Tropical Journal of Pharmaceutical Research May 2021; 20 (5): 947-952
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i5.9

Original Research Article

Ormosanine confers protection on neuronal function via regulation of inflammatory cytokine levels and oxidative stress

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Sent for review: 24 March 2020 *Revised accepted: 18 April 2021*

Abstract

Purpose: *To investigate the neuroprotective effect of ormosanine on rat model of Alzheimer's disease (AD), and the mechanism(s) of action involved.*
Methods: *Rats were randomly assigned to 4 groups (10 rats/group): control group, AD group, 25 mg ormosanine/kg group, and 50 mg ormosanine/kg group. Alzheimer's disease (AD) was induced in the rats via intracerebroventricular (ICV) injection of amyloid-β (Aβ)25-35 at a concentration of 1 mg/mL. Cognitive function was determined by Morris water maze test (MWMT), while lipid profile, oxidative stress parameters and cytokine level were assayed using their respective assay kits. The levels of monoamines were determined in brain tissues using high-performance liquid chromatography (HPLC), while terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was used to determine apoptosis in neuronal cells.*
Results: *Cognitive function was significantly improved ($p < 0.05$) in ormosanine treated group than AD group of rats. Ormosanine significantly and dose-dependently reduced lipid and cytokines levels as well as activities of AST and ALT, but it significantly increased the level of HDL-C in a dose-dependent fashion ($p < 0.05$). Moreover, ormosanine significantly and dose-dependently increased SOD activity, but reduced MDA level and neuronal cell apoptosis in brain tissues of AD rats ($p < 0.05$). Treatment of AD rats with ormosanine led to significant and dose-dependent increase in the levels of monoamines and BDNF in rat brain tissues ($p < 0.05$).*
Conclusion: *These results show that ormosanine confers protection on neuronal function via regulation of inflammatory cytokine levels and oxidative stress, and therefore, could potentially be developed for the management of Alzheimer's disease.*

Keywords: *Amyloid-β peptide, Apoptosis, Cognitive function, Neuro-inflammation, Alzheimer's disease, Ormosanine*

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INTRODUCTION

Alzheimer's disease (AD), a chronic brain degeneration, is a major cause of dementia. It destroys memory and other important mental functions [1]. A report reveals that AD is the third most cause of death of older population of United State [2]. In most patients with AD, symptoms first appear in the mid-60s. Although statistics vary, experts suggest that

more than 5.5 million Americans, most of them aged 65 and above, may have AD-related dementia [2]. The pathogenesis of AD is complex and has not been fully elucidated. However, it may be due to a combination of genetic, environmental and lifestyle factors. Hyper-phosphorylation of tau protein (p-tau) and Aβ peptide deposition are the major pathogenic changes observed clinically in the brain of population suffer from AD. However exact pathogenesis of AD is still not known. Dysregulation in the metabolism of amyloid precursor protein (APP) causes Amyloid-β (Aβ) peptide plaque formation [3]. Abnormal p-tau protein causes the formation of Neurofibrillary tangles (NFTs). Hippocampus and entorhinal cortex dysfunction occurs in the Tau pathology is followed by APP [3]. Moreover, neurotoxicity in AD is due to increased oxidative stress [4]. The formation and deposition of Aβ increases oxidative stress and causes impairment of cognitive functions [5,6].

Studies have shown that amyloid-β $(Aβ)_{25-35}$ causes neuronal injury via oxidation/nitration of proteins, induction of nitric oxide synthase and long-term potentiation (LTP)-like changes [7]. Deposition of Aβ leads to microglia stimulation and enhanced release of inflammatory cytokines [8]. Several signals activate the microglia cells for neurotoxicity or neuroprotective effect under pathological condition [9]. Mitogen-activated protein kinase (MAPK)-dependent pathways have been demonstrated to contribute to neuro-inflammation [9]. The conventional drugs used for the management of neuro-inflammation and AD have several limitations.

