Fermented seeds of *P. macrophylla* seeds mitigate against memory deficit and restored altered enzymatic in the brain of streptozotocin-diabetic rats

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Research Article

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Abstract

Memory deficit has been known as one of the complications of diabetes. Fermented *P. macrophylla* seeds have been used in folklore for various metabolic diseases. The research aims to evaluate the impact of diets with the inclusion of the fermented seed of *P. macrophylla* on memory deficit in diabetes and its underlying mechanisms. Before the induction, the rats were subjected to training sessions. Thereafter, administration of streptozotocin (50 mg/kg body weight) to the trained rats via intraperitoneal (i.p). 72 hours after, the rats blood glucose level was checked, and rats with a blood glucose level greater than 250 mg/dl were selected for the memory index evaluation study. The induced rats were randomly distributed into groups: Normal rats (group 1), untreated diabetic rat (Group 2), acarbose treated diabetic rats (group 3); All were fed with basal diet and those placed on diet supplemented with fermented seed of *P. macrophylla* (10 & 20% inclusion) were allotted to group 4 & 6. Then, evaluation of memory retention capacity on day 14 of the experiment. Thereafter, experimental rats were sacrificed, tissue of interest (brain) was excised, homogenized and homogenates were used for biochemical analysis. The cholinergic, angiotensin-1-converting enzyme (ACE), arginase activity and biomarkers for oxidative stress were significantly altered in untreated diabetic rats when compared with non-diabetes rats. Conversely, the memory capacity of the diabetic rats was observed less in comparison to the non-diabetes rats. Meanwhile, diabetic rats placed on diet with fermented seeds of *P. macrophylla* (10 & 20% inclusion) exhibited significantly higher memory capacity, lower activity of cholinergic, ACE, arginase activity in relation to untreated diabetic rats while the antioxidant status of the brain was enhanced. Nevertheless, fermented seeds of *P. macrophylla* ameliorated memory deficit in STZ induced diabetes rats. This gave credence to its nutraceutical potential as claimed in folk medicine.

Background

Diabetes, a metabolic disease, has numerous complications such as hypertension, retinopathy and neuropathy (ADA, 2009). Interestingly, these complications are manifested as a result of uncontrol or badly regulated glycemic levels (van Harten et al., 2006). Studies have shown that diabetes increases the rate of brain damage such as brain atrophy and lacunar infarcts. These complications are evident via deficits in learning, memory index and movement disorders (van Harten et al., 2006). Research findings have shown that diabetes in humans or animals causes dysfunction to the central nervous system, cognitive, behavioral, and an increased risk of vascular abnormalities in the brain (Moheet et al., 2015; Munshi, 2017). Also, there is an increase in hippocampal astrocyte reactivity, decreased dendritic complexity and disturbing neurotransmission (van Elderen et al., 2010; Moran et al., 2013). The distortion in the neurotransmission could be linked to the upregulation of cholinesterase (Agunloye and Oboh, 2020), angiotensin-1-converting enzymes (Zou et al., 2009), arginase, and oxidative stress in the brain of diabetes subjects (Agunloye and Oboh, 2020).

Also, research findings have shown that hyperglycemia promotes the generation of quantum of free radicals in the brain and these cause peroxidation of the brain lipid component and nerve cells which eventually lead to cell death and impairment in cognitive function (Sun et al., 2016). Interestingly, brain

tissue and neurons are susceptible to oxidative damage due to their high lipid content, huge energy need and very low antioxidant prowess predisposing neurons and brain tissue to damages evident by functionality decline (Niedzielska et al., 2016; Li et al., 2013)

African oil bean seed [*Pentaclethra Macrophylla (P. macrophylla*) Benth], commonly called “Ugba” or Ukpaka” has been used by the locals for the preparation of a delicious meal called African salad. Fermented seed of *P. macrophylla* has been shown to exhibit anti-anaemic, (Nwanjo et al., 2006), anti-hyperlipidemia (Anioke, 2019), blood pressure-lowering (Okwuonu et al., 2013) as well as being a source for phytochemicals and nutritional elements (Anioke, 2019; Igwenyi et al., 2015). Nevertheless, their paucity of information on the ameliorative effect of fermented seed of *P. macrophylla* on memory index, brain cholinergic and antioxidant potentials. Therefore, this study aims to evaluate the nutraceutical prowess of fermented seed of *P. macrophylla* on memory index, cholinergic system, ACE, arginase and antioxidant status in the STZ induced diabetes rats.

Material And Methods

Sample preparation

Mature seeds of *P. macrophylla* were sourced locally. The seeds were prepared into powdery form as described by Onyenibe et al., 2019. The supplemented diets were formulated according to the procedure of Agunloye & Oboh, 2020.

Animal grouping and treatment

Male adult albino Wistar rats were purchased from the animal colony of the Department of Biochemistry, University of Ibadan. Meanwhile, before the start of the study, Departmental and Institution ethical clearance was obtained. The rats were adapted to the new environment for 14 days. Then, streptozotocin (STZ) (50 mg/body weight) was used to induce diabetes through i.p administration. The blood glucose level was checked after 72 hr. Rats having elevated blood glucose above 250 mg/dl were selected for the feeding trial. Table 1 represents feed formulation and animal grouping.

Evaluation of memory index

The memory index in the normal and diabetic rats (untreated and treated) was evaluated via the Morris Water Maze test according to the procedure of Tuzcu and Baydas (2006)

Biochemical assay

Determination of AChE activity

Assessment of AChE activity in the brain homogenate as described by Ellman et al., 1961. The reaction was commenced by adding phosphate buffer pH 7.4 of 0.1 M to 100 mM of DTNB. This was followed by the addition of 100 µL of tissue homogenate. The entire reacting mixture was incubated with 0.8 mM acetylthiocholine iodide (AcSCh) as substrate. The rate of forming yellow anion of 5, 5'- dithio-bis-acid-

nitrobenzoic was monitored at the absorbance of 412 nm for 3 min. The result was presented as µmol.AChE/h/mgprotein

Determination of ACE activity

The brain ACE activity was determined as described by Cushman and Cheung, 1971. 50 µL of brain homogenate and 150 µL of 8.33 mM of hippurylhistidylleucine (Bz-Gly-His-Leu) in 125 mM Tris-HCl buffer (pH 8.3) were incubated at 37 °C for 30 min. After incubation, the reaction was arrested by adding 250 µL of 1 M HCl. The Gly–His bond was then cleaved, and the hippuric acid produced by the reaction was extracted with 1.5 mL ethyl acetate. Next, the mixture was centrifuged to separate the ethyl acetate layer; then, 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was redissolved in distilled water, and its absorbance was measured at 228 nm. The brain ACE activity was expressed as µmol HHL cleaved/min.

