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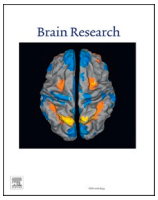

Investigation of therapeutic effect of curcumin α and β glucoside anomers against Alzheimer's disease by the nose to brain drug delivery

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ABSTRACT

Alzheimer's disease (AD) is one of the greatest geriatric medicinal challenges of our century and is the main disease leading to dementia. Despite extensive scientific research advances, available disease-modifying treatment strategies remained limited; thus, increasing demand for new drugs. In recent years, medicinal plants attracted attention due to their potential role in dementia. In the present study, α and β anomers of curcumin glucosides (CGs) were synthesized and evaluated for Alzheimer's treatment. CGs were synthesized by fusion reaction as a novel and easy method with more advantages (high yield, short reaction time, and low chemicals), and the products were characterized using HNMR. Wistar male rats were used to administer different treatments. They divided into control, sham, Alzheimer, and test groups (Alzheimer + α anomer and Alzheimer + β anomer). Animals received normal saline, Scopolamine (1 mg/kg), high dose anomers, scopolamine, and two doses (12.5 and 25 mg/kg) of anomers, respectively, for 10 days. Then the Morris Water Maze (MWM) test was performed on all animals. Finally, the animals' brains were extracted and homogenized for glutathione, acetylcholine esterase activity, protein carbonyl, and lipid peroxide level detection. The escape latency and the distance towards the hidden platform in Morris water maze in the Alzheimer group were significantly higher than both the control and test groups. Besides, there were no significant differences between sham and control groups in all tests. Both anomers led to a significant increase in glutathione, and acetylcholine levels while they caused a decrease in lipid peroxidation and protein carbonyl levels in brain tissue. It seems that intranasal administration of both anomers positively influenced maze learning in scopolamine receiving subjects. Although both anomers resulted in similar biochemistry tests, a higher dose of β anomer indicated better results than α anomer not only in behavioral tests but also in biochemical tests.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and cognitive decline that is the most common form of dementia affecting elderly people. Although the exact etiologic factor of AD is unknown, evidence has been mounting that supports the hypothesis that free radical-induced oxidative damage may play a role in the development of AD. AD also characterized by an excess of β amyloid proteins, lipid peroxides, ventricular enlargement, cortical atrophy, and progressive degeneration of neurons of the basal forebrain cholinergic system (Möller and Graeber, 1998; Citron, 2010). According to the cholinergic hypothesis, memory loss and deterioration of cognitive functions in AD are attributed to the loss of acetylcholine (ACh) (Cummings and Back, 1998; Bowen et al., 1992; Bartus et al., 1982). The protection or replacement of neurotransmitters, therefore, becomes the basis for the treatment of AD. Nowadays, the most efficient strategy to enhance cholinergic transmission is through acetylcholine esterase (AChE) inhibitors. AChE inhibitors donepezil, rivastigmine, and galantamine are approved for the treatment of AD. These drugs are the first-line agents in the treatment of AD (Farlow, 2002). The use of AChE inhibitors helps in increasing and maintaining the levels of ACh in the CNS. However, most of these drugs lead to several unwanted side effects. Hence, there is an urgent need for novel therapeutic molecules with neuroprotective capacity that could be employed as independent or adjunctive therapy along with neurotransmitter replacement therapy in AD. Since neurodegeneration in AD involves multiple pathways,

