ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study The Effect Of *Withania somnifera* Dry Fruit Ethanolic Extract On Cognitive Function In High Fat Diet Induced Obesity Rats.

Prameelarani A[1], and Ahemadi Banu*.

*Research Scholar, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.
[1]Department of Pharmaceutical technology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.

ABSTRACT

Currently, the cognitive function of high fat diet- induced obesity rats was investigated in the presence of Withania somnifera dry fruit ethanolic extract. Obesity rats were prepared by feeding 30 days of high fat diet, confirmed obesity rats were divided into groups and Withania somnifera was then treated. Obesity rats were prepared by feeding of high fat diet for 30 days, confirmed obesity rats were divided in into groups, and then treated Withania somnifera fruit ethanolic extract (100 and 200 mg/Kg B.wt.) for 30 days. Cognitive function was assessed at the time of 25th, 27th, 30th, 42th, 44th and 45th day and lipid profile and body weight was determined on 30th and 45th day, antioxidant activity was estimated at the end of 45th day, results were interpretated using graph pad prism 5. Results were concluded that rats significantly increased body weight, cognitive impairment and increased total cholesterol, triglycerides, LDL and decreased HDL in high fat diet rats, that was ameliorated by treatment of Withania somnifera fruit ethanolic extract. Conclusion that the Withania somnifera fruit treatment enhanced cognitive function as well as maintained balanced lipid profile in obesity rats.

Keywords: Obesity, Withania somnifera fruit ethanolic extract (Ws Et), High fat diet (HFD), Cognitive function, Lipid profile.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Obesity is one of the main health concerns in today's society. It is mainly described as an excessive increase in body weight, with disproportionate accumulation of body fat mass, caused by excess energy intake over energy expenditure over a long period of time (Paternain L et al., 2011). Besides the well-recognized set of metabolic alterations, obesity is also suggested to be associated with psychiatric disorders, such as anxiety and depression2 (albeit not all studies show such association) (Kress AM et al., 2006; Ohayon MM and Hong, 2006; De wit L et al., 2010). Depression in itself is a serious chronic mental disorder characterized by health complications including, among others, anhedonia and changes in appetite and pattern of food intake (Wong ML et al., 2001; Grippo AJ et al., 2003; Elizalde N et al., 2008; Bekris S et al., 2009). Mild environmental stressors, to which individuals are exposed daily, can be both triggers of depression and alter feeding behaviors. Thus, these may, directly or indirectly, contribute to the metabolic changes triggered by elevated glucocorticoids, as well as to the onset of obesity (Stone A et al., 1994; Epel E et al., 2001) However, it is interesting to note that the individual's response to stress depends on various factors, including the type of stressors, their intensity, frequency and duration (Diane A et al., 2008) In fact, the individual specificities in the stress response, namely in terms of metabolism, may explain some of the controversies in the literature. Studies in animal models of stress showed that while intense and painful stressors result in inhibition of food intake and exposure to chronic mild stressors leads to a reduction in food intake (Marin MT et al., 2007) other mild stressors induce spontaneous feeding. Still, most of the studies have focused on the acute effect of stress on animals' feeding behavior and are mostly conducted using healthy animals displaying normal body weight and fed with regular rodent diet. Therefore, information is lacking on the effects of stress in animal models under other types of diets, namely high-fat diet (HFD). Here we used a model of HFD-induced obesity to evaluate the consequences of HFD on mood and cognition.

Withania somnifera (L.) Dunal is an important medicinal plant of family Solanaceae. It is commonly called as Ashwagandha, Asgandh, or Indian ginseng and has been extensively used in Indian, Unani, and African traditional medicine (Mishra et al., 2000). Withania somnifera possesses immense therapeutic potential and is known for its immunomodulatory (Rasool and Varalakshmi, 2006), anti stress (Archana and Namasivayan, 1998), cardioprotective (Mohanty et al., 2004), anti aging, antioxidant, anti-inflammatory (Mishra et al., 2000), anti-tumor (Wadhwa et al., 2013), neuroprotective, and anti-brain cancer activities (Kataria et al., 2011). The fruits and seeds of Withania somnifera are used as a diuretic, hypnotic and also used in curdling plant milk to prepare vegetarian cheese (Saritha and Naidu, 2007). The protective effects of Prunus Withania somnifera fruit ethanolic extract on cognitive impairments in rats fed with HFD were investigated in this study.