Estimation of activity of arginase

Brain arginase activity was evaluated as reported by Kaysen & Strecker, 1973. 50 µl of the tissue homogenate was added to admixture of 75 µl of Tris-HCl (50 mmol/l, pH 7.5) containing 10 mmol/l $MnCl_2$. The reaction was initiated upon the introduction of 0.1 M of arginine solution as substrate. The resulting mixture was incubated at 37 °C for ten minutes. Thereafter, 2500 µl of Erhlich reagent was introduced to stop the reaction. The admixture was then incubated for 20 minutes at 37 °C. The absorbance was read at 450 nm. The amount of urea produced was extrapolated from urea curve prepared with concentrations of urea varying from 0.1-1.0 mol. The result was present µmol urea produced/min/mg protein.

Estimation of the total thiol level

The total thiol level was determined in the head regions of the control and treated flies according to the method previously described by Ellman (1959). The reaction system was made up of 170 mL of 0.1 M potassium phosphate buffer (pH 7.4), 20 mL of sample, and 10 mL of 10 mM DTNB. At the end of 30 min incubation at room temperature, the absorbance was measured at 412 nm. A standard curve was plotted for each measurement using reduced glutathione (GSH) as a standard and the results were expressed as mmol/ mg protein.

Catalase (CAT) Activity

Catalase activity in the brain homogenate was evaluated as described by Aebi (1984).. 20 µL of the tissue homogenate was added to a reaction mixture of 0.1 M potassium phosphate buffer (pH 7.4), 10 mM H_2O_2. The change in absorbance at 240 nm due to degradation of H_2O_2 was monitored for 120 seconds. CAT activity was expressed in units/mg protein

Malondialdehyde (MDA) level determination

Brain MDA was determined as described by Ohkawa *et al.,* 1979. 0.1 ml of the tissue homogenate was added to mixture of 0.35 ml of 0.8% TBA, .35 ml of acetic acid/HCl solution and 0.1 ml of 8.1% sodium dodecyl sulfate (SDS). The reacting mixture was incubated at 100°C for 1 hr 30 min. Then, the mixture was allowed to cool, absorbance was read at 532 nm. The result was presented as µmol MDA/mg protein.

2.7.10 Quantification of total protein level

Protein content of plasma, heart, and kidney homogenate was determined as described by Bradford (1976) using bovine serum albumin (BSA) as standard.

Results

Figure 1 represents the mean escape latency of normal rats, diabetic rats, acarbose (25 mg/kg body weight) treated diabetic rats and diabetic rats placed on fermented seeds of *P. macrophylla* supplemented diets (10 and 20% inclusion). The mean escape latency of untreated diabetic rats was significantly ($P < 0.05$) higher in comparison with the non-diabetic rats. Meanwhile, feeding diabetic rats with the fermented seeds of *P. macrophylla* supplemented diets significantly ($p < 0.05$) reduced the mean escape latency in second when compared with untreated induced rats.

Interestingly, induced diabetic rats placed on diet with 20% inclusion of fermented seeds of *P. macrophylla* had the least mean escape latency in comparison with other treated groups. Also, Fig. 2 (a-b) represents activity of AChE and BChE in non-diabetic rats, untreated diabetic rats, diabetic rats treated with acarbose and those diabetic rats fed with diets supplemented with fermented seeds of *P. macrophylla*. The brain cholinesterase (AChE and BChE) activity of untreated diabetic rats was significantly higher when compared with the non-diabetic rats (normal rats) as well as when compared with supplemented diets (10 & 20% fermented *P. macrophylla* inclusion) fed diabetic rats. Meanwhile, 20% supplementation with fermented *P. macrophylla* significantly reduced brain BChE activity when the effect of diet supplementation on cholinesterase was compared. Figure 3 represents activity of ACE in the brain of non-diabetic rats, untreated diabetic rats and in the brain of diabetic rats fed with the supplemented diets (10 and 20% inclusion of fermented seeds of *P. macrophylla*). Activity of ACE was observed significantly ($p < 0.05$) higher in the brain of untreated diabetic rats in comparison with non-diabetic rats and those diabetic rats placed on diets with *P. macrophylla* supplementation. Also, activity of arginase in the brain of non-diabetic rats, untreated diabetic rats and diabetic rats placed on acarbose as well as those fed with diet supplemented with fermented *P. macrophylla* (10 & 20% inclusion) are presented in Fig. 4. Arginase activity was significantly lower in the brain of non-diabetic rats, diabetic rats administered with acarbose (25 mg/kg body weight) as well as those fed with supplemented diets in comparison with untreated rats. However, diet supplementation with 20% inclusion of *P. macrophylla* reduced arginase activity less when all the treatment groups were compared but the difference were not significant. Figure 5 (a-b) represents total thiol and non-protein thiol levels in the brain of non-diabetic rats, untreated diabetic rats, diabetic rats fed with diet with fermented seed of *P. macrophylla* (10 & 20%

inclusion). The level of total thiol and non-protein thiol in the brain of untreated diabetic rats were significantly lower when compared with what is obtainable for the non-diabetic and those in the treated groups. Meanwhile, diet with 20% supplementation enhanced the level of total thiol and non-protein thiol than those fed with diet with 10% inclusion of *P. macrophylla.* More so, Fig. 6 depicts the activity of superoxide dismutase (SOD) in the brain of diabetic rats. Interestingly, SOD activity was significantly lower ($p < 0.05$) in untreated diabetic rats when compared with the diabetic rats placed on diets supplemented with *P. macrophylla* (10 & 20% inclusion). Meanwhile, diet with 20% supplementation enhanced activity of SOD in the brain of diabetic rats more than others in the treated groups.

Furthermore, Fig. 7 depicts catalase activity in the brain of non-diabetic rats, untreated diabetic rats as well as diabetic rats treated with supplemented diets (*P. macrophylla* 10 & 20% inclusion). The activity of catalase was observed significantly ($p < 0.05$) lower in the brain of untreated diabetic rats in comparison with non-diabetic rats. Nevertheless, feeding induced diabetic rats with the supplemented diets (*P. macrophylla* 10 & 20% inclusion) caused an elevation in the activity of diabetic rats placed on diet with the inclusion of *P. macrophylla* when compared with diabetic rats (untreated). Nevertheless, Diabetic rats fed with 20% supplemented diet had higher catalase activity than those fed with 10% supplementation. Also, MDA equivalent compounds were quantified in the brain of non-diabetic rats, untreated diabetic rats and diabetic rats fed with supplemented diets (*P. macrophylla* 10 & 20% inclusion respectively) as presented in Fig. 8. The brain MDA level of diabetic rats (untreated) was observed to be significantly ($p < 0.05$) higher when compared with MDA equivalent compounds of non-diabetic rats and those fed with diet supplemented with 10 & 20 fermented seeds of *P. macrophylla.* Nonetheless, diabetic rats that were placed on diet with 20% supplementation of fermented seed of *P. macrophylla* had lesser MDA equivalent compounds when the effect of the treatments was compared.

