Original Research Paper

Volume-9 | Issue-3 | March-2019 | PRINT ISSN - 2249-555X

Human Genetics

# STUDY THE EFFECT OF *PHYLLANTHUS EMBLICA* 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 *Phyllanthus emblica* dry fruit ethanol extract. Obesity rats were prepared by feeding 30 days of high fat diet, confirmed obesity rats were divided into groups and *Phyllanthus emblica* 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 *Phyllanthus emblica* fruit ethanolic extract (100 and 200 mg/Kg B.wt.) for 30 days. Cognitive function was assessed at the time of 25$^{th}$, 27$^{th}$, 30$^{th}$, 42$^{th}$, 44$^{th}$ and 45$^{th}$ day and lipid profile and body weight was determined on 30$^{th}$ and 45$^{th}$ day, antioxidant activity was estimated at the end of 45$^{th}$ 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 *Phyllanthus emblica* fruit ethanolic extract. Conclusion that the *Phyllanthus emblica* fruit treatment enhanced cognitive function as well as maintained balanced lipid profile in obesity rats.

**KEYWORDS :** Obesity, *Phyllanthus emblica* fruit ethanolic extract (PE Et), High fat diet (HFD), Cognitive function, Lipid profile.

## INTRODUCTION

Overweight and obesity are estimated to affect over 2 billion people worldwide (Ng et al., 2014), which has resulted in an enormous strain on healthcare systems (Petrov et al., 2015). Human obesity is associated with the consumption of high-fat diets (HFDs). Although the relationship between obesity and its adverse effects on the brain remains unclear, studies have suggested that obesity and body fat deposition play an important role in the pathogenesis of certain brainrelated disorders (Kim et al., 2015). Furthermore, increasing evidence suggests that obesity and HFD can also cause long-term memory loss, neuronal damage, and cognitive impairment (Kim et al., 2015).

Alzheimer's disease (AD) is the most common form of dementia and is a significant worldwide health problem. AD is one of the most common neurodegenerative diseases, and is characterized by progressive functional disturbances in cognition and memory (Peng et al., 2013). AD has characteristic molecular and biochemical abnormalities, including cell loss, amyloid-β (Aβ) deposits, chronic oxidative stress, and DNA damage (Suzanne and Wands, 2008). There are several factors that can increase AD risk, including diabetes, stroke, atherosclerosis, obesity and HFD consumption (Knight et al., 2014). Moreover, in transgenic mouse models, high-fat diets increase the deposition of Aβ peptides (Levin-Allerhand et al., 2002). HFD in animal models of AD is associated with an increased accumulation of the toxic Aβ peptide and impaired behavior (Asadbegi et al., 2017)

*Phyllanthus emblica* L., commonly known as Indian gooseberry, is widely distributed in China, India, Indonesia, Malaysia, and Thailand, and its fruit has been used in many traditional medicines for atherosclerosis, diabetes, upset stomach, diarrhea, and skin problems (Barthakur & Arnold, 1991). Due to its high levels of vitamin C (412–900 mg/100 g) and minerals, Indian gooseberry fruit is used in juices, jams, and cosmetics (Jain & Khurdiya, 2004). Phytochemical investigations have reported that Indian gooseberry fruit extract contains tannins and various phenolic compounds, including ellagic acid, gallic acids and corilagin (Poltanov et al., 2009). In addition, Indian gooseberry has been shown to have antimicrobial, adaptogenic, antiatherogenic, antitussive, hepatoprotective, immunomodulatory, and chemoprotective activities (Liu, Zhao, Wang, Yang, & Jiang, 2008). Interestingly, Indian gooseberry is used as a tonic and collyrium for eye disorders, and such use improves sight, reduces cataracts, and minimizes infections (Srivasuki, 2012). A recent report showed that beta-glucogallin, a compound isolated from Indian gooseberry, had inhibitory effects on inflammatory eye-diseases such as uveitis by inhibiting aldose reductase (Chang et al., 2013). Other berries, such as blueberries, blackberries, and goji berries, have been considered beneficial to eye health because their constituent flavonoids, including anthocyanins, interact directly with rhodopsin and protect the retina from oxidative stress (Kalt, Hanneken, Milbury, & Tremblay, 2010). Indeed, oxidative stress plays a critical role in many age-related retinal diseases including age-related macular degeneration (AMD), and glaucoma (Wang, Kim, & Sparrow, 2017). Berry fruits also have neuroprotective effects on the brain, and have been shown to enhance neuroplasticity, neurotransmission, and calcium buffering (Miller & Shukitt-Hale, 2012). In the present study we examined the protective and therapeutic effects of *Phyllanthus emblica* fruit methanolic extract on the cognitive impairments in HFD fed rats.