Medicines from the natural origin shows promising effect in the management of several disorders. Ormosanine is a pentacyclic alkaloid isolated from *Akebia quinata* [10]. It possesses antimalarial, analgesic, sedative, hypnotic and liver- protective effects [11,12]. The hepatoprotective effect of ormosanine is exerted via modulation of MAPK pathway as well as regulation of oxidative stress [13]. Investigated report presented here shows the beneficial effect of ormosanine on rat model of AD, and the mechanism(s) involved.

EXPERIMENTAL

Rats

Forty male Sprague Dawley rats (250 – 300 g) were housed (Temperature: 24 ± 3 °C, 12-h light/12-h dark cycle and Humidity: 60 ± 5 %) as per the guidelines of Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) [14].The study protocol was approved by the Institutional Animal Care and Use Committee of Wuwei People's Hospital, China (approval no. IAEC/WPH/09/2018).

Experimental design

The rats were randomly assigned to 4 groups (10 rats/group): control group, AD group, 25 mg ormosanine/kg group and 50 mg ormosanine/kg group. Alzheimer's disease (AD) was induced in the rats via ICV injection of $Aβ_{25-35}$ at a concentration of 1 mg/mL under ketamine (90 mg/kg bwt) anesthesia. The rats were placed on stereotaxic apparatus to maintain their body temperature while being anesthetized. The injection point was stereotaxically located: 3 mm dorsal, ± 2.3 mm lateral and -3.6 mm posterior. The $Aβ_{25-35}$ was administered bilaterally and reflux was prevented by keeping the needle in place for 2 min. After AD induction, the rats were placed on warm pad for 14 days to recover and maintain their sternal recumbencies. Rats in the treatment groups received ormosanine at doses of 25 and 50 mg/kg *p.o.* for 21 days. Behavioral analysis was carried out using MWMT.

Morris water maze test (MWMT)

MWMT was used to determine the spatial learning, MWMT apparatus was divided into equal quadrants. Platform was placed in such a manner so that rat is not able to see the platform on the surface of water. Rats were trialed for a week and between 3-4 h each day trail was given in two session. Rats were allowed to swim, and data was observed by determining the time spent in the target quadrant after removing the platform [14].

Measurement of serum markers of atherosclerosis

Peripheral venous blood was drawn from retro-orbital plexus of each rat and serum was separated by centrifuge the blood for 10 min at 13,000 rpm at 4 °C. The levels of TC, LDL-C, and HDL-C were assayed in serum using automated biochemical analyzer, ALT and AST activity were estimated using their respective assay kits.

Determination of levels of inflammatory cytokines and BDNF

Levels of NF-kB, IL-1β, IL-6 and TNF-α in the serum and level of BDNF in the tissue were determined using their respective ELISA kits.

Measurement of oxidative status of AD rats

Standard assay method was used to observe the activity of SOD and level of MDA in AD rats.

Determination of levels of monoamines

The levels of monoamines in brain tissue were determined using HPLC. High-performance liquid chromatography (HPLC) setup with autosampler AS-1555 and RP18 analytical column (C-18 guard column made of Thermosil) was used for the separation of monoamines. A mixture of dibutyl amine (0.01 %, v/v), 0.2 M EDTA, 0.055 % heptane sulphonic acid, 16 % methanol and 0.02 M sodium acetate was used as mobile phase. The resolution peaks were recorded with Borwin chromatographic software. The levels of 5-HT, DA and NA were determined using a fluorescent detector at 280 and 315 nm.

Neuronal apoptosis assay

Apoptosis in the hippocampal tissue was assessed by TUNEL assay. The excised brain tissues were fixed in formaldehyde at 4 ˚C for 5 h and subsequently embedded in paraffin. The embedded tissues were then sliced into thin 2-µm sections using a refrigerated microtome. Fluorometric dead end TUNEL kit was used for the determination of TUNEL-positive cell population. Inverted Eclipse Ti2 microscope was used for analysis of the TUNEL-positive cells in rat brain tissues**.**

Statistical analysis

The data are presented as mean ± standard error mean (SEM, n = 10). One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was applied for statistical analysis by using GraphPad Prism software (ver 6.1; San Diego, CA, USA). Statistical significance was indicated having the $p < 0.05$.