Discussion

The study sought to unravel the protective effect of the fermented seed of *P. macrophylla* supplemented diet against cognitive deficit in streptozotocin-induced diabetic rats. In this study, memory deficit, cholinergic dysfunction as well as antioxidant status were assessed in STZ induced diabetic rats. Studies have shown that diabetes causes dysfunction to the neurological architecture of the central nervous system with the symptomatic expression of low learning and weak memory (Babaei-Balderlou et al., 2010; Nedzvetsky et al., 2003). Interestingly, STZ administration causes neuroinflammation, oxidative stress as well as biochemical alterations in the brain of induced rats. Perhaps, these manifest at the early stages of the pathogenesis of neurogenerative disease in diabetes (Kamatet al., 2014).

Neurotransmitters are biomolecules that involve in the neurotransmission of information signalling across the synaptic nerves end, that is from one target neuron to another target neuron (Lodish et al., 2000). Interestingly, these biomolecules are prone to enzymatic degradative effect of cholinesterase, monoamine oxidase and so on. Prolonged degradation of neurotransmitters causes impairment in memory and cognitive functions (Agunloye and Oboh, 2019). As presented in this study, untreated diabetic rats exhibited below par memory index (ability to escape to a safe platform) as well as elevated

cholinesterase activity in the brain of STZ induced diabetes. Cholinesterase (AChE and BChE) contribute prominently to the manifestation of cognitive dysfunction based on their hydrolytic degradative effect on the neurotransmitter, acetylcholine (ACh) is a neurotransmitter that resides at the synaptic end of each nerve ending where it involves in the neurotransmission of signals across neurons. Meanwhile, previous studies have shown that activity of cholinesterase is grossly elevated in diabetes rats (Agunloye and Oboh, 2019; Okesola et al., 2020). Interestingly, elevated AChE and BChE activity alongside evidence of memory deficit in the diabetic rats as presented in Figs. 1 & 2 confirmed the neuropathic effect of diabetes. This observation agreed with previous research findings suggesting that diabetes can initiate neurogenerative diseases (Agunloye and Oboh, 2019; Okesola et al., 2020). This study gave credence to the nutraceutical potential of diet supplementation with the fermented seed of *P. macrophylla*. It is noteworthy that induced rats that were placed on diet with the inclusion of fermented seed of *P. macrophylla* exhibited an improved memory index (ability to move faster to the safe platform) which could be linked to the observed lower activity of cholinesterase in the brain of the induced rats (Figs. 1 & 2). Meanwhile, reduced brain AChE and BChE activity imply that an ample amount of ACh could be amassed at the synaptic junction to ensure ease neurotransmission of signals across neurons and prevent neuronal damage (Okesola et al., 2020). In the same vein, cognitive dysfunction has been linked to an upregulated renin-angiotensin system in the brain (Hernandez et al., 2003). Interestingly, research outcomes have shown that ACE inhibitors ameliorate and enhance cognitive function (Hernandez et al., 2003). It is noteworthy that brain ACE activity contributes to dementia through beta-amyloid formation (Miners et al., 2008), initiation of inflammatory processes (Goel et al., 2015) and promotion of oxidative stress (Chan et al., 2005). Interestingly, the observed reduction in the activity of ACE might responsible for the overall improvement in the memory index of the diabetes rats placed on the fermented seed of the *P. macrophylla* diet. Meanwhile, ACE activity has been linked to memory deficit based on the effect of angiotensin II on ACh concentration in the cortex. Since research find had shown that angiotensin II impairs the biochemicals process involved in the release of acetylcholine in the cortex (Barnes et al., 1989). Therefore, reduction in the brain ACE and cholinesterase activity will ensure adequate availability of ACh at the synaptic junction of neurons which consequently improves cognitive function in diabetic state. Also, minimizing activity arginase in diabetic brain has been sought to provide symptomatic relief in memory deficit as well as in Alzheimer's disease (AD) individuals (Chang et al., 1998). This ameliorative effect could be linked to the observed decrease in the utilization of L-arginine by brain arginase. The resultant effect is the bioaccumulation of L-arginine for nitric oxide production via enzymatic activity of endothelial nitric oxide synthase. The has shown that blockage of L-arginine utilization by arginase halt memory loss and minimize known symptoms of AD. Interestingly, the bioavailability of nitric oxide (NO) enhances neuroprotection via vasodilation and improve the supply of blood to the neurons and prevent the manifestation of oxidative stress (Durante et al., 2007). As shown in this study, feeding diabetic rats with fermented seed of *P. macrophylla* supplemented diets caused brain arginase activity to be minimal. This minimal arginase correlate to the proportion of fermented seed of *P. macrophylla* in their respective diets. Interestingly, bioactive compounds in fermented seed of *P. macrophylla* could be responsible for the reduced arginase activity.

Hyperglycemia has been linked to the manifestation of free radical generation and this contribute to the manifestation of cognitive and memory deficit in diabetes mellitus (Kamat et al., 2014; Sánchez-Chávez and Salceda, 2000). Meanwhile, excessive liberation of hyperglycemia mediated free radical in the brain of causes oxidative stress in brain of diabetes rats (Sánchez-Chávez and Salceda, 2000). The occurrence of oxidative stress causes an increase in brain MDA level while antioxidant status activity of enzymic and non-enzymic antioxidant are significantly compromised. It is worth note that hyperglycemia elevated ROS level which cause reduction in NO availability and inactivate its vasodilatory effect. Also, hyperglycemia upregulate activity of inducible nitric oxide synthase (iNOS) and the resultant effect is the production of peroxinitrite, a compound with great potential to initiate oxidative imbalance (Nash and Fillit, 2006; Ganguli et al., 2000). In this study, the reduced redox state in the brain of STZ induced diabetes rats was restored adjudged by an increase in the concentration of total thiol, non-protein thiol as well as enhanced activity of antioxidant enzymes in the brain of diabetes rats placed on fermented seed of *P. macrophylla* supplemented diets. These antioxidant prowess of fermented seeds of *P. macrophylla* supplemented diets might be as result of presence of different constituents of health benefit in the diet (Chukwuma and Chigozie, 2016). The antioxidant potential of *P. macrophylla* offer complementary effect against memory deficit in induced rats. As revealed in Figs. 4, 5 & 6 respectively, the first line defense mechanism against oxidative stress (SOD, catalase and thiols) were all enhanced after feeding the induced rats with the *P. macrophylla* supplemented diet. In the same vein, lower MDA level implies that the integrity of the brain tissue was protected against hyperglycemia and angiotensin II induced oxidative imbalance could be prevented (Agunloye and Oboh, 2020). Interestingly, one of the justifications for the elevated MDA level in the diabetes is the observed reduction in the activity SOD, catalase and total thiol as shown in the brain of untreated diabetes rats. These observations are consistence with previous findings on the role of antioxidant deficit on the manifestation of chronic diabetic neuropathy (Baydas et al., 2005; Alvarer et al., 2009).