MATERIAL AND METHODS

Plant material and its identification

Dried ripe fruits of Withania somnifera were procured from the local market, Guntur, Andhra Pradesh. Fruits were found to contain 20.2% husk, 41.2% seeds and 31%pulp. Drug was first crushed and reduced to coarse powder.

Preparation of fruits of Withania somnifera ethanolic extract

Withania somnifera fruit powder was extracted with 7 volumes of 95% ethanol in Soxhlet apparatus at 60-70°C for 6 h. The filtrate was distilled and concentrated under reduced pressure at low temperature (40°C) in rotaevapour. A dark brown, semi-solid residue was obtained. It was stored at 4°C and used for further studies.

Experimental procedure for evaluate cognitive behavior on high fat diet induced obesity animal model Animals

Adult Wistar rats (180-220g) either sex was procured from MKM, Hyderabad, India. Animals were kept in an ambient temperature (24±1°C) colony room under a light / dark cycle of 12/12 hours. Animals were provided with an adequate supply of food and water. Animals have received adequate food and water supplies. Animals have been taken care of in accordance with the CPCSEA, New Delhi and

experimental protocols with the approval of the Committee on Institutional Animal Ethics (345/IAEC/SICRA/PhD/2017).

Preparation of high fat diet

The normal pellet diet (40%) was grinded to its fine state and sieved. The animal fat procured from the local market, cut into small pieces and placed in a clean and neat tray. Small quantity of the powdered pellet diet was spread onto the animal fat (25%) and added to proportion of coconut oil (6%) and mix well. Other ingredients (Fructose (10%), Casein (6%), Egg protein (12%), Minerals and vitamins (0.5%), sodium chloride (0.5%)) were added one by one and prepare a dough mass. It is stored in a well sealed container for preventing fungal attack and it is freshly prepared each day. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats.

Experimental design

The rats were divided into 3 groups with 6 in each group.

Group I- Normal rats
Group II-Hyperlipidemic rats treated with Sd.CMC
Group III-Hyperlipidemic rats treated with 100 mg WsEt/kg b.w./day for 30 days.
Group IV- Hyperlipidemic rats treated with 200 mg WsEt/kg b.w./day for 30 days.

The vehicle or WSEt were administered to the rats using a gastric force feeding needle. Both groups of rats were fed HFD during the first 30 days of the treatment, after that HFD was replaced with normal standard diet for the second 30 days of the treatment. Body weights, serum lipids, and lipoprotein levels were measured on the 30^{th} day and 45^{th} day after the treatment and also assess behavior pattern on day of 25^{th}, 27^{th}, 30^{th}, 42^{th}, 44^{th} and 45^{th} such as special learning and memory in the Morris water maze test and step-down avoidance test (Gacar N et al., 2011).

Biochemical parameters

At the completion of the experiment, blood samples were collected by puncture from the retro orbital plexus and transferred to heparinated tubes and centrifuged at 3000 rpm for 10 min at 4 ° C. To measure total cholesterol, HDL cholesterol and triglycerides (TG), the plasma was used.

Twenty four hour after the last treatment, all the animals were euthanized by cervical dislocation and the brain was dissected out from the cranial cavity. The brain was washed in 0.9 % NaCl solution and kept in an ice cold PBS (pH 7.4) in a petriplate and was minced into small pieces. It was further homogenized immediately in Teflon homogenizer under the cold condition and cold centrifuged at 4°C to obtain 10 % w/v brain tissue homogenate was subjected for estimation of total protein, reduced glutathione (GSH) (Prince PSM. and Menon VP 1999), superoxide dismutase (SOD) (Huang HF 2012), inflammatory mediators such as TNF α, IL 1β (Cui G 2012) and acetyl choline esterase (AchE) (Pohanka 2011) and also performed behavioral pattern of mice.