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including oxidative stress, mitochondrial damage, neuroinflammation, protein aggregation among others, the therapeutic molecules should be able to simultaneously target multiple pathways and be non-toxic to humans at high concentrations. Research evidence has highlighted the therapeutic potential of dietary polyphenols. Among these, Curcumin, obtained from the spice turmeric (Curcuma longa) possesses anti-oxidative, anti-inflammatory (da Costa et al., 2019), anti-carcinogenic, hypocholesterolemia and wound healing properties (Anand et al., 2008; Shishodia et al., 2008). Curcumin displays neuroprotective effects in experimental models of CNS diseases (da Costa et al., 2019; Lim et al., 2001; Thiyagarajan and Sharma, 2004; Yang et al., 2005). Dietary supplementation with Curcumin reduces neuroinflammation, astrocytic proliferation, oxidative stress, and amyloid pathology in the AD model (Grundman and Delaney, 2002; Ringman et al., 2005; Singh et al., 2014). Curcumin can scavenge reactive species, prevent protein aggregation and induce neurogenesis in vivo (Xu et al., 2007; Priyadarsini et al., 2003; Kim et al., 2008), and exhibits neuroprotective properties in different experimental models of AD (Mythri and Srinivas Bharath, 2012; Mythri et al., 2011). However, in vivo application of Curcumin is limited due to rapid metabolism and/or biotransformation, systemic elimination, inefficient cellular uptake, and transport across the blood–brain barrier (BBB), decreased bioavailability, and stability in the CNS (Garcea et al., 2004; Pan et al., 1999). To circumvent this, liposomes, and micelles have been employed to increase the bioavailability of Curcumin (Gupta and Dixit, 2011a, 2011b; Li et al., 2012, 2014; Ray et al., 2011). Nanoparticle formulations have shown promising results in vitro, but their efficacy in vivo is limited (Bisht et al., 2007; Tiyaboonchai et al., 2007). We tried to synthesize Curcumin-glucosides (CGs) as a novel synthetic system in which glucose helps to deliver curcumin to the brain via solution in water. There are a few references on the synthesis of curcumin glycoside by plant cell suspension cultures (Kaminaga et al., 2003) and by chemical methods (Masada et al., 2007; Vijayakumar and Divakar, 2005) but there is no report on the synthesis of fusion reaction so far. Hence, the present study reports for the first time, curcumin- β and α-D-glucosides using fusion reaction. We also investigated their effect on spatial learning and memory in a rat model of the Morris water maze. Finally, glutathione, protein carbonyl, ACh, lipid peroxide, and antioxidants level were measured in brain tissue samples in all groups.

2. Results

2.1. Synthesis of curcumin glucosides (CGs)

The fusion reaction (Marco, 2016) is employed in the synthesis of curcumin α and β glucosides. α anomer was an orange-red shiny soft powder but β anomer was an orange-red turbid hard powder. Both of them were soluble in water. The experimental yield was 79% for α anomer and 87% for β. Analysis techniques is also shown + 3.5° and + 30.3 optical rotation for α and β respectively. Melting point obtained 110-112℃ for 145–148 for α and β. We employed NMR spectra for characterizing these two anomers. You can find with following.

2.1.1. Curcumin tetrahydro β -D-glucoside

Orange amorphous powder, yield: 87%; **m.p.** = 145–148 °C; **optical rotation**: $[\alpha]_D^{25}$ =+ 3.5° (c 0.1 in CHCl3).**^{1}HNMR** (400 MHz, CDCl3): δ = 7.62 (d, J = 15.7 Hz, H4), 7.15 (d, J = 8.1 Hz, H6), 7.08(d, H10), 6.99 (t, J = 17.6 Hz, H7), 6.51 (d, J = 15.8 Hz, H3),5.88 (d, J = 39.6 Hz, H11), 5.75 (d, J = 8.3 Hz, 8H), 5.63 (s, 6H), 5.29 (t, J = 9.4 Hz, H1), 3.98 (s, CH3), 3.60 (d, J = 5.9 Hz, H14- H13), 3.94 – 3.78 (m, H12), 3.83 – 3.65 (m, H15), 1.64 (s, OH). **^{13}C NMR** (100 MHz): δ = 182.46(C2,C2′), 149.75(C9,C9′), 144.81(C8,C8′), 140.48(C4,C4′), 127.37(C5,C5′), 125.84(C3,C3′), 119.41(C6,C6′), 115.27(C7,C7′) 111.91(C10,C10′), 100.59(C11,C11′), 77.52(C15,C15′), 69.76(C12,13,C12, 13′), 69.15 (C14,C14′), 61.41(C16,C16′), 58.05(OCH3), 52.64(C1).

