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Research Article

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Study the Effect of *Prunus amygdalus* Dry Fruit Ethanolic Extract on Cognitive Function in High Fat Diet Induced Obesity Rats

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ABSTRACT

Currently, the cognitive function of high fat diet- induced obesity rats was investigated in the presence of Prunus amygdalus dry fruit ethanol extract. Obesity rats were prepared by feeding 30 days of high fat diet, confirmed obesity rats were divided into groups and Prunus amygdalu 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 Prunus amygdalus 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 Prunus amygdalus fruit ethanolic extract. Conclusion that the Prunus amygdalus fruit treatment enhanced cognitive function as well as maintained balanced lipid profile in obesity rats.

Keywords: Obesity; *Prunus amygdalus* fruit ethanolic extract (PA Et); High fat diet (HFD); Cognitive function; Lipid profile

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INTRODUCTION

Overweight and obesity are evaluated to influence more than 2 billion people worldwide, which has caused an enormous strain on the framework of human services [1]. Human obesity is linked to the use of high fat weight management plans (HFDs) [2]. Although the relationship between overweight condition and its adverse effects on the brain remains unclear, studies have shown that obesity and deposition of body fat play an important role in the pathogenesis of certain brain - related diseases [3]. Moreover, increasing evidence suggests that overweight and HFD conditions can also lead to long - term memory loss, neuronal damage and cognitive weakness [2]

Alzheimer's disease (AD) is the most common form of dementia and an important global problem in health. AD is one of the most common neurodegenerative diseases with progressive cognitive and memory disorders [4]. AD has molecular and biochemical abnormalities, including cell loss, deposits of amyloid-β (Aβ), chronic oxidative stress and damage to DNA. A number of factors, including diabetes, stroke, atherosclerosis, obesity and HFD, may increase the risk of AD [5]. Furthermore, high fat diets in transgenic mouse models increase Aβ peptides. HFD is associated with an increase in toxic Aβ peptide accumulation and impaired behavior in animal AD models [6].

The balance between oxidation and antioxidation is key to maintaining a healthy biological system [6,7]. Oxidative stress refers to the imbalance between reactive oxygen production and the antioxidant defense system, which prevents oxidative damage to the system. Pathogenesis of various diseases is affected by oxidative stress [8]. Natural antioxidant-enriched diets have a positive effect on oxidative stress, blood pressure and serum lipid composition [9]. Obesity and AD markers are associated with oxidative stress and mitochondrial dysfunction, which can lead to neurodegeneration. Oxidative stress may be an important risk factor in AD pathogenesis underlying causal mechanisms [10].

Prunus amygdalus Batsch (Rosaceae) is a mediterranean indigenous tree. *Prunus amygdalus's* edible part is a popular nutritious food known as amonds. *Prunus amygdalus* nuts are referred to in classical ayurvedic tests as medhyarasayana (nootropic agent) and are also used in folklore practice [11]. Many of the biological properties of *Prunus amygdalus* fruit are mediated by its antioxidant effects, including its toxicity protection. *Prunus amygdalus* can help to prevent the progress of various oxidative stress- related diseases and justify their use in traditional medicine [12]. The protective effects of *Prunus amygdalus* fruit on cognitive impairments in rats fed with HFD were investigated in this study.

MATERIALS AND METHODS

Extract Preparation

Fresh and dry fruit were purchased from local markets, pooled and considered as a single sample of that market. *Prunus amygdalus* dry fruit powder was extracted with petroleum ether followed by methanol in Soxhlet apparatus at 60-70°C for 6 h. The filtrate was distilled and concentrated at low temperature (40°C) in rotavapour under reduced pressure. After extraction, 25 % and 5 % percent in petroleum extract and methanol extract were evaporated to dryness.

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 IV 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 PAEt/kg orally/day for 30 days.

Group IV-Hyperlipidemic rats treated with 200 mg PAEt/kg orally/day for 30 days.