Statistical analysis

All data are expressed as the means ± SEM. Statistical differences among the experimental groups were tested by using a one way analysis of variance (ANOVA) and Dunnet test was employed for multiple comparisons. P-values less than 0.05 were accepted as significant

RESULTS

Table: 1, 2; Fig 1,2 Showed that the , after feeding HFD, there was a significant rise in the levels of serum TG, TC, LDL along with a decrease in HDL cholesterol in the rats. Treatment with Ws.Et for first 30 days resulted in a significant decrease in the levels of serum TG, TC, LDL and increased HDL, despite feeding on HFD during the period of treatment. After withdrawal of HFD, continuation of the treatment of group III and IV rats with Ws.Et for the next 15 days has resulted in a further significant decrease in the levels of serum TG, TC, LDL

ISSN: 0975-8585

cholesterol to normal levels whereas in group II rats serum lipids and lipoprotein levels remained higher than those in the controls.

Table 1 : Effect of Ws Et on lipid profile in obesity and HFD feeding rats.

Group	After 30 days treatment of Ws Et. With HFD				
	TG	TC	HDL	LDL	Bd wt.
I	129±1.8	101±1.3	35±1.3	44.3±1.4	161±2.5
II	434.8±6.5***	376.8±3.8***	20.3±0.9***	269.4±3.8****	424.8±0.7***
III	332.8±4.2	325.2±0.8	29.4±0.9	238.9±4.1	399.89±2.1
IV	262.6±2.8*	274.7±2.3*	32±1.3*	187.3±2.4*	349.8±3.5*

Values are expressed as mean +S.E.M of 6 animals. The plasma lipid parameters and bd.wt. significantly (P< 0.001****) increased in HFD feeded rats compared to normal rats similararly lipid parameters and bd.wt were significantly (P< 0.05^{b*}) decreased in Ws.Et. treated rats

Fig1: Effect of Ws Et on lipid profile in obesity and HFD feeding rats.

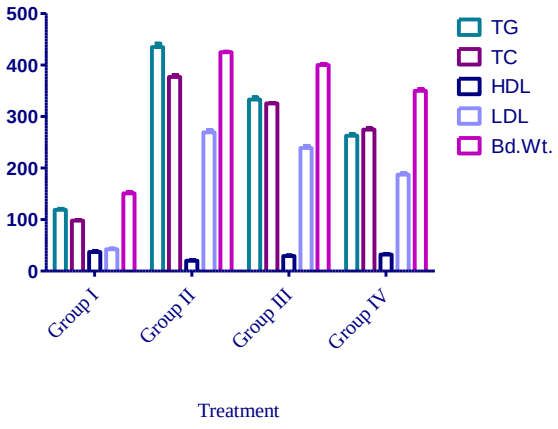

Table 2: Effect of Ws Et on lipid profile in obesity and without HFD feeding rats.

Group	After second 15 days treatment with Ws et. without HFD feeding				
	TG	TC	HDL	LDL	Bd wt.
I	129±1.8	118±1.3	39±1.3	48.3±1.4	171±2.5
II	345.8±2.4***	352.6±2.2***	24.5±0.8***	249.9±1.9***	398.9±4.9***
III	274.7±2.8	233.9±3.9	28.5±1.8	223.6±3.5	332.8±5.5
IV	202.9±1.9**	198.8±1.8**	32.5±0.9**	194.7±3.5**	286.8±6.4**

Values are expressed as mean +S.E.M of 6 animals. The plasma lipid parameters and bd.wt. Significantly (P< 0.001****) increased in HFD feeded rats compared to normal rats similararly lipid parameters and bd. wt were significantly (P< 0.01*) decreased in Ws.Et. treated rats

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Fig 2: Effect of Ws Et on lipid profile in obesity and without HFD feeding rats.