In conclusion, STZ administration caused an impairment to learning and memory in an induced rats through elevated cholinesterase, ACE, arginase activity and imbalance reductive state in the brain. Meanwhile, *P. macrophylla* supplemented diets ameliorated memory deficit in induced rats while elevated activity of AChE, BChE, ACE, arginase was restored. Also, brain antioxidant status was enhanced adjudged by elevated thiol concentration, SOD activity, catalase activity as well as reduced MDA equivalent compounds. Therefore, fermented seed of *P. macrophylla* offer protection against neurological disorder in diabetes.

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o Code availability: Not applicable
o Authors' contributions:

Agunloye Odunayo M: Conceived, designed collect data, analyzed and draft the Manuscript

Oboh G: Provide logistics and vetting of the draft manuscript.

** Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals
o Ethics approval: Approved by University ethical committee with ethical number FUTA/ETH/21/02
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Tables

Table 1: Diet formulation and animal grouping

Treatment	Group 1 Basal	Group 2 Control	Group 3 + Acarbose 25 mg/kg body weight	Group 4 + 10% supplementation	Group 5 + 20% sumg/kg CAA
Skim milk	28	28	28	28	28
Oil	10	10	10	10	10
Primix	4	4	4	4	4
Corn starch	58	58	58	48	38
. macrophylla	-			10	20

Figures

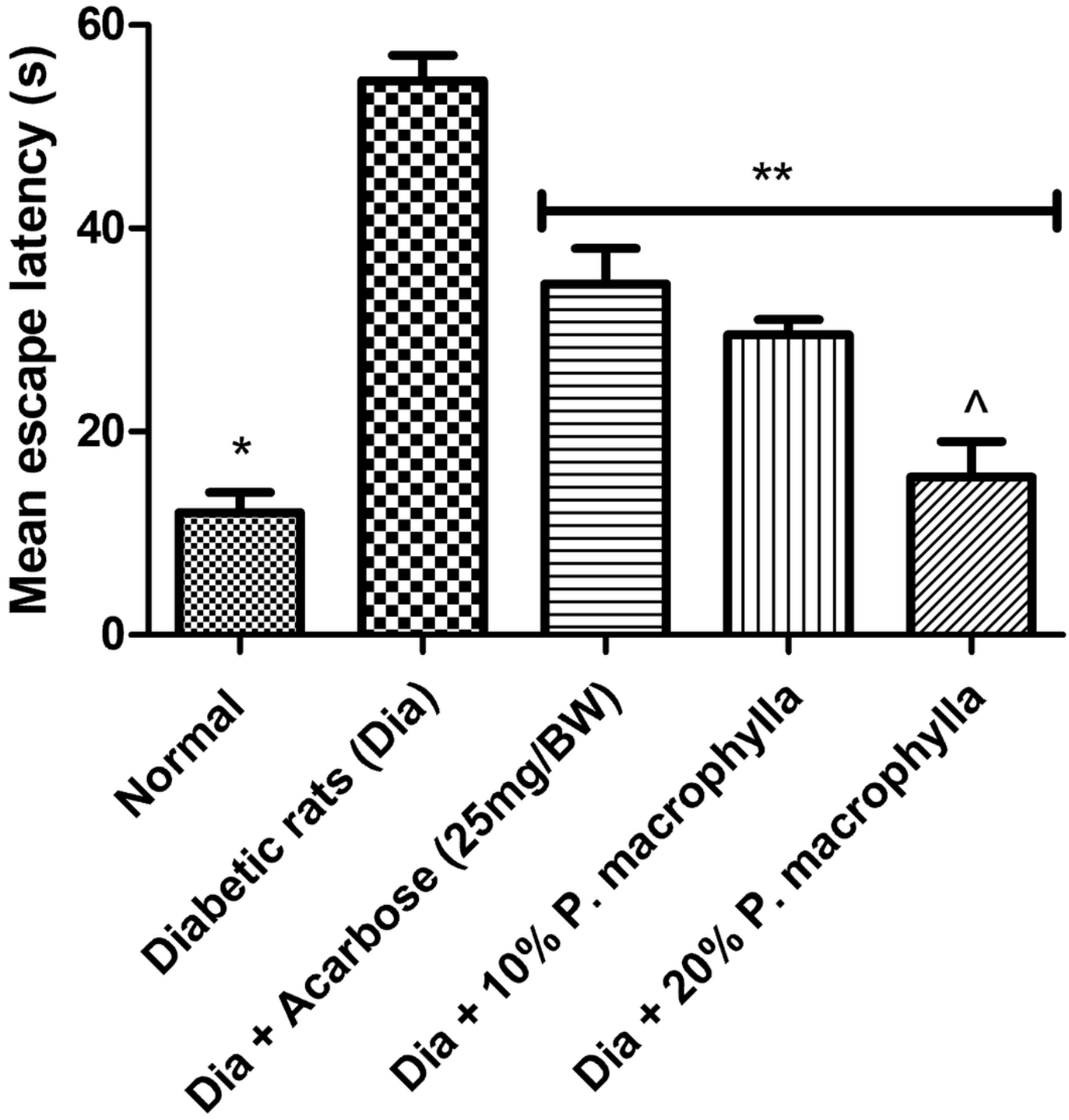

Figure 1

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20 mean escape latency in seconds in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic Values are significantly ($p < .05$) different when diet supplemented with 10% with 20% of *P. macrophylla*.