2.1.2. Curcumin tetrahydro α-D-glucoside

Orange amorphous powder, yield: 79%; **m.p.** = 110–112 °C; **optical rotation**: $[\alpha]_D^{25}$ =+30.3°. (c 0.14 in CHCl3).**^{1}HNMR** (400 MHz, CDCl3): δ = 7.63 (d, J = 15.8 Hz, H4), 7.16 (dd, H6), 7.09 (dd, H10),6.96(dd, H3), 6.46 (t, J = 33.5 Hz,H7), 5.87 (d, J = 24.3 Hz, H11), 5.51 (t, H), 3.94 (d, J = 33.3 Hz, H17), 3.90 (s, H12), 3.06 – 2.87 (m, 2H), 2.81 – 2.58 (m, 2H), 2.19 (d, J = 26.5 Hz, 5H), 1.88 (H, OH). **^{13}C NMR** (100 MHz): δ = 182.46(C2,C2′), 149.75(C9,C9′), 144.81(C8,C8′), 140.48(C4, C4′), 127.37(C5,C5′), 125.84(C3,C3′), 119.41(C6,C6′), 115.27(C7,C7′) 111.91(C10,C10′), 100.59(C11,C11′), 77.52(C15,C15′), 69.76(C12,13, C12, 13′),69.15(C14,C14′), 61.41(C16,C16′), 58.05(OCH3), 52.64(C1).

2.2. Spatial learning and memory

Curcumin α and β-D-glucose were investigated for their effect on spatial memory using the Morris water maze behavioral test. CG's were administered with two different doses (12.5 and 25 mg/kg; intranasal). After the intraperitoneal (IP) injection of scopolamine (1 mg/kg), rats showed impairment in spatial memory compared to that of the control group in which there was a significant increase in the time percent that rats spend in the target quarter to find the hidden platform. As shown in Fig. 2, the learning process in both the control and donepezil treated Alzheimer group has been occurring and Q2 presences percent to time total are significantly higher than the scopolamine received group (45.7% ± 7.2, 44.5% ±5.4, and 35.7% ± 9.7 respectively, $P < 0.01$) in the last day of the trial process. Rat's groups involve the low and high doses of curcumin α and β-D glucosides (23.7%±0.02, 27.6%±3.2, 33.6%±4.5, and 28.6%±4.3) as indicated in Fig. 3 have changed the learning process in the last day to the first day. This result indicated that the learning process was impaired in this group. On the test day, rats' presence in the Q2 quarter (target quadrant) differs again with a similar pattern as trial days. In other words, on the test day, the presence percentages of Alzheimer rats in the Q2 quarter were significantly lower than the other groups (Fig. 4).

2.3. Biochemical assays

The mean brain tissue and plasma concentration of all groups include the low and high doses of curcumin α and β-D glucosides, scopolamine (Alzheimer), and controls in rats following glutathione level, antioxidant level, lipid peroxide level, protein carbonyl, and acetylcholine esterase activity are illustrated. The amount of curcumin glucosides, scopolamine, and donepezil reaching the brain via intranasal administration.

The respective concentration mean of glutathione level in the brain tissue in the control(sham), Alzheimer, donepezil, the high and low doses of α anomer, the high and low doses of β anomer groups were 111.1 ± 47.7, 66.03 ± 15.9, 336.8 ± 12.04, 264.9 ± 50.8, 164.04 ± 24.3, 151.56 ± 17.9, and 276.07 ± 55.7 μM/g tissue(Fig. 5). The antioxidant activity in the brain tissue following in the control (sham), Alzheimer, donepezil, the high and low doses of α anomer, the high and low doses of β anomer groups were 962.5 ± 29.5, 416.6 ± 4.5, 1225.3 ± 52.9, 1241.4 ± 14.3, 963.9, 754.02 ± 15.5, and 739.02 ± 29.8 mM/g tissue(Fig. 6). The lipid peroxide level that of groups were 18.7 ± 1.4, 39.6 ± 12.1, 36.96 ± 15.6, 34.17 ± 5.9, 27.33 ± 4.9, 37.78 ± 8.02, and 15.46 ± 1.02 μM/g tissue (Fig. 7). The carbonyl protein level for all groups of aforesaid were 1.09 ± 0.5, 0.92 ± 0.5, 1.09 ± 0.17, 1.3 ± 0.3, 1.12 ± 0.3, 1.04 ± 0.2, and 0.99 ± 0.4 nmol/g tissue (Fig. 8). The percent of the acetylcholine esterase level is measured in plasma for all samples were 86.5 ± 12.6, 17.29 ± 4.2, 46.95 ± 13.7, 46.5 ± 5.6, 113.65 ± 2.08, 34.59 ± 3.8, and 13.59 ± 4.04 (Fig. 9). The percent of the acetylcholine esterase level is calculated in brain tissue for all above samples were 42.4 ± 12.6, 41.8 ± 4.1, 55.8 ± 13.7, 35.3 ± 5.6, 36.24 ± 2.08, 36.4 ± 3.8, and 45.5 ± 4.04.