The vehicle or PAEt was given to the rats by means of a gastric force feeding needle. Both rats were fed HFD during the first 30 days of therapy, after which HFD was replaced with normal standard diet for the second 30 days of therapy. Body weights, serum lipids, and lipoprotein levels were measured on the 30th day and 45th day after the treatment and also assess behavior pattern on day of 25th, 27th, 30th, 42th, 44th and 45th in Morris water-maze (MWM) model and step down avoidance method

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.

RESULTS

Acute Toxicity Profile

The rats treated with the PA Et, 5 to 2,000 mg/kg, p.o., exhibited normal behavior. They were alert, with normal grooming, touch response and pain response. There was no sign of passivity, stereotypy and vocalization. Their motor activity and secretory signs were also normal. The animals showed no signs of depression. Alertness, limb tone and grip strength as well as the gait of the animals were normal. The paste of PA was found to be safe up to a dose 2,000 mg/ kg in Figure 1.

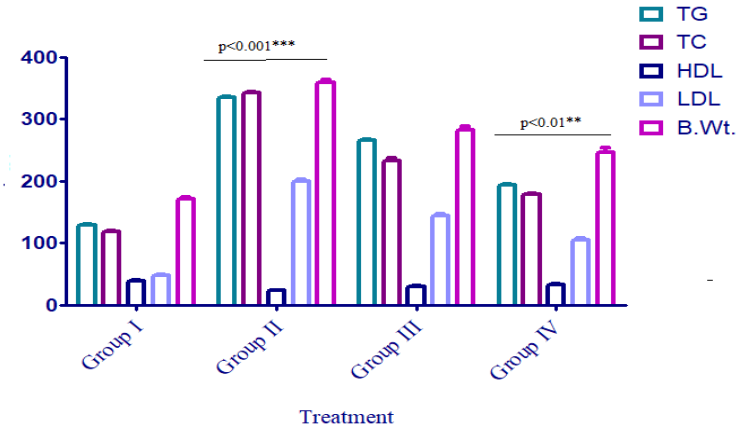

Figure 1. Effect of PA Et on lipid profile hyperlipedimic rats

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 PA Et.treated rats.

Lipid Profile

Lipid profiles such as triglyceride, total cholesterol, LDL and body weight were significantly increased 129±1.8 to 335.8±1.4 ($p<0.001^{***}$), 118±1.3 to 342.6±1.2 ($p<0.001^{***}$), 48.3±1.4 to 199.9±1.9 ($p<0.001^{***}$), 171±2.5 to 358.9±4.9 ($p<0.001^{***}$) respectively, decreased HDL levels 42±1.3 to 23.5±0.8 in high fat diet fed rats, while PA Et 200 mg/kg Orally. Treated rats were exhibited significant decrease triglyceride 335.8±1.4 to 192.9±1.9 ($p<0.01^{**}$), total cholesterol 342.6±1.2 to 178.8±1.8 ($p<0.01^{**}$), LDL 178.8±1.8 to 104.7±3.5 ($p<0.01^{**}$), B. wt.171±2.5 to 246.8±6.4 ($p<0.01^{**}$) and significantly increased HDL 23.5±0.8 to 32.5±0.9 ($p<0.01^{**}$) compare to high fat diet rats (Figure 2).

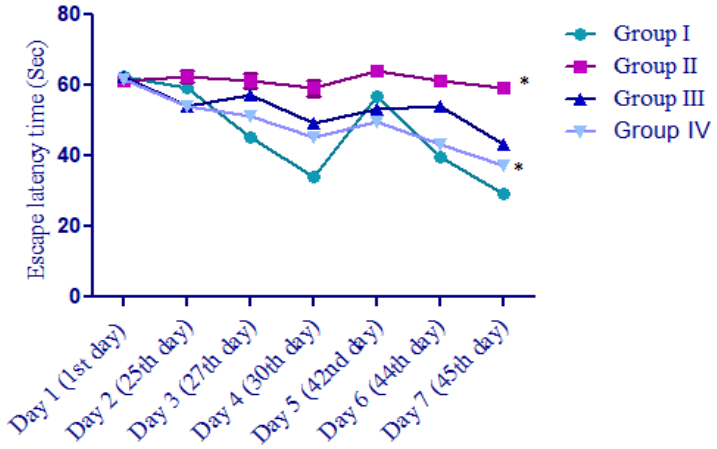

Figure 2. Effect of PA Et. on special learning and memory in hyperlipedimic rats using Morris water maze method

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.