## MATERIALS AND METHODS

### Extract preparation

Fresh and dry fruit were purchased from local markets, pooled and considered as a single sample of that market. *Phyllanthus emblica* 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 PE Et/kg orally/day for 30 days.

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

The vehicle or PE Et 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.

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.

## RESULTS

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

| Group | After 30 days treatment of PA Et. With HFD | | | | |
|---|---|---|---|---|---|
| | TG | TC | HDL | LDL | B.Wt. |
| I | 133±0.8 | 128.6±0.9 | 42.4±0.3 | 64.2±0.4 | 159±1.5 |
| II | 334.9±6.5*** | 337.8±5.9*** | 19.7±1.9*** | 234.3±1.8*** | 394.8±5.7*** |
| III | 224.4 ±4.2 | 243.5±1.8 | 28.9 ±0.9 | 176.8 ±2.1 | 297.9 ±3.1 |
| IV | 187.4 ±2.1* | 174.5±2.4* | 33.7 ±1.3* | 108.2±2.1* | 197.3 ±1.5* |

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< 0.05b*) decreased in PA Et.treated rats

**Fig: 1 Effect of PE Eton lipid profile in obesity and HFD feeding rats.**

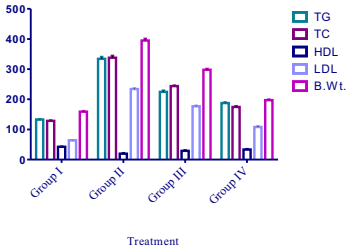



**Table 2 Effect of PE Eton lipid profile in obesity and without HFD feeding rats.**

| Group | After second 15 days treatment with PA Et. without HFD feeding | | | | |
|---|---|---|---|---|---|
| | TG | TC | HDL | LDL | Bd wt. |
| I | 149±2.8 | 128±2.3 | 44±2.3 | 47.3±1.4 | 187±2.5 |
| II | 298.4 ± 6.4*** | 298.2 ±1.2*** | 19.8 ±0.8*** | 212.4 ±1.9*** | 339.4 ±4.9*** |
| III | 212.4 ±4.2 | 223.4 ±2.1 | 31.2 ±1.8 | 154.3 ±1.6 | 248.6 ±2.8 |
| IV | 154.5±2.1** | 168.4±2.4** | 34.2 ±1.2** | 143.2±2.1** | 178.3±2.1** |

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

**Fig: 2 Effect of PE Eton lipid profile in obesity and without HFD feeding rats.**

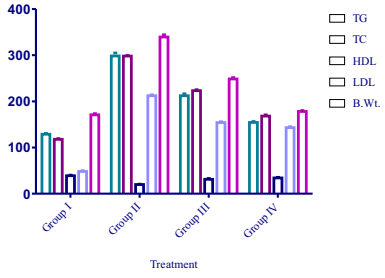



**Table 3: Effect of PE Et. on special learning and memory in high fat diet feeded rats using Morris water maze method.**

| Group | Escape latency time (Sec) | | | | | | |
|---|---|---|---|---|---|---|---|
| | After 30 days treatment of PA Et. With HFD feeding | After second 15 days treatment with PA 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±0.2 | 60.4±0.4 | 46±1.2 | 35±1.2 | 57±1.3 | 40±0.6 | 30.1±1.2 |
| II | 61.3±0.3 | 62.4±1.7 | 61.2±1.9 | 59.2±2.2a* | 64.2±1.2 | 61.2±0.8 | 59.3±1.2*** |
| III | 62.3±1.2 | 50.3±1.1 | 54.2±1.9 | 51.2±1.2 | 50.3±0.2 | 52.4±0.2 | 44.3±0.3 |
| IV | 61.5±0.7 | 54.2±0.8 | 55.3±1.2 | 42.3±0.9b* | 39.4±0.4 | 36.3±0.3 | 33.2±0.2*** |