RESULTS

Effect of ormosanine on rat cognitive function

In AD group rats, time spent in the target quadrant, number of crossings and escape latency were reduced significantly than in control group (Figure 1). However, ormosanine treatment significantly ($p < 0.05$) and dose dependently enhances these parameters.

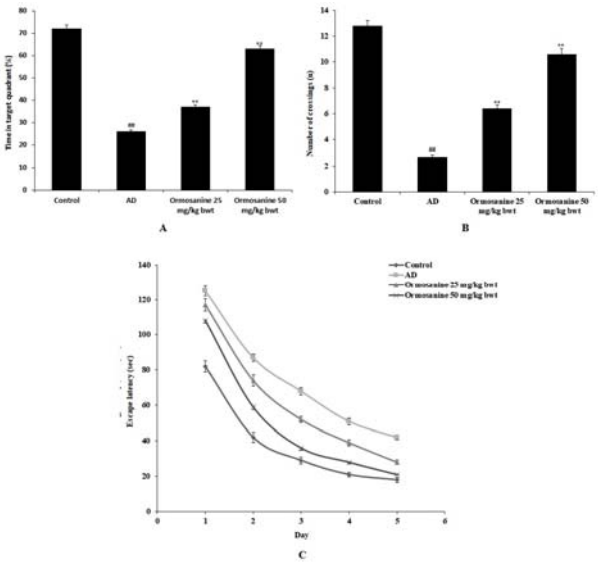

Figure 1: Effect of ormosanine on rat cognitive function. **(A):** Time spent in target quadrant; **(B):** Number of crossings; and **(C):** Escape latency of rats. $^{\#\#}P < 0.05$, compared with control group; $^{**}p < 0.05$, than AD group

Effect of ormosanine on liver function indices and lipid profile

Ormosanine significantly and dose-dependently reduced the levels of LDL-C, TC as well as activities of AST and ALT, but it significantly increased ($p < 0.05$) the level of HDL-C in a dose-dependent fashion (Table 1).

Table 1: Effect of ormosanine on liver function indices and lipid profiles

Group	HDL-C (mg/dL)	LDL-C (mg/dL)	TC (mg/dL)	AST (U/L)	ALT (U/L)
Control	17.80 ± 1.04	76.40 ± 4.20	503.90 ± 14.80	114.60 ± 4.27	38.72 ± 1.63
AD	8.46 ± 0.86$^{\#\#}$	251.90 ± 10.50$^{\#\#}$	1206.00 ± 37.10$^{\#\#}$	239.30 ± 8.61$^{\#\#}$	107.40 ± 3.82##
Ormosanine (25 mg/kg)	11.50 ± 0.92**	171.00 ± 8.20**	901.50 ± 20.90**	186.40 ± 6.92**	71.37 ± 3.16**
Ormosanine (50 mg/kg)	14.90 ± 0.63**	96.20 ± 6.80**	658.40 ± 17.30**	137.10 ± 4.95**	51.69 ± 2.03**

$^{\#\#}p < 0.05$, than control group; $^{**}p < 0.05$, than AD group

Effect of ormosanine on indices of oxidative stress

As shown in Figure 2, ormosanine significantly and dose-dependently increased SOD activity, while it significantly reduced MDA level in AD rats ($p < 0.05$).

