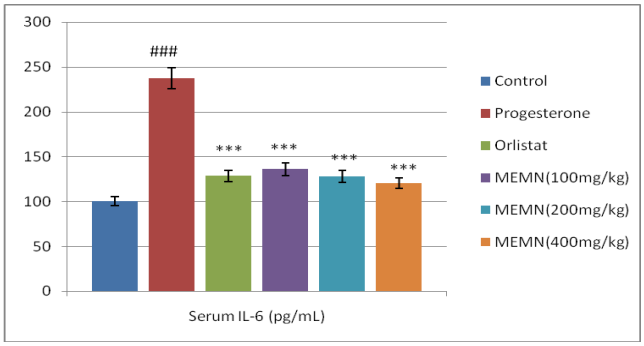


Figure 3. Effect of MEMN on IL-6.

3.15 Estimation of IL-6

The effect of progesterone on plasma IL-6 levels was monitored (Figure 3). The mice in Group II exhibited a significant increase in plasma IL-6 (***p<0.001) compared to those in the control group; this result demonstrates the considerable impact of progesterone on IL-6 levels. The mice treated with the extract at doses of 100, 200, and 400 mg/kg exhibited a significant decrease (**p<0.01) in IL-6 levels compared with Group II mice.

MEMN, methanolic extract of *M. nagi*. ###p < 0.001 when compared with the normal control group, ***p < 0.001 when compared with the inducer group.

3.16 Histopathology

Histopathology results revealed uniform spherical cells with normal shape and structure in Group I; decreased membrane integrity, accumulation of fat, and remarkable cell size reduction in Group II; and commendable and effective decrease in cell size and reduction in membrane damage in Groups III, IV, V, and VI. MEMN (400 mg/kg) showed a more relevant result than the control (Figure 4).

Histopathological analysis of the liver revealed necrosis in all the groups. The liver tissue of the progesterone-treated mice showed a high level of necrosis and that of the MEMN (400mg/kg)-treated mice exhibited minimal damage to the cells. This result revealed that the extract protected against liver damage (Figure 5).

Group I had uniformly spherical cells with normal shape and structure, Group II exhibited decreased membrane integrity, the accumulation of fat, and a notable change in cell size. Groups III, IV, V, and VI exhibited an effective decrease in cell size, reduction in membrane damage, and decreased fat accumulation.

MEMN, methanolic extract of *M. nagi*.

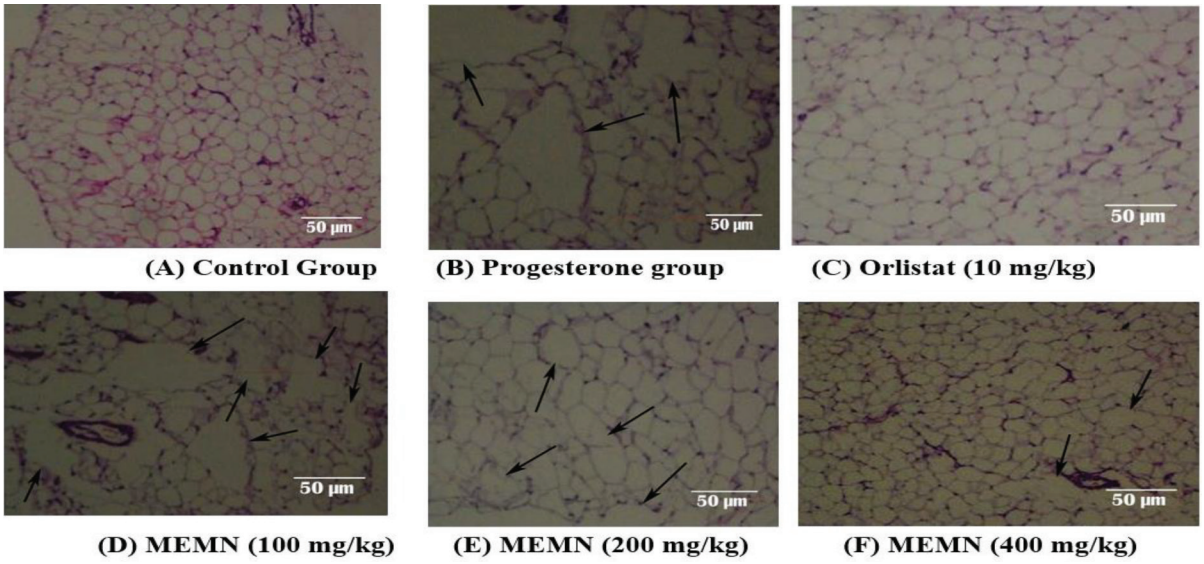


Figure 4. Histopathology of the mesenteric fat pad of mice.