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

**Fig: 3 Effect of PE Et. on special learning and memory in high fat diet feeded rats using Morris water maze method.**

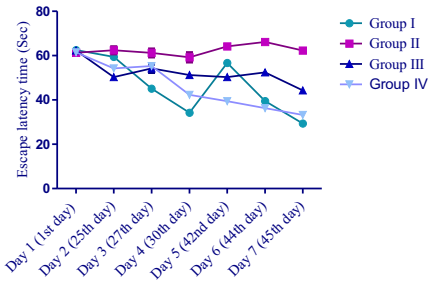


**Table 4: Effect of PE Et. on special learning and memory in high fat diet feeded rats using step down avoidance method**

| Group | Latency time (Sec) | | | | | | |
|---|---|---|---|---|---|---|---|
| | After 30 days treatment of PA Et. With HFD feeding | After second 15 days treatment with PA 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 | 261.2 ± 1.5 | 245.4 ± 0.9 | 255.6 ±1.1 | 267.6 ±1.4 | 259.6 ±1.3 | 261.3 ±2.1 | 243.5 ±6.6 |
| II | 240.3±1.3 | 233.4±1.4 | 176.3±1.3 | 161.5±1.9*** | 178.2±1.9 | 176.4±2.1 | 172.4±2.2a*** |
| III | 222±2.1 | 230±2.2 | 169 ±3.6 | 177.4±1.5 | 187.4±1.7 | 199.8±1.4 | 201.2±1.4 |
| IV | 210.5 ±1.1 | 199.4±1.4 | 176.2±2.4 | 192.7±1.8* | 201.8±1.1 | 210.4±1.2 | 215.3±2.1b*** |

Values are expressed as mean +S.E.M of 6 animals. p<0.001a*** compared to normal rats; p<0.001a*** compared to HFD feeding rats, p<0.05* Compare to HFD feeding rats

**Fig: 4 Effect of PE Et. on special learning and memory in high fat diet feeded rats using step down avoidance method**

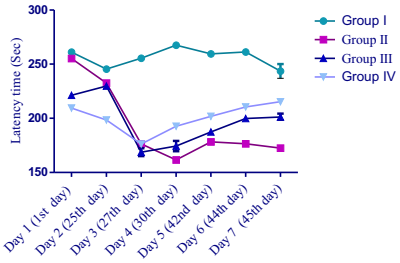



**Table: 5 Effect of PE Et. on brain parameters in high fat diet feeding rats**

| 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 | 52.1± 1.2 | 62.5± 0.9 | 0.090±0.002 | 1.45± 0.02 |
| II | 24.3± 1.9**a | 32.1± 1.2***a | 0.040±0.002***a | 4.23± 0.01***a |
| III | 29.1± 2.1 | 39.2± 1.5 | 0.058±0.003 | 3.21± 0.05 |
| IV | 32.5± 1.9**b | 41.3± 1.9**b | 0.062±0.001**b | 2.97± 0.02**b |

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 compared to HFD feeding rats.

**Fig: 5 Effect of PE Et. on brain GSH in high fat diet feeded rats**

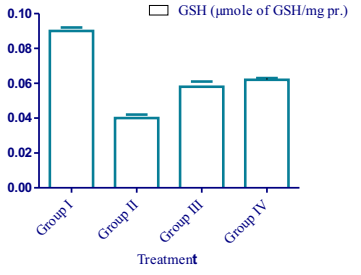



**Fig: 6 Effect of PE Et.on brain Ach E in HFD feeding rats**

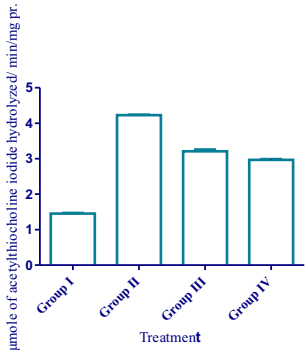
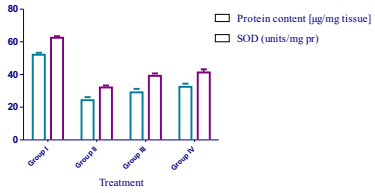