Behavior Study

After 30 days, high fat diet rats were exhibits significantly ($p<0.05$) increases (34.2±1.2 to 59.2±2.2) escape latency time, indicating impairment of memory. There is slightly decreases (59.2±2.2 to 45.3±0.7 ($p<0.05$)) after treatment of PA Et. (100 and 200 mg/kg. orally) for 30 days as compared with the high fat diet fed rats (Figure 3).

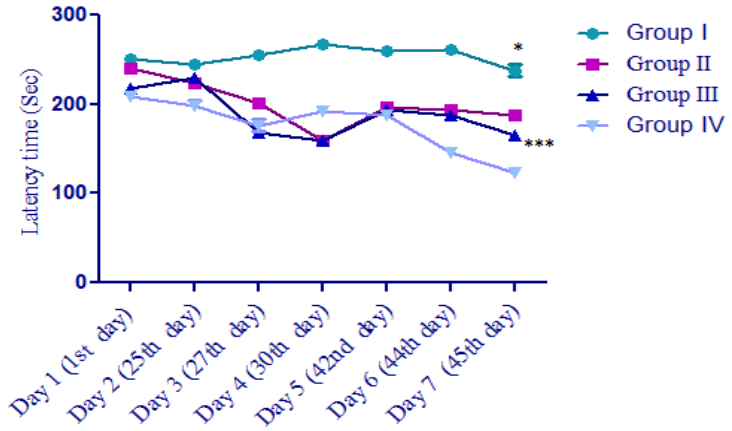

Figure 3. Effect of PA Et. on special learning and memory in hyperlipedimic rats using step down avoidance method

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

After 30 days, high fat diet rats were exhibits significantly ($p<0.001$) decreases (267.6 ±1.4 to 210.2±3.2)

latency time, indicating impairment of memory. There is slightly increases (210.2±3.2 to 173.5±0.8 ($p<0.05$)) after treatment of PA Et. (100 and 200 mg/kg. orally) for 30 days as compared with the high fat diet fed rats.