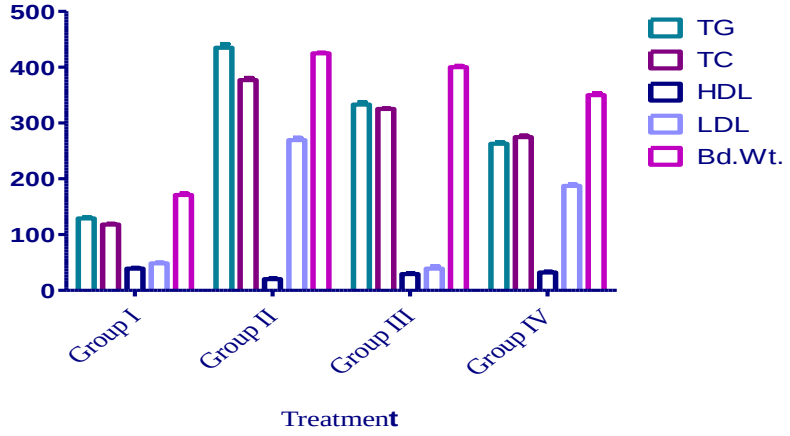

Table: 3; Fig: 3 showed that Spatial learning ability was measured using the Morris water maze test. The escape latency time (in seconds) is demonstrated for the 7 days of reference memory testing in Ws.Et with HFD feeding rat and Ws.Et. treated rats without HFD treated rats. The escape latency (sec) of the 7-day trial was significantly increased in the HFD group as compared to normal group rats. Treatment with Ws.Et for first 30 days resulted in a significant decreased escape latency time compared to normal group rats, similarly after withdrawal of HFD, continuation of the treatment of group III and IV rats with Ws.Et for the next 15 days has resulted in a further significant decreased in latency time compared to HFD feeding rats

Table 3: Effect of Ws Et. and obesity on special learning and memory in the Morris water maze test.

Group	Escape latency time (Sec)						
	After 30 days treatment of Ws Et. With HFD feeding				After second 15 days treatment with Ws Et. without HFD feeding		
	Day 1 (1st day)	Day 2 (25th day)	Day 3 (27th day)	Day 4 (30th day)	Day 5 (42nd day)	Day 6 (44th day)	Day 7 (45th day)
I	61.5±1.2	60.4±0.4	55.1±1.2	53.2±1.2	52.7±0.3	48.5±0.6	44.3±1.2
II	60.3±1.3	60.4±0.7	61.2±0.9	60.2±1.2[a]*	64.2±1.2	59.2±0.8	58.3±1.2
III	62.3±1.2	61.3±1.2	58.3±0.3	51.3±1.1	45.3±1.3	41.2±1.2	39.4±0.7
IV	63.5±1.6	58.2±0.9	56.4±0.4	48.3±0.7[b]*	35.6±0.8	33.2±0.4	29.3±0.7***

Values are expressed as mean +S.E.M of 6 animals. p<0.0001*** compared to HFD feeding rats, p<0.05[a]* Compare to normal rats; p<0.05[b]* compare to HFD feeding rats

Fig 3: Effect of Ws Et. and obesity on special learning and memory in the Morris water maze test.

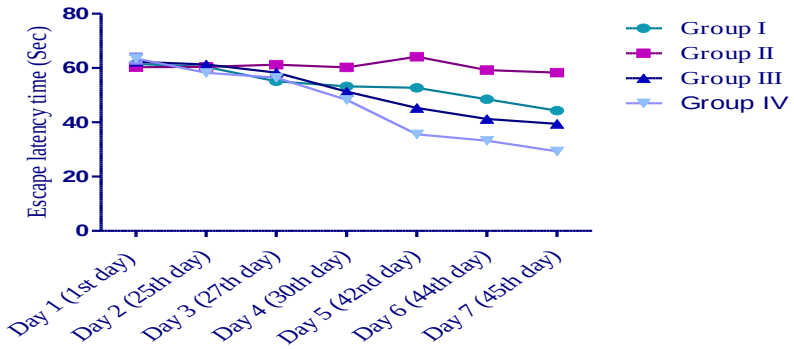

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Table 4; Fig: 4 Short-term memory was measured using the step-down avoidance test. The latency time (sec) was significantly decreased to in the HFD group as compared to the control group (P<0.05). After 30 days of treatment Ws Et. Group was exhibited significant increased in latency time compared to the normal group. Similarly after withdrawal of HFD, continuation of the treatment of group III and IV rats with Ws.Et for the next 15 days has resulted in a further significant increased in latency time compared to HFD feeding rats.