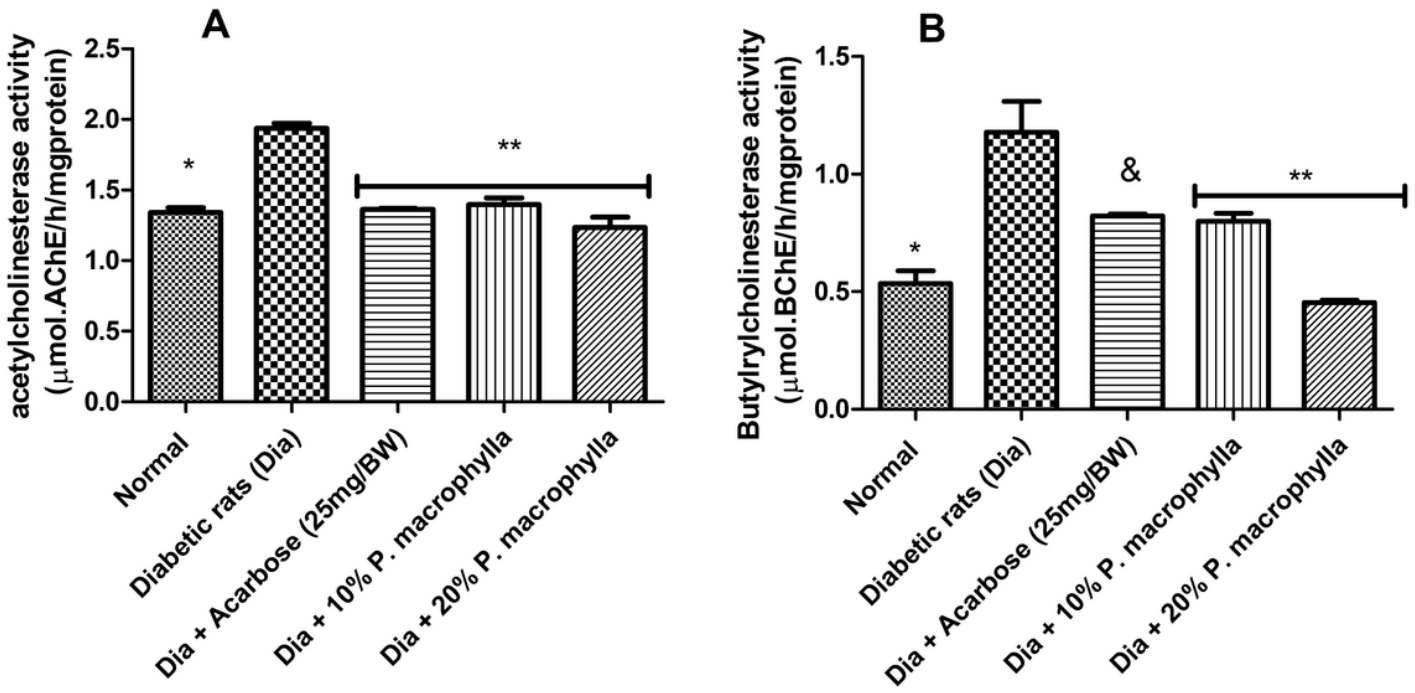

Figure 2

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20) on activity acetylcholinesterase (A) and butytrylcholinesterase (B) in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic, Values are significantly ($p < .05$) different when diet supplemented with 10% with 20% of *P. macrophylla*, $^{\&}$Values are non-significantly ($p < .05$) different when compared with diabetic rats.

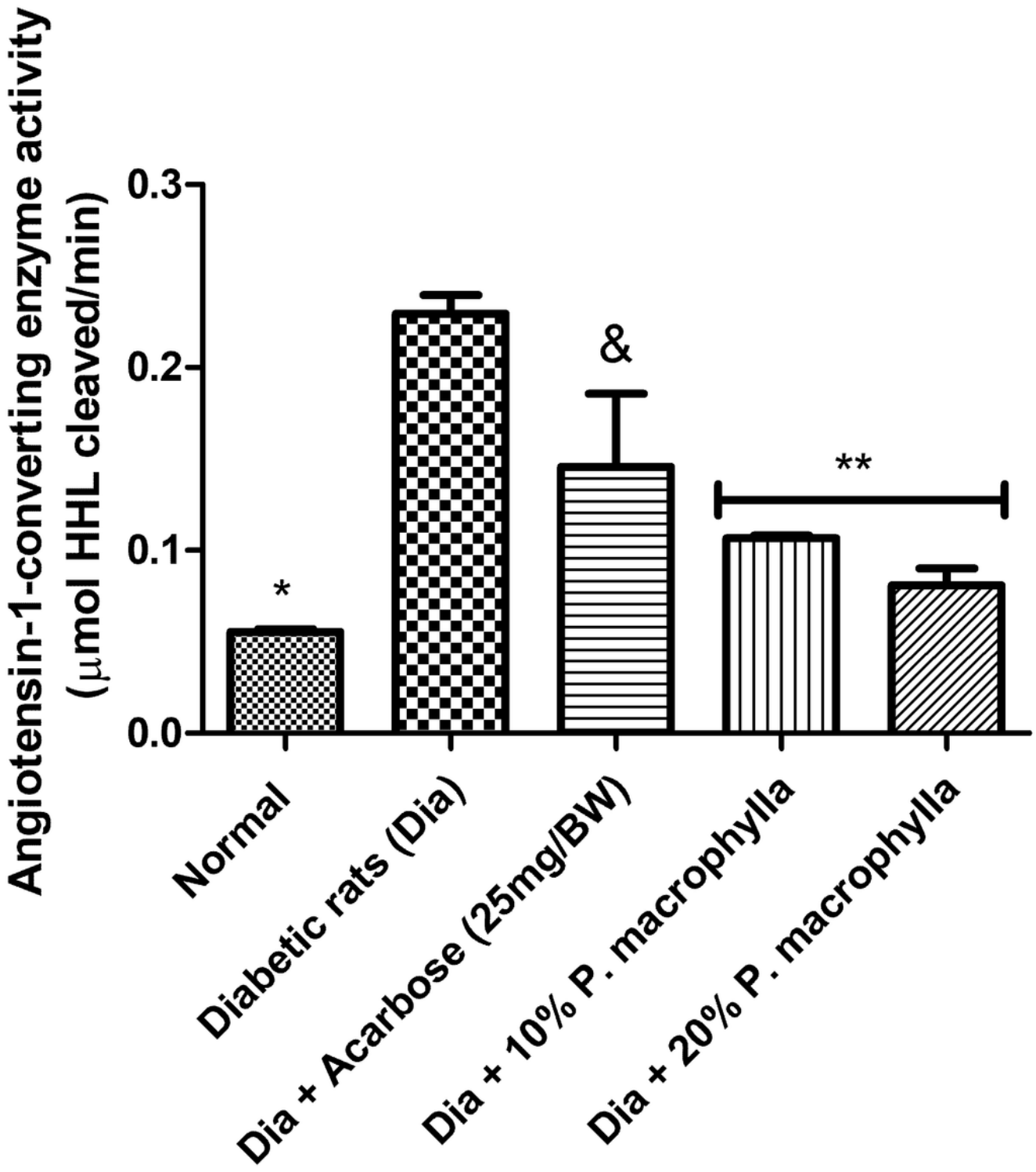

Figure 3

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20) on brain ACE activity in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic, Values are significantly ($p < .05$) different when diet

supplemented with 10% with 20% of *P. macrophylla*, [&]Values are non-significantly ($p < .05$) different when compared with diabetic rats.