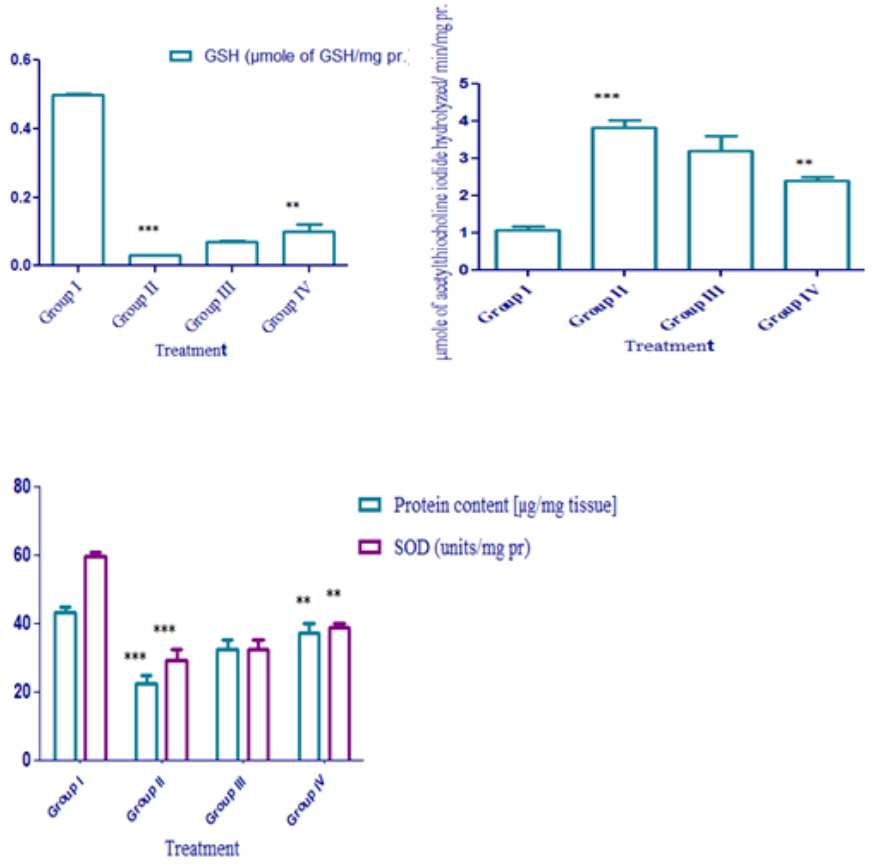

Figure 4. Effect of PA Et on brain biochemical parameters in hyperlipidemic rats

Values are expressed as mean +S.E.M of 6 animals . $p<0.001^{***a}$; $p<0.01^{**b}$ compared to normal rats; $p<0.01^{**}$ Treatment group (Group IV) compared to HFD feeding rats (Group II).

Superoxide Dismutase and Reduced Glutathione Levels

High fat diet rats were exhibited significant ($p<0.001^{***}$) decreases brain protein, SOD and GSH 43.3±1.5 to 22.6±2.1, 59.8±1.2 to 29.4±3.2, 0.5±0.002 to 0.03±0.00 respectively, acetyl choline esterase activity was significantly increases 1.08±0.1 to 3.82±0.2 compare to normal rats, while PA Et. Treated rats were showed significantly ($p<0.01^{**}$) increases brain protein (22.6±2.1 to 37.3±2.6), SOD (29.4±3.2 to 38.9±1.3), GSH (0.03±0.001 to 0.1±0.021) and acetyl choline esterase decreases (3.82±0.2 to 2.4±0.1) compared to high fat diet rats (Figure 4).

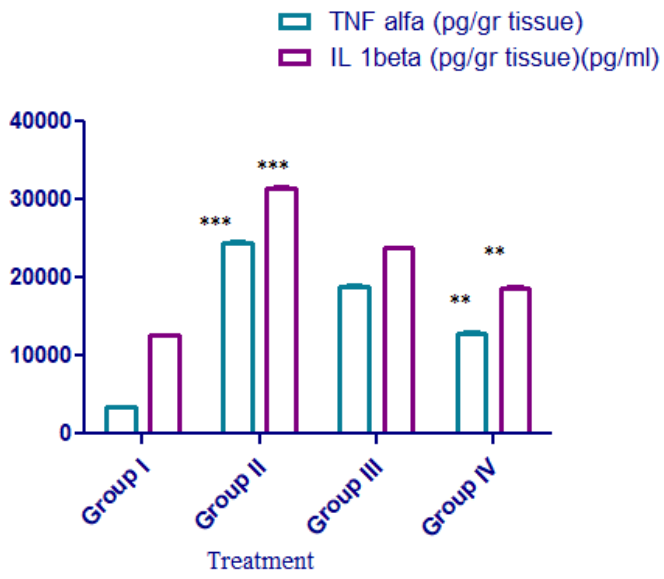

Figure 5. Effect of PA.Et. on brain TNF alfa, IL 1 beta in hyperlipedimic rats

Values are expressed as mean +S.E.M of 6 animals. $p<0.001^{***a}$; $p<0.01^{**b}$ compared to normal rats; $p<0.01^{**}$ Treatment group (Group IV) compared to HFD feeding rats (Group II).