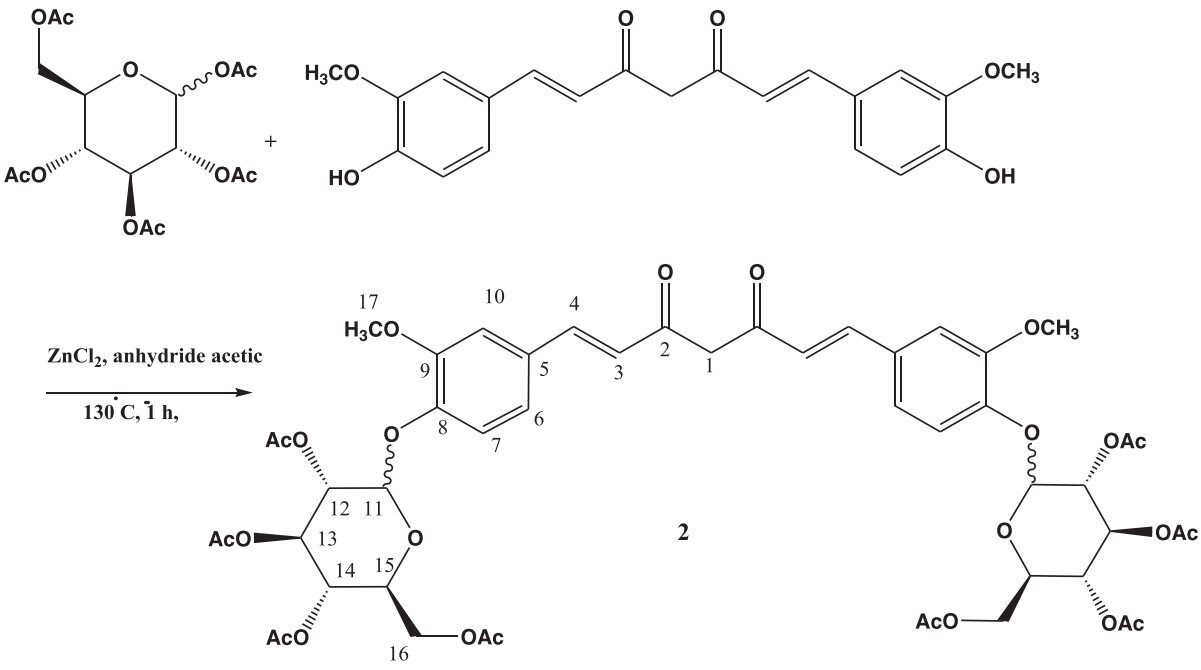


Fig. 1. Synthetic method of curcumin tetra-o-acetyl α and β-D-glucopyranose.