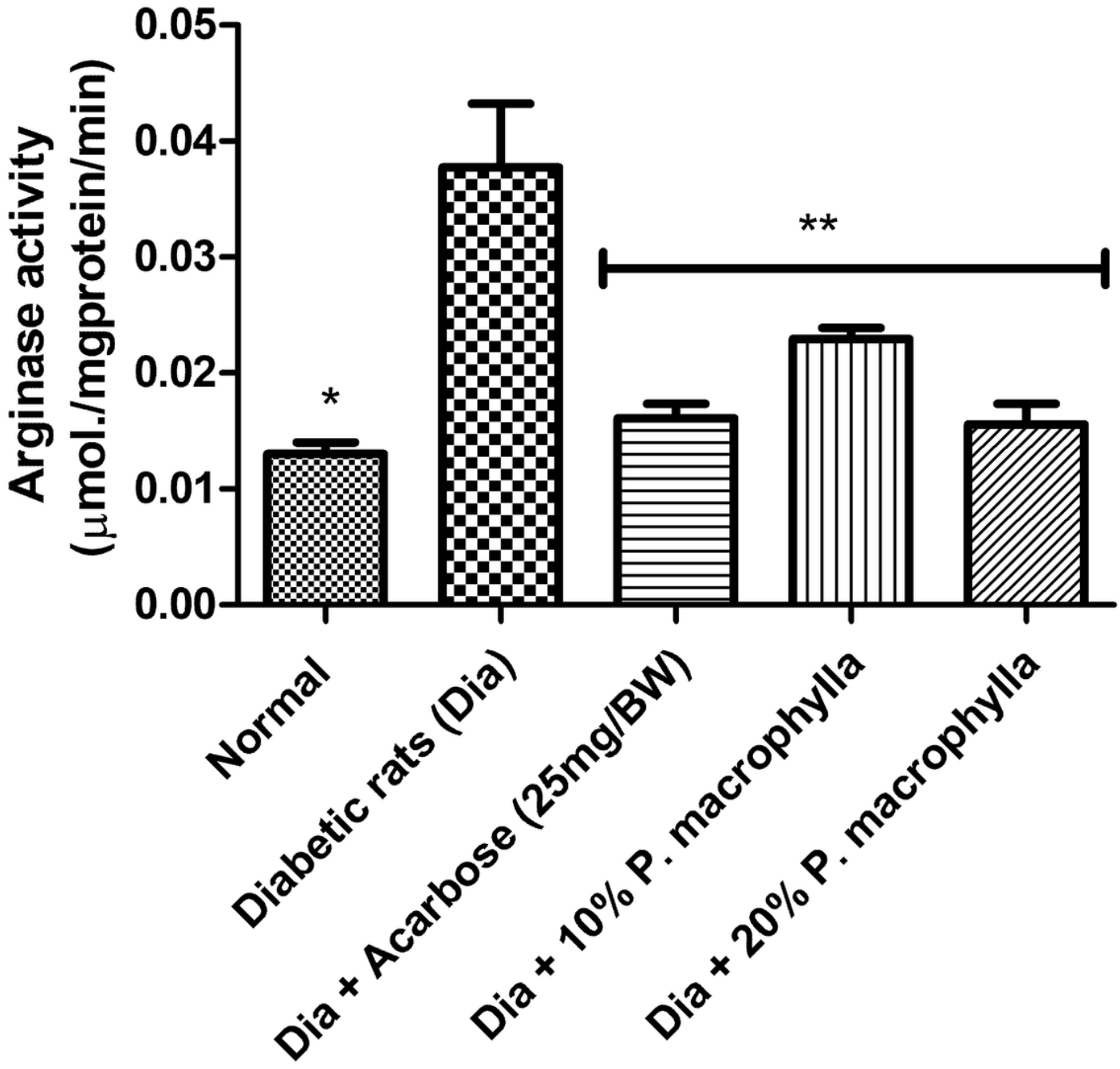

Figure 4

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20) on brain arginase activity in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic.