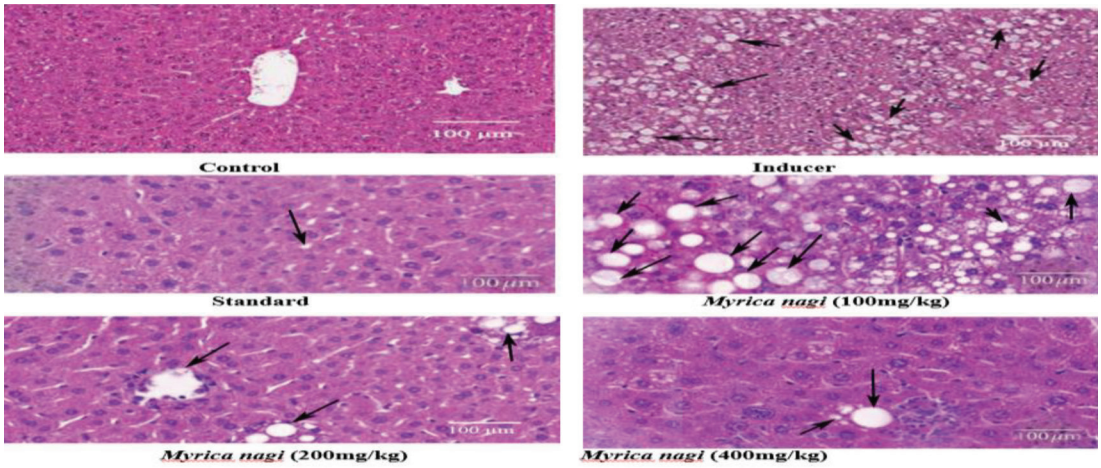


Figure 5. Histopathology of mouse liver.

No Necrosis in Group I. Group II Liver tissues from progesterone-treated mice showed high levels of necrosis, No cell damage in Group III, Group IV showed lipid droplets with necrosis, Group V hepatic protection was observed; Group VI minimal damage to liver cells.

MEMN, methanolic extract of *M. nagi*.

4. Discussion

Hyperphagia induced by progesterone may be mediated by the γ-aminobutyric acid type A (GABAA) receptor system[30]. Our data on the effect of progesterone on dopamine levels suggest a pivotal role of neurosteroids in hyperphagia and reinforce the role of phytochemicals in regulating feeding behavior.

The reason for weight gain is the imbalance between calorie intake and expenditure[31,32]. The female hormone progesterone is known for maintaining pregnancy. However, the progesterone metabolite allopregnanolone acts as a male hormone, as the adrenal glands produce it in both sexes. Additionally, the synthesis of both these hormones occurs in the nervous system in association with adaptation to stress. Moreover, the increase in progesterone production may be due to the response of neural cells to injury in the brain. It is quite possible that non-reproductive progesterone may act through allopregnanolone, which without binding to PR, may

act as a positive modulator of the GABAA receptor[33,34]. Allopregnanolone (GABAA receptor steroid) increases dopamine release in the nucleus accumbens[35]; thus, GABA and allopregnanolone might indirectly influence dopamine-induced food intake.

The fruit of *M. nagi* has recently attracted great interest due to its consumption by the local population for relieving stomach pain and vertigo. A phytochemical investigation revealed that it contains carbohydrates, flavonoids, glycosides, saponins, tannins, and alkaloids. Flavonoids, saponins, alkaloids, and tannins have been reported to reduce cholesterol levels and exhibit antioxidant activity[20]. In this study, *M. nagi* was found to be useful for controlling hyperphagia and hyperlipidaemia due to the presence of the aforementioned phytoconstituents. The present study is a continuation of our previous *in vitro* study that proved the presence of phytoconstituents like myricetin, quercetin, gallic acid, and caffeic acid in the fruit of *M. nagi*. These phytocompounds can act on various enzymes that play a strong role in obesity and diabetes[36].

In agreement with our findings, previous reports have indicated that sodium-dependent vitamin C transporter 1 (intestinal transporter for vitamin C) and glucose transporter 2 are inhibited by flavonoids, leading to decreased absorption of glucose. Additionally, flavonoids reduce hyperglycemia by inhibiting glucose reabsorption in the kidneys through inhibition of the symporters (sodium–glucose) located in the proximal convoluted tubule of the kidneys[19].