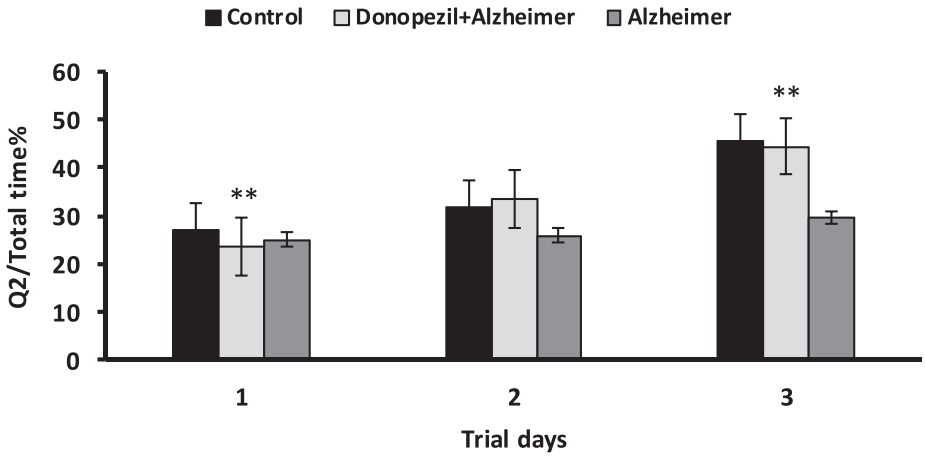
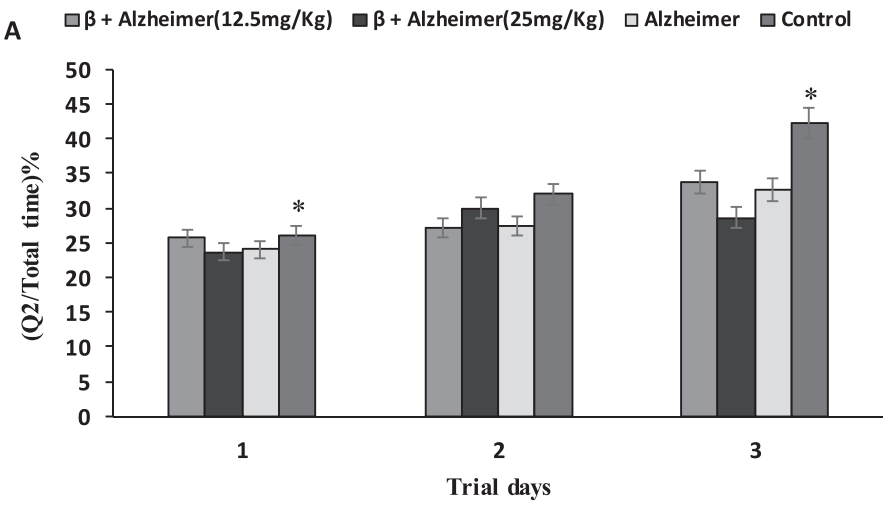


Fig. 2. Comparison of control (normal saline), Alzheimer (scopolamine 1 mg/kg) and standard treatment (donepezil 1 mg/kg) groups for spatial memory and learning using Morris water maze test in trial days. Both control and standard treatment groups differ in the last trial day with the Alzheimer group (scopolamine) significantly ($P < 0.01$). Error bars represent mean ± SEM, n = 8.

3. Discussion

Curcumin is an insoluble compound in water so researchers have been tried to improve this problem with different methods such as coated, esterification and conjugated. In hence, we tried to conjugate curcumin with glucose by esterification. This work lead to increase curcumin solubility in water. Previous studies have shown that Curcumin α and β-D-glucosides could be synthesized by three methods. In the enzymatic synthesis, the yield only reached about 55% of curcumin-β-D-glucose after 2 h (Masada et al., 2007) and it was 48% for curcumin-α-D-glucose after 72 h (Vijayakumar and Divakar, 2005). In addition to low yield, the separation remaining enzyme from the final products is difficult in this method. The Koenig–Knorr reaction is another way to prepare curcumin β-glucoside. The yield of the reaction is 95% (Parvathy et al., 2009); 64% (Bhaskar Rao et al., 2014), and 51% (Mohri et al., 2003) but this way needs a lot of chemicals and also a phase transfer catalyst. Suspension culture was also employed for the synthesis of CGs. This method is time-consuming and takes a lot of time for synthesis at least 12 days with 3% of yield (Kaminaga et al., 2003). Hence we tried to synthesize both anomers (α and β) of the CGs using fusion reaction (Marco, 2016) and characterized by NMR (Figs. 10 and 11). In this reaction, zinc chloride as a catalyst plays a key role. The reaction was performed at 130℃ in the presence of the catalyst for 1 hr. The fusion reaction requires a high temperature, but meanwhile, it has a shorter reaction time than the other methods. The results showed the method could improve the yield of the reaction for curcumin α and β-D-glucosides to 78% and 87%, respectively.