**Fig: 7. Effect of PE Et.on brain protein and SOD in HFD feeding rats**




**le :6 Effect of PE.Et. on brain TNF α and IL 1 β in high fat diet feeded rats.**

| Group | TNF α (pg/gr tissue) | IL 1 β (pg/gr tissue) |
|---|---|---|
| I | 3100± 29.4 | 12000± 45.3 |
| II | 32000±50.4***a | 29000± 35.3***a |
| III | 21000± 65.5 | 19500±40.5 |
| IV | 18500± 54.4**b | 17500±65.5**b |

All values are expressed in Mean± SEM. p<0.001a*** ; p<0.01a** compared to normal rats; p<0.01** Treatment group compared to HFD feeding rats

**Fig: 8 Effect of PE.Et. on brain TNF α and IL 1 β in high fat diet feeded rats.**

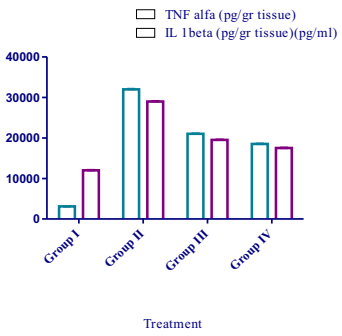



Table: 1, 2; Fig 1,2 Showed that the , after feeding HFD, there was a significant (P<0.001 ***) rise in the levels of serum TG, TC, LDL along with a decrease (P<0.001 ***) in HDL cholesterol in the rats. Treatment with PE .Et for first 30 days resulted in a significant decrease (P<0.5*) in the levels of serum TG, TC, LDL and increased HDL (P<0.5*), despite feeding on HFD during the period of treatment. After withdrawal of HFD, continuation of the treatment of group III and IV rats with PE.Et for the next 15 days has resulted in a further significant decrease (P<0.1**) in the levels of serum TG, TC, LDL cholesterol to normal levels whereas in group II rats serum lipids and lipoprotein levels remained higher than those in the controls.

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 PE.Et with HFD feeding rat and PE.Et. treated rats without HFD treated rats. The escape latency (sec) of the 7-day trial was significantly (P<0.001***) increased in the HFD group as compared to normal group rats. Treatment with PE.Et for first 30 days resulted in a significant decreased (P<0.01**) escape latency time compared to normal group rats, similarly after withdrawal of HFD, continuation of the treatment of group III and IV rats with PE.Et for the next 15 days has resulted in a further significant decreased (P<0.01**) in latency time compared to HFD feeding rats

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 (P<0.05*) in latency time compared to HFD feeding rats.

Table 5; Fig 5,6,7 showed that the HFD feeding rats were exhibited significant increase protein (52.1± 1.2 to 24.3± 1.9 (p < 0.01**)), SOD (62.5± 0.9 to 32.1± 1.2 (p<0.001***). GSH levels from 0.09 ±0.002 to 0.04 ±0.002 (p< 0.001***) and increased Ach E activity from 1.45± 0.02 to 4.23± 0.01 (p< 0.001***) compared to normal rats. At the treatment of PA.Et (200 mg/kg bd.wt.) rats exhibited significant increased Protein (32.5± 1.9; p<0.01**), SOD (41.3± 1.9; p<0.01**), GSH (0.062±0.001; p<0.01**) and decreased Ach E (2.97± 0.02; p<0.01**) compared to normal rats.

Table 6; Fig: 8 revealed that TNF α and IL 1 β levels were significantly (P< 0.001***[a]) increased from 3100± 29.4 to 32000±50.4, 12000± 45.3 to 29000± 35.3 respectively, in high fat diet feeding rats compared to normal rats, similararly TNF α, IL 1 β levels were significantly decreased to 18500± 54.4 (P < 0.01**[b]), 17500±65.5 (P < 0.01**[b]) respectively, in PA.Et. (200 mg/kg B.wt.) treated rats.

## DISCUSSION

Present research examined the effects of *Phyllanthus emblica* fruit on cognitive deficits caused by HFD, which were correlated with different behavioral paradigms, and evaluated antioxidant activity.These findings suggest that *Prunus amygdalus* has prevented cognitive impairment and improved antioxidant activity, which is typically caused by HFD. HFD has multiple associations with cognitive impairment (Komaki et al., 2015). HFD can also increase the disease neuropathology and cognitive deficits in AD mouse models (Knight et al., 2014). Accordingly, it has been reported that maternal over-nutrition due to HFD during pregnancy and lactation in an animal model of AD accelerated AD disease pathology and impaired behavior in their offspring (Martin et al., 2014). It has been revealed that a HFD and Aβ injection decreased LTP induction in rats (Asadbegi *et al.,* 2016b). In line with this, the numbers of pyramidal neurons in rat hippocampal CA1 subfield were significantly decreased in Aβ-injected rats fed an HFD (Asadbegi *et al.,* 2016a). Impaired spatial memory and hippocampal synaptic plasticity has also been reported in genetically obese animal models (Hwang *et al.,* 2010).