Based on previous studies, the present work was advanced to include *in vivo* studies. Initially, toxicity testing was performed on mice by treating them with a sample (methanolic extract) at an LD_{50} of 2000mg/kg, p.o. dose, which clearly showed no toxicity in the mice. In the present study, subchronic treatment, i.e., 28 days of progesterone treatment (10mg/kg, s.c.), elicited marked hyperphagia and weight gain compared to the vehicle treatment (control group) at all time intervals.

Our results established the effect of progesterone (10mg/kg) on hyperphagia in female rats. This effect was possibly due to the anti-oestrogen action of progesterone, resulting in increased food consumption[37]. A high dose of progesterone (10mg/kg) produces an anorectic response, which shows that in low doses, progesterone may not modulate feeding behavior[38]. Thus, our results are consistent with the literature. In the present study, progesterone treatment in mice resulted in increased food consumption at 30min, 1h, and 2h; however, MEMN administration (100, 200, and 400 mg/kg) resulted in reduced food consumption.

Further, the progesterone-treated mice exhibited a significant decrease in exploratory behavior compared to the control group; however, mice treated with MEMN at doses of 100, 200 and 400 mg/kg showed a significant increase in the number of ambulatory, rearing, and grooming movements compared to the disease control mice.

The thermogenic effect of natural progesterone is well known, and progesterone treatment has been reported to increase the body temperature; therefore, measurement of the rectal temperature was considered as one of the parameters in this study[7]. In our study, we observed a significant increase in the body temperature of progesterone-treated mice compared to that of normal control mice. However, treatment with MEMN decreased the body temperature to the normal range.

Serotonin, dopamine, and GABA imbalances lead to changes in feeding behavior[39-41]. Hyperphagia due to progesterone occurs by the inhibition of 5-hydroxytryptamine (5-HT; serotonin) reuptake at the hypothalamic site; this suggests the possible existence of a relationship between progesterone and the serotonin receptor system in the regulation of food intake and body cfweight[42]. In this study, administration of MEMN (100, 200, and 400 mg/kg,p.o.) led to a reduction in food intake and weight through the alteration of dopamine and serotonin levels.

Based on these results, we hypothesize that after the extract is absorbed through the gastrointestinal tract, it crosses the blood–brain barrier and enters the brain. Further, it may lead to the amplification of signaling in the hypothalamus (the master regulator of food intake and energy expenditure) and may inhibit the reuptake of 5-HT, leading to the activation of β-adrenergic receptor, which is associated with the promotion of fat loss[7,43].

Moreover, the investigation revealed that this model is associated with an increase in serum lipids, including TC, TG, LDL, and VLDL, and a decrease in HDL. The liver function test also showed an increase in ALP and SGPT levels. However, there was a significant decrease in TG, TC, LDL, VLDL, ALP, and SGPT and an increase in HDL. This result may be due to the protective effect of MEMN at 100, 200, and 400 mg/kg, p.o. A significant (**p <0.01) increase in serum HDL levels was observed in mice treated with MEMN at doses of 100, 200, and 400

mg/kg, p.o. Considering this result, it can be concluded that MEMN is a cardio-protective agent.

Leptin and adiponectin are the key hormones in the treatment of obesity. Leptin, which is secreted by fat cells and encoded by the LEP gene, is a satiety hormone that helps in maintaining a normal weight by regulating food intake and body weight. Circulating adiponectin can target multiple tissues and regulate insulin sensitivity as well as fatty acid oxidation; low levels of adiponectin lead to insulin resistance[44-47]. In this study, *M. nagi* at doses of 100, 200, and 400 mg/kg induced a decrease in leptin levels and an increase in adiponectin levels; this signifies its potential for treating insulin resistance. Histopathology revealed fat accumulation that indicated steatosis, which produced a yellow and bulky appearance. However, the condition was reversed in rats treated with MEMN at doses of 100, 200, and 400 mg/kg,p.o. and in those treated with orlistat.

MEMN exhibited anti-obesity, hepatoprotective, and anti-hyperlipidaemic effects in our study. Additionally, the plant extract lowered the liver enzymes ALP and SGPT. To accomplish a successful pharmaceutical intervention using the methanolic extract of *M. nagi* to reverse cholesterol transport and inhibit increased lipogenesis, it must be further evaluated in clinical studies.

5. Conclusion

Collectively, the results from the present study suggested the use of herbal extracts in the treatment of hyperphagia associated with obesity and diabetes. Moreover, it is well-established over decades that herbal-based drug candidates show fewer side effects than allopathic drugs. The methanolic extract of this fruit has been suggested to exhibit beneficial effects against obesity; thus, it could potentially serve as an effective agent for the treatment or prophylaxis of obesity.

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