In order to investigate the effects of the synthesized compounds in reversing scopolamine-induced amnesia, both α and β anomers were administered intranasal in the low and high doses after IP injection of scopolamine (1 mg/kg) and data compared to that of the control group (Normal saline). The findings revealed that both α and β anomers were significantly effective in decreasing memory impairment induced by scopolamine injections, with some differences on the day of their effectiveness. The percentages of presence in the Q2 quarter, for groups, received α anomer showed interesting results (Fig. 3, A). On the second



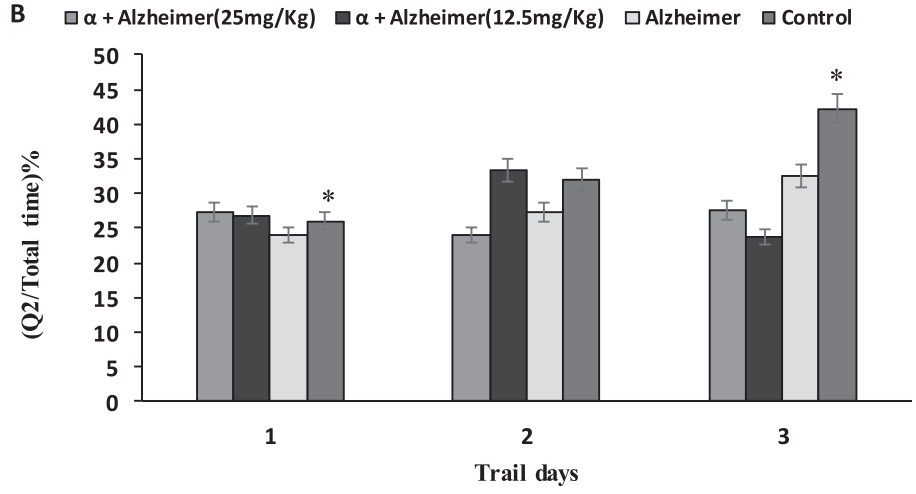


Fig. 3. (A and B): Comparison the effects of different doses (12.5 and 25 mg/kg) of Curcumin Glucosides (α-D glucoside in 3A and β-D glucoside in 3B) on scopolamine-induced amnesia using Morris water maze test in rats. For all groups: error bars represent Mean ± SEM; and n = 8. 3A: Low dose of α-D glucoside showed better results than Alzheimer group on the second day ($P < 0.01$) but not on the last day; which represents state dependent learning rather than spatial memory trial. 3B: Low dose of β -D glucoside showed much better results than Alzheimer group on the last trial day ($P < 0.01$).

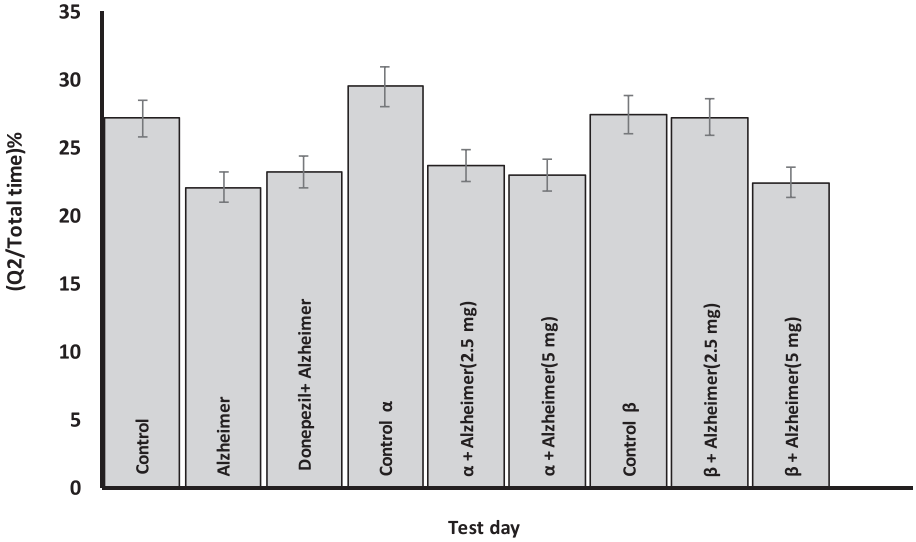


Fig. 4. Comparison of spatial memory among in all groups on test day. CGs were studied for their effect on spatial memory in scopolamine-induced amnesia at two doses (12.5 and 25 mg/kg; intranasal) except control groups for each anomer with solely the high dose of the anomer. Data are presented as the mean ± SEM. All groups were compared to Scopolamine received group($P < 0.05$).