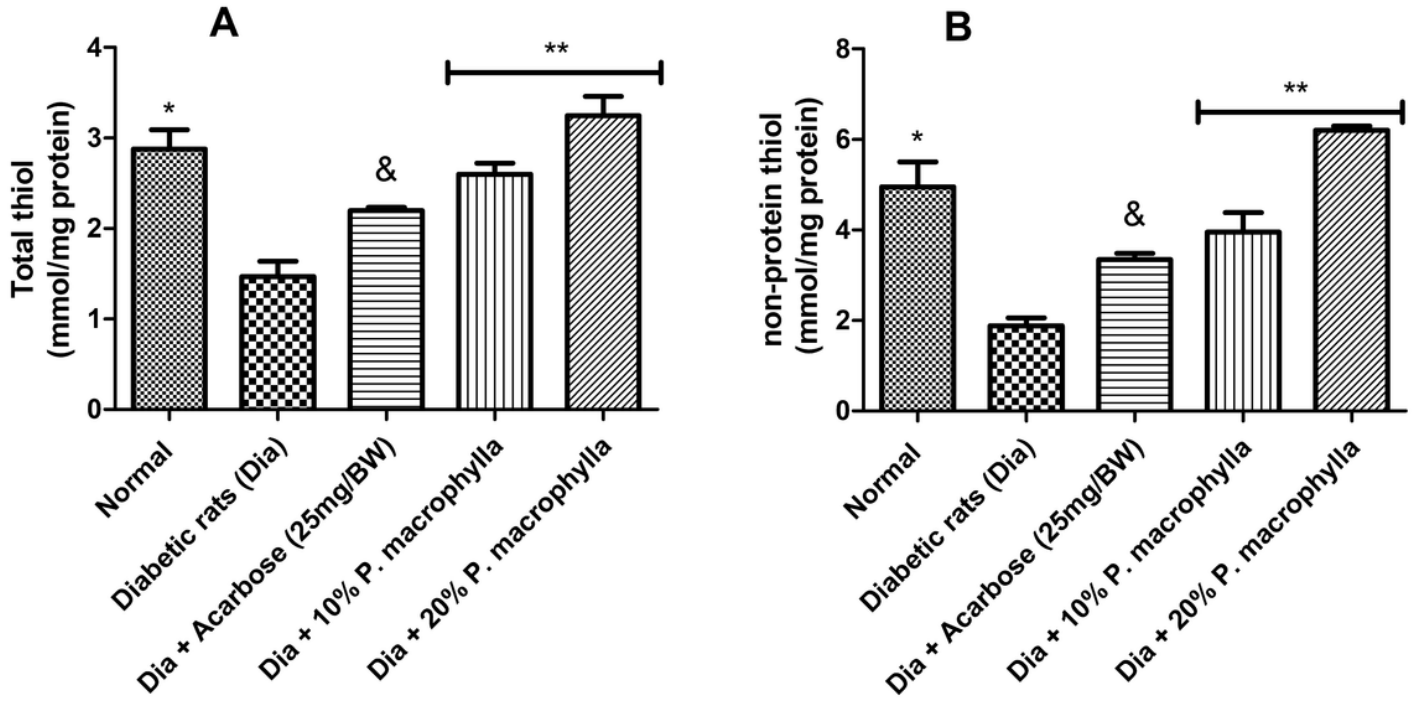

Figure 5

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20) on brain total thiol (A) and non-protein thiol (B) in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic, $^{\&}$Values are non-significantly ($p < .05$) different when compared with diabetic rats.

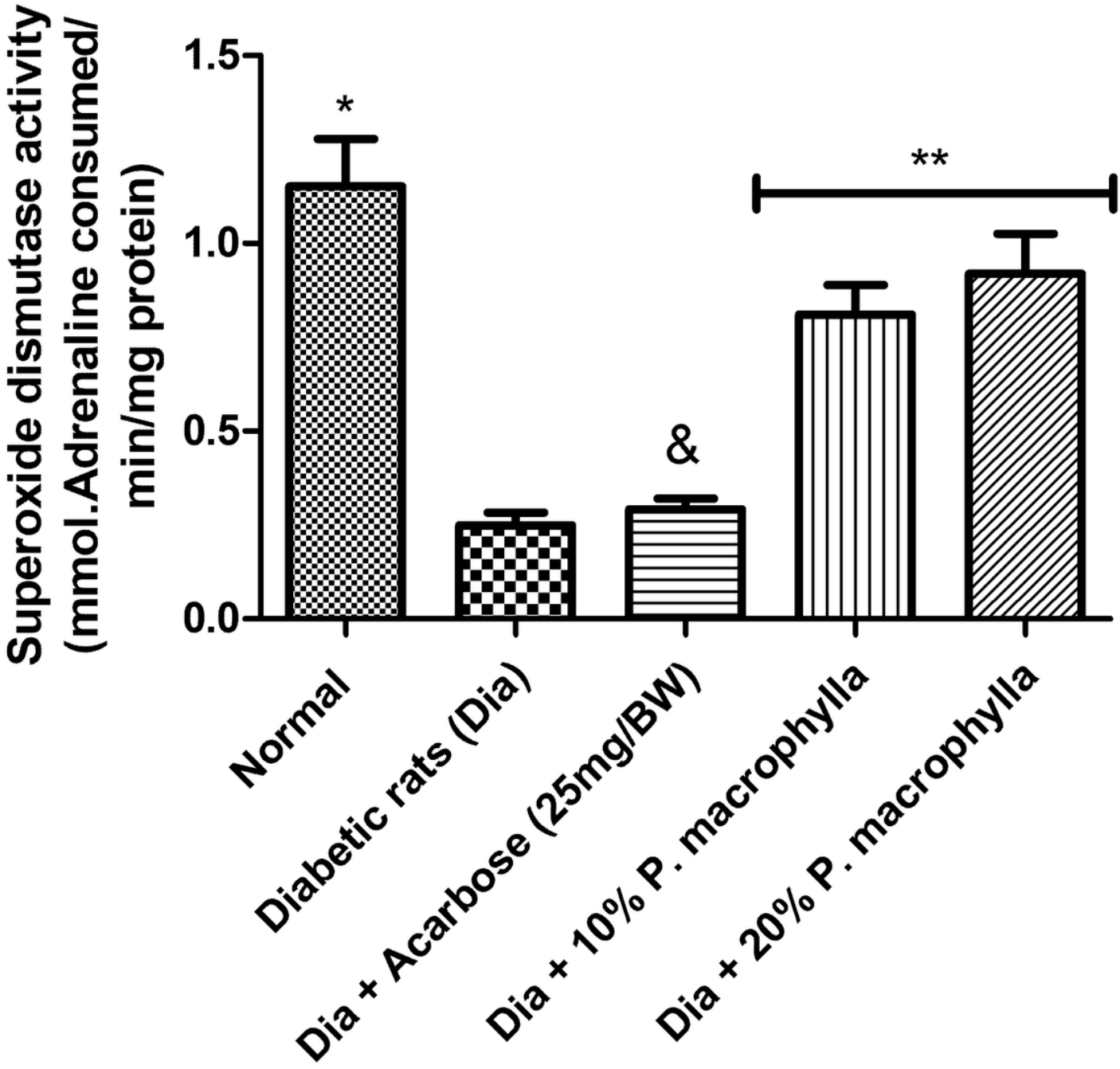

Figure 6

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20) on brain superoxide dismutase activity in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic, $^{\&}$Values are non-significantly ($p < .05$) different when compared with diabetic rats.