Table 4: Effect of Ws Et. and obesity on special learning and memory in the step-down avoidance test.

Group	Latency time (Sec)						
	After 30 days treatment of Ws Et. With HFD feeding				After second 15 days treatment with Ws Et. without HFD feeding		
	Day 1 (1st day)	Day 2 (25th day)	Day 3 (27th day)	Day 4 (30th day)	Day 5 (42nd day)	Day 6 (44th day)	Day 7 (45th day)
I	250.5 ± 2.5	245.4 ± 0.9	255.6 ±2.1	267.6 ±2.4	269.6 ±2.3	271.3 ±2.1	277.4±1.6
II	220.3±2.3	223.4±2.7	181.2±3.9	170.2±3.2^{a***}	187.2±3.2	193.2±3.8	198.3±4.2^{a***}
III	218.8±1.9	219.8±3.8	198.7±2.6	199.4±2.1	212.8±2.8	221.3±2.8	220.7±2.9
IV	209.4±1.3	208.4±2.9	210.4±3.2	213.5±1.8*	221.5±1.2	230.5±1.9	234.6±1.9^{b***}

Values are expressed as mean +S.E.M of 6 animals. p<0.0001^{a***} compared to normal rats; p<0.0001^{a***} compared to HFD feeding rats, p<0.05* Compare to HFD feeding rats

Fig 4: Effect of Ws Et. and obesity on special learning and memory in the step-down avoidance test.

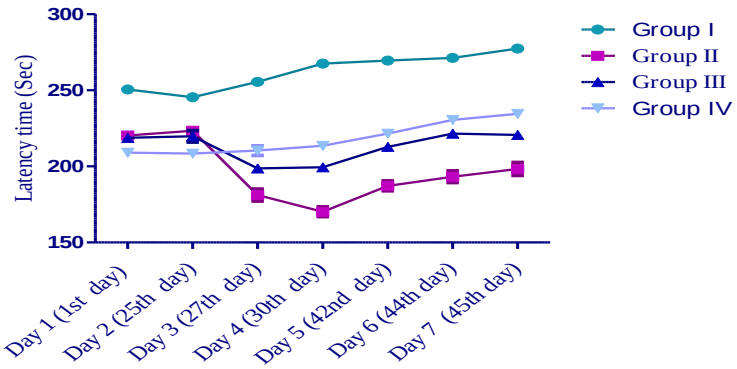


Table 5; Fig 5,6,7,8 showed that the HFD feeding rats were exhibited significant increase protein content from 35.3 ± 0.5 to 12.6 ± 2.1 (p < 0.01), SOD from 61.3±2.2 to 19.32±4.2 (p<0.0001). GSH levels from 0.09±0.01 to 0.02±0.001 (p< 0.001) and increased Ach E activity from 0.05±0.01 to 1.82±0.02 compared to normal rats. At the treatment of Ws.Et (200 mg/kg bd.wt.) rats exhibited significant increased Protein (21.3±1.6), SOD (35.98±1.3), GSH (0.05±0.021) and decreased Ach E (0.54±0.01) compared to normal rats.

Table: 5 Effect of Ws.Et. on brain parameters in HFD feeding rats

Group	Protein content [µg/mg tissue]	SOD (units/mg pr)	GSH (µmole of GSH/mg pr.)	µmole of acetylthiocholine iodide hydrolyzed/ min/mg pr.
I	35.3±0.5	61.3±2.2	0.09±0.01	0.05±0.01
II	12.6±2.1**a	19.32±4.2***a	0.02±0.001***a	1.82±0.02***a
III	15.6±1.8	27.34±2.1	0.03±0.002	0.87±0.04
IV	21.3±1.6**	35.98±1.3**	0.05±0.021**	0.54±0.01**

ISSN: 0975-8585

Values are expressed as mean +S.E.M of 6 animals. p<0.0001[a***] ; p<0.01[a**] compared to normal rats; p<0.01[**] Treatment group compared to HFD feeding rats.