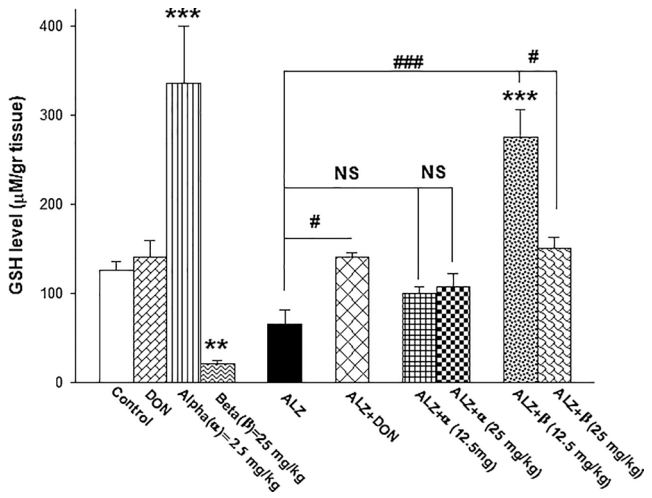


Fig. 5. Comparison of glutathione level in brain tissue for all groups consists of Control, Alzheimer(ALZ), Donepezil (DON), Alzheimer + Donepezil(Alzheimer group receiving Donepezil), α and β curcumin glucosides(contain control and Alzheimer groups), Beta(control with 25 mg/kg), Alpha(control with 25 mg/kg), Alzheimer group receiving β curcumin glucosides(ALZ + β) and Alzheimer group receiving α curcumin glucosides (ALZ + α). CGs evaluated in every two doses (12.5 and 25 mg/kg). The differences in the mean values among the treatment groups are greater than would be expected; there is a statistically significant difference (P = <0.001). Error bars represent mean ± SEM; n = 5.

day of the trial process, the low dose α anomer caused a significant augmented spatial memory similar to that of state-dependent learning which followed by a diminished memory again according to what we can obtain in state-dependent learning memory. However, the high dose of this anomer did not have any recognizable effect on learning and memory. The presence percentages of the low and high doses of β anomer were a bit different on the first and third day (Fig. 3, B) but the results were similar to α anomer.

The results of the test day indicated, only the low dose of β anomer was significantly effective ($P < 0.05$) in retrieving memory impairment caused by scopolamine (Fig. 4). According to both trial and test days' results, it can be deduced that α anomer in the presence of scopolamine seems to have some effect on the amygdala and it is effective in the retrieval of state-dependent learning impairment which can be caused by scopolamine. Because it showed a very good effect on the second day of the trial process (Fig. 3) whereas, no positive effect on the test day (Fig. 4). On the other hand, β anomer seems to be effective in spatial memory impairment caused by scopolamine in the hippocampus, because of its gradual augmenting effect on learning in trial tests and spatial memory on the test day (Fig. 4); which in turn needs to be proved by further histochemical investigations. In comparison with other reports, our data indicated better results than learning memory. They showed after three-month of treatment with curcumin (da Costa et al., 2019), rats find platform sooner while with our protocol found platform just only after 14 days of treatment. Therefore, in this study not only synthesized components (curcumin glucoside) but also employed method and protocol seem to be better.

Curcumin has poor oral bioavailability, but the synthesis of CGs improved this problem. Therefore, using curcumin or nanocarriers such as NLC (Meng et al., 2015) and metals (Dakhel et al., 2015) for drug delivery was a good decision. However, reports indicated negligible effects for them both treatment and prevention (Potter, 2013; Hamaguchi et al., 2010; Lee et al., 2013; Ahmed and Gilani, 2009). But we observed the different effects for CGs.

Glutathione level of the brain in AD is decreased as reported in the literature (Lovell et al., 1998). There is a significant difference between the Alzheimer group and both of the anomer doses of CGs as well as donepezil groups (Fig. 5). They increased the level of glutathione in the test group, but it is inordinate. It could be because of the excess of CGs and donepezil, which react with DTNB as Michael's addition and lead to add intensity of curves.

Using of GSH level