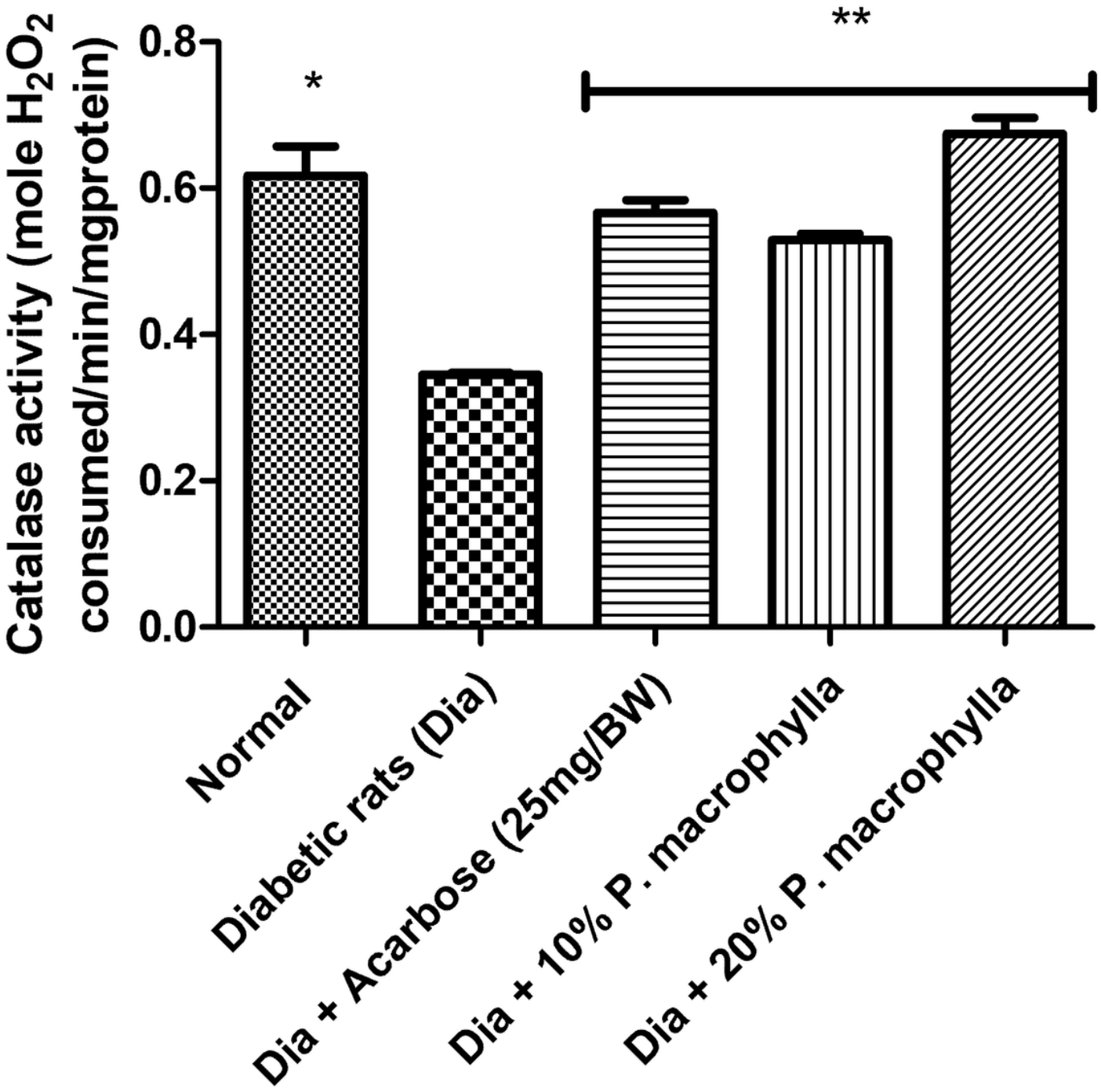

Figure 7

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20) on brain catalase activity in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic, [&]Values are non-significantly ($p < .05$) different when compared with diabetic rats.

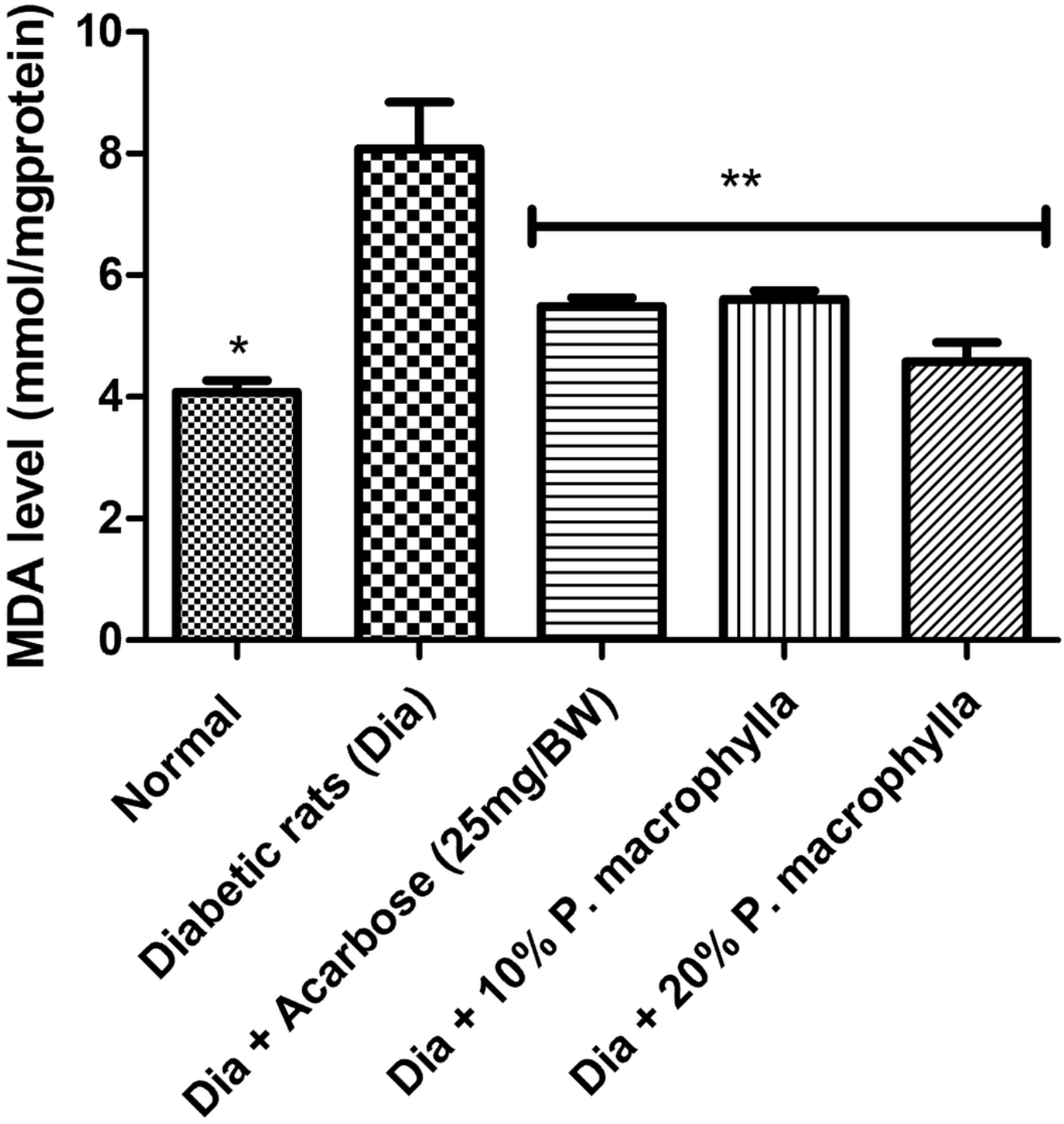

Figure 8

Effect of diets supplemented with *P. macrophylla* inclusion (10 & 20) on brain malondialdehyde level in streptozotocin-induced diabetic rats. Values represent mean ± SD (n = 6). *Values are significantly ($p < .05$) different when compared control with diabetic, **Values are significantly ($p < .05$) different when compared to treated group with diabetic.