A maternal HFD can decrease memory formation in mice (Martin *et al.,* 2014). Accordingly, there is increasing evidence that suggests that HFD in animal models of AD is associated with an increased accumulation of the toxic Aβ peptide and impaired behaviors (Barron et al., 2013).

The early phases of obesity are characterized by an increased production of reactive oxygen species and decreased nitric oxide bioavailability (Vargas Robles *et al.,* 2015). Obesity-induced blood-brain barrier damage was associated with an upregulation of pro-inflammatory cytokines and increased oxidative stress (Tucsek et al., 2014). The excessive production of oxidants can cause imbalances, termed oxidative stresses (Ganji et al., 2017b). The resulting neuroinflammation and oxidative stress in the mouse hippocampus is likely to contribute to the cognitive decline observed in aged obese animals (Tucsek et al., 2014). Increased antioxidant availability may be helpful in preventing or slowing the progress of various oxidative stress-related diseases (Rangasamy and Namasivayam, 2014). Antioxidant and free radical scavenging can protect cell membranes against free radical damage (Di Pasqua et al., 2010). HFD-evoked oxidative stress and mitochondrial damage mechanisms that can lead to neurodegeneration (Nuzzo et al., 2015).

In our study, the rats in the HFD groups exhibited significantly decrease TAC and TTG when compared to those in thymol groups. In our experiment, the MDA and TOS levels in the HFD groups were significantly higher than those in the thymol groups. Oxidative stress markers and antioxidant concentrations were evaluated in several animal studies exploring the role of oxidative stress in neurodegenerative diseases. Oxidative stress is a major risk factor for AD, and has been suggested to be a trigger for AD pathology (Hernández-Zimbrón and Rivas-Arancibia, 2015; Markesbery, 1997). One hypothesis is that oxidative stress and inflammation are the underlying causal mechanisms of AD pathology (Holmes, 2013; Mecocci et al., 1994; Morris and Tangney, 2014). However, whether oxidative damage precedes and directly contributes to the intracellular accumulation of the Aβ1–42 peptide remains a matter of debate (Hernández-Zimbrón and RivasArancibia, 2015). 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 *Phyllanthus emblica* 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. (Hernandez Zimbrón and Rivas Arancibia, 2015). Today, many studies have been conducted to replace chemicals with natural substances, including natural antioxidants from plant sources (Di Pasqua et al., 2010; Ganji et al., 2017b). Herbs contain large amounts of antioxidants, such as secondary metabolites that play an important role in absorbing and neutralizing free radicals (Rangasamy and Namasivayam, 2014). *Phyllanthus emblica* fruit has antioxidant activity and neuroprotective effects. The anti-oxidative effects of *Phyllanthus emblica* fruit might be due to the presence of antioxidant phenolic compounds and tannins (Mayachiew & Devahastin, 2008a). In this sense, in our experiment a significant decrease was observed in the level of TG, LDL and cholesterol in the HFD group. Cholesterol in the brain is involved in a series of interdependent metabolism processes of Aβ including the synthesis, aggregation, neurotoxicity, and elimination. The phosphorylated tau is also considered to be related to cholesterol metabolism (Sun et al., 2015). Accordingly, it has been reported that Aβ would accumulate remarkably in the brain of rabbits fed a high-cholesterol diet (Sparks et al., 1994). 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 (Kuo et al., 2015; Osada et al., 2006). In accordance with this, it has been shown that consuming polyphenol rich foods such as *Phyllanthus emblica* fruit may prevent the onset of AD (Lau et al., 2005).

## CONCLUSION

Our results highlight the potential benefit of the dietary antioxidant *Phyllanthus emblica* fruit 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.

## ACKNOWLEDGEMENT

Authors thankful to University College of Pharmaceutical Sciences, Acharya Nagarjuna University and Sicra labs for providing lab facilities.

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