Fig 5: Effect of Ws.Et. on brain GSH in HFD feeding rats

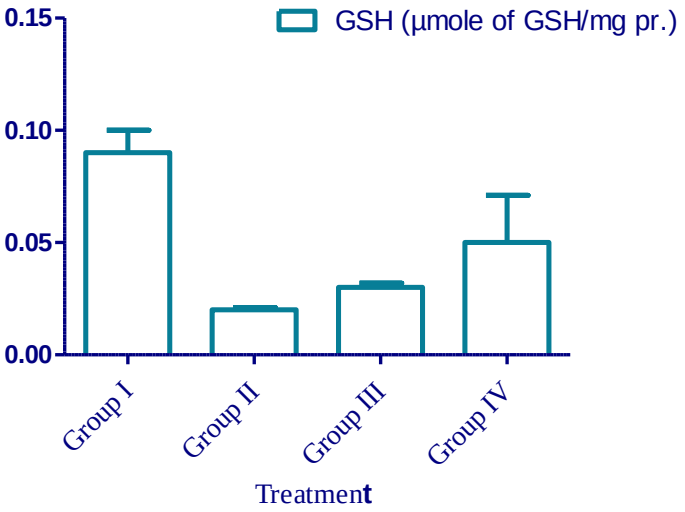


Fig 6: Effect of Ws.Et. on brain Ach E in HFD feeding rats

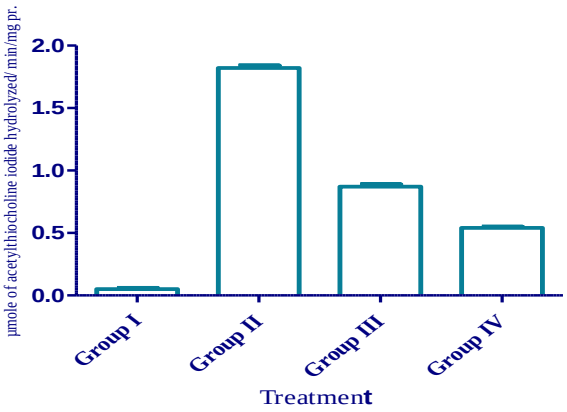


Fig 7: Effect of Ws.Et. on brain protein and SOD in HFD feeding rats

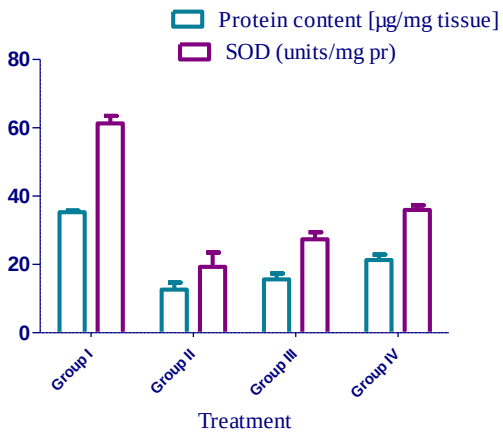


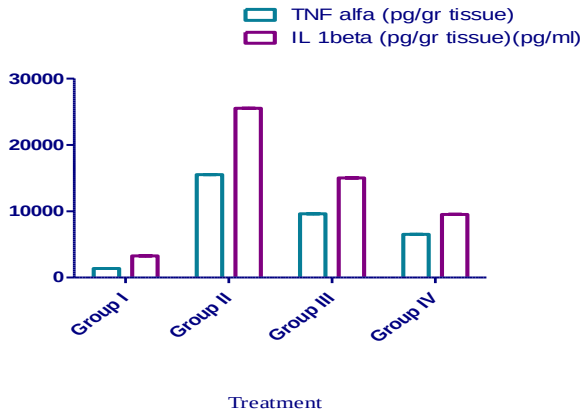
Table 6; Fig: 8 revealed that TNF alfa and IL 1beta levels were significantly (P< 0.001) increased from 1350±12.3 to 15500±22.4, from 3250±21.5 to 25500±13.5 respectively, in high fat diet feeding rats compared to normal rats, similararly TNF alfa levels, IL 1beta levels were significantly decreased to 6500±15.2 (P < 0.01), 9500±25.3 respectively, in Ws.Et. (200 mg/kg B.wt.) treated rats.