Inflammatory Mediators Levels

High fat diet rats were exhibited significant ($p<0.001^{***}$) TNF alfa and IL 1 beta 3400 ±32.9 to 24500±54.4, 12650±61.5 to 31500±53.5 respectively, while PA Et. Treated rats were showed significantly ($p<0.01^{**}$) reduced TNF alfa and IL 1 beta 24500±54.4 to 12900±95.8, 31500±53.5 to 18700±87.3 respectively compared to high fat diet rats (Figure 5).

DISCUSSION

The current study examined the effects of *Prunus amygdalus* on cognitive deficits caused by HFD, which were correlated with different behavioral paradigms, and evaluated antioxidant activity. These novel findings suggest that Prunus amygdalus has prevented cognitive impairment and improved antioxidant activity, which is typically caused by HFD [9]. Obesity-induced blood-brain barrier damage was associated with an up-regulation of pro-inflammatory cytokines and increased oxidative stress [13]. The resulting neuro-inflammation and oxidative stress in the mouse hippocampus will probably lead to the cognitive decline seen in aged obese animals. Increased antioxidant availability can help prevent or slow the development of various oxidative stresses-related diseases [14]. Free radical scavenging and antioxidants can prevent free radical damage to the cell membranes [15]. The mechanisms that can cause neuro degeneration are oxidative stress and mitochondrial damage caused by HFD [10]. In our study, rats in the HFD groups showed significantly decreased antioxidants GSH, SOD and increased inflammatory mediators TNF alfa, IL 1beta compared to normal groups, which are normalized by *Prunus amygdalus* fruit ethanol extract treatment. In a number of animal studies examining the role of oxidative stress in neurodegenerative diseases, oxidative stress markers and antioxidant concentrations were evaluated. Oxidative stress is an important risk factor for AD and a trigger was suggested for AD pathology [16].

The HFD- induced rodent model has been used extensively to investigate obesity pathophysiology, insulin resistance and its complications [17]. Recent studies have however shown that HFD caused cognitive impairment due to

hippocampal synaptic plasticity significantly impaired [18] and increased tau protein expression [19], mitochondrial dysfunction [20], brain inflammation and decreased brain derived neurotrophic factor (BDNF) [21], disturbances in lipid metabolism that impair glucose transporters and the amyloid precursor protein [22], it can decrease memory function in animals. In the current study, HFD fed rat shows an increase in latency of escape in MWM, a decrease in latency in the step - down method of avoidance, which indicates that impairment in learning and thinking capacity was improved by the treatment of *Prunus amygdalus* fruit ethanol extract.

Prunus amygdalus prevents HFD-induced obesity in murine models through several mechanisms, including visceral fat accumulation attenuation, lipid reduction, improved insulin and leptin sensitivity and increased antioxidant potential [23]. The consumption of foods rich in antioxidants, such as certain fruits, legumes, nuts and vegetables, can therefore counteract obesity [9]. In this sense, a significant decrease in the level of TG, VLDL and cholesterol in the *Prunus amygdalus* group was observed in our experiment. Brain cholesterol is involved in a number of interdependent processes of Aβ metabolism, including synthesis, aggregation, neurotoxicity and elimination. The phosphorylated tau is also considered to be related to cholesterol metabolism [24]. Accordingly, it has been reported that Aβ would accumulate remarkably in the brain of rabbits fed a high-cholesterol diet [25]. In agreement with our results, it has also been demonstrated that blood cholesterol concentrations in rats can be reduced by consuming a polyphenol rich diet [26]. This shows that consuming polyphenol rich foods such as Oriental plum can prevent the onset of AD [27].

CONCLUSION

Our results highlight the potential benefit of the dietary antioxidant *Prunus amygdalus*. Antioxidant activity may contribute to the beneficial effects of this model, which is used to prevent and treat oxidative stress - related diseases such as AD. Further investigations are however necessary to determine its effectiveness and potential toxicity in clinical trials**.**

CONFLICTS OF INTEREST

None.

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