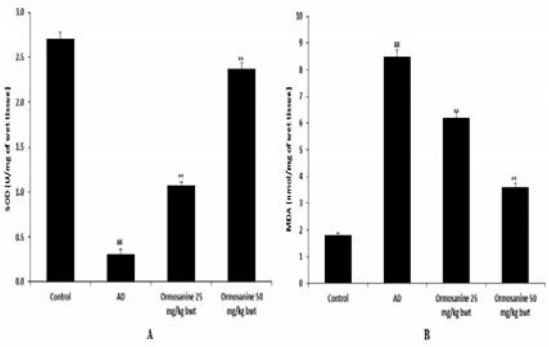

Figure 2: Effect of ormosanine on indices of oxidative stress. (A): Activity of SOD in brain tissue; (B): Level of MDA in brain tissue. $^{\#\#}P < 0.05$, compared with control group; $^{**}p < 0.05$, than AD group

Effect of ormosanine on levels of inflammatory cytokines

Treatment of AD rats with ormosanine led to significant and dose-dependent reductions in the levels of circulating IL-1β, IL-6, TNF-α and NF-kB ($p < 0.05$; Figure 3).

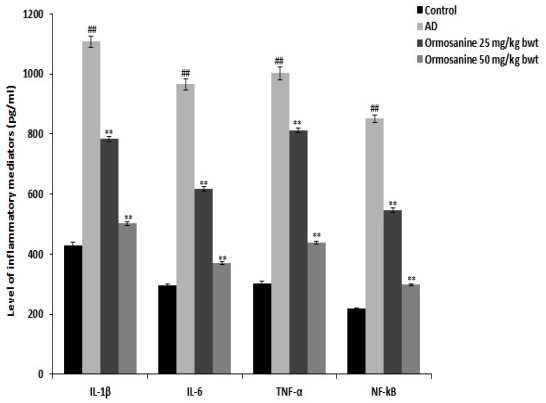

Figure 3: Effect of ormosanine on levels of inflammatory cytokines. $^{\#\#}P < 0.05$, than control group; $^{**}p < 0.05$, compared with AD group

Effect of ormosanine on the levels of monoamines in rat brain tissues

As shown in Figure 4, treatment of AD rats with ormosanine led to significant and dose-dependent increases in brain tissue levels of NA, DA and 5-HT ($p < 0.05$).

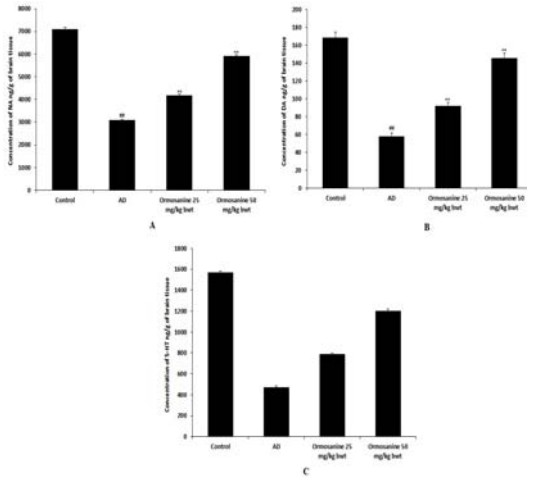

Figure 4: Effect of ormosanine on the levels of monoamines in rat brain tissues. **(A):** NA; **(B):** DA; and **(C):** 5-HT. $^{\#\#}P < 0.05$, compared with control group; $^{**}p < 0.05$, compared with AD group

Effect of ormosanine on neuronal cell apoptosis

Neuronal cell apoptosis was significantly and dose-dependently reduced by ormosanine treatment ($p < 0.05$; Figure 5).