Table 6: Effect of Ws.Et. on brain TNF alfa and IL 1 beta in HFD feeding rats.

Group	TNF alfa (pg/gr tissue)	IL 1beta (pg/gr tissue)(pg/ml)
I	1350±12.3	3250±21.5
II	15500±22.4***	25500±13.5***a
III	9600±15.8	15500±15.5
IV	6500±15.2**	9500±25.3**b

All values are expressed in Mean±SEM. p<0.0001^{a***}; p<0.01^{a**} compared to normal rats; p<0.01** Treatment group compared to HFD feeding rats

Fig 8: Effect of WA.Et. on brain TNF alfa and IL 1 beta in HFD feeding rats.



DISCUSSION

The principal finding of the current study is that withania somnifera prevents memory impairment induced by HFD. In correlation, melatonin prevented levels of major oxidative stress biomarkers in the brain such as GSH, inflammatory mediators and cognitive impairment induced by HFD.

Currently, there is growing interest in clarifying the roles of life style and dietary habits in neural health. Several studies have shown that diet rich in saturated fat and refined sugar can decrease cognitive functions [Pintana H et al., 2012]. HFD aggravates cognitive function impairment during other conditions/diseases such as aging (Soontornniyomkij V et al., 2016), chronic stress (Allegra M 2003), sleep deprivation (Alzoubi KH et al., 2013), and can accelerate the course of dementia in Alzheimer's disease (Vandal M et al., 2014). In accordance, current work showed that HFD consumption impairs memories.

HFD was shown to induce increased production of free radicals that leads to cell damage (Zhang X et al., 2005). HFD leads to an imbalance between reactive oxygen species and antioxidant enzyme/species (Ribeiro MC et al., 2009). It has been reported that HFD increases brain oxidative stress via decreasing the levels of GSH and increasing the levels of GSSG, leading to reduced GSH/GSSG ratio (Alzoubi KH et al., 2013). This occurs alongside, a reduced activity of major antioxidative enzymes such as superoxide dismutase, reduced acetyl choline esterase enzymes and also increased inflammatory mediators such as TNF α and IL 1β. These results support the current study findings that showed that HFD caused significant changes in brain oxidative stress biomarkers. The activity of antioxidant enzyme, SOD was reduced by HFD. In addition, the GSH/GSSG ratio was reduced, decreasing the scavenging effect of glutathione in the brain. The reduction in the

antioxidant defense mechanisms increases oxidative stress in the brain and provides a reasonable explanation for memory deficits accompanying HFD. In fact, oxidative stress is associated with cognitive functions impairment in several other health conditions such as Alzheimer's disease (Markesbery WR et al., 1998), sleep deprivation [Alzoubi KH et al., 2018], and aging [Nicolle MM et al., 2001]. Withania somnifera possesses immense therapeutic potential as neuroprotective and studies have shown that Withania somnifera has a significant and positive effect on the cognitive functions (Kataria et al., 2011) and also exhibited antioxidant activity (Mishra et al., 2000).

In this study, we showed that administration of Withania somnifera fruit ethanolic extracts prevents memory impairment induced by HFD. Previous studies have documented the protective effect of Withania somnifera on learning and memory deficits induced by a number of pathological or physiological conditions such as Alzheimer's disease [Jayaprakasam et al., 2010], aging and brain tumor [Kataria et al., 2011].

In conclusion, Withania somnifera prevents memory impairments induced by HFD, probably via preventing oxidative stress and inflammatory mediators levels in the brain.

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