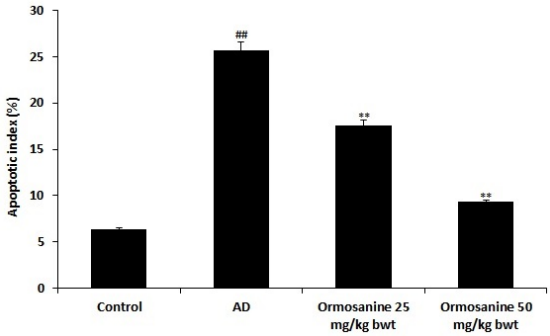

Figure 5: Effect of ormosanine on neuronal cell apoptosis. $^{\#\#}P < 0.05$, compared with control group; $^{**}p < 0.05$, than AD group

Effect of ormosanine on BDNF

As shown in Figure 6, treatment of AD rats with ormosanine led to significant and dose-dependent increase in BDNF levels ($p < 0.05$).

DISCUSSION

Cognitive dysfunction occurs due to neurodegeneration in AD. Studies have shown that neuro-inflammation contributes significantly to the pathogenesis of AD [15]. The limited effectiveness of drugs currently used for the management of AD has necessitated the search

for novel compounds that can effectively alleviate the symptoms of AD.

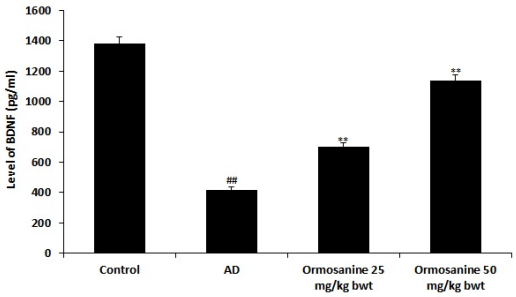

Figure 6: Effect of ormosanine on BDNF level. $^{\#\#}P < 0.05$, compared with control group; $^{**}p < 0.05$, than AD group

The present study investigated the neuroprotective effect of ormosanine on a rat model of AD, and the mechanism(s) involved. It has been reported that deposition of Aβ peptide produces toxic effect on neurons and leads to impairment of cognitive functions [16]. Amyloid plaque contains Aβ peptide in the brain tissue of patients suffering from AD 17]. Amyloid-β $(Aβ)_{25-35}$ is used experimentally to induce AD since it is well established that it causes cognitive impairment. The results of MWMT demonstrated that ormosanine significantly enhanced cognitive function in brain tissues of rats with AD.

There are several pathogenic factors involved in the development of AD including cytokine and oxidative stress [18]. Neuronal injury occurs due to imbalance between levels of antioxidant molecules and reactive oxygen species. Molecules that can significantly reduce oxidative stress have been shown to exert neuroprotective effects [19]. In this study, ormosanine significantly reversed the AD-induced changes in oxidative stress. These results agree with those from previous reports [19]. It is likely that the neuroprotective effect of ormosanine is attributable to its antioxidant property. Oxidative stress enhances the synthesis and release of inflammatory cytokines, which in turn cause neuronal cell injury and apoptosis [18]. The results of this study showed that ormosanine reduces the levels of cytokines, an indication that the crude drug may possess anti-inflammatory effect. Monoamines are vital for maintenance of cognitive function [20]. They are implicated in various cognitive processes such as memory and executive functions. In this study, treatment of AD rats with ormosanine led to enhancement of levels of NA, DA and 5-HT.

During inflammation, BDNF, a relatively mature neurotrophic factor, promotes the proliferation of neurons and glial cells via various molecular mechanisms. It plays an important role in brain development. The level of BDNF is usually altered when there is cognitive impairment [21]. The results of this study indicate that ormosanine may enhance the level of BDNF in brain tissues of AD rats and are in agreement with those of previous reports.

CONCLUSION

The results obtained in this study show that ormosanine confers protection on neuronal function via regulation of inflammatory cytokine levels and oxidative stress. Thus, ormosanine can potentially be developed for the treatment of AD.

DECLARATIONS

Acknowledgement

The authors are thankful to Wuwei People's Hospital, China for providing the facilities to conduct this work.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Juping Liang performs the experimental work and contributes in the development of protocol. Jicun Dong contributes in the data collection, designed the work, write the manuscript and supervised it. Yang Yang performs stastical analysis and histopathology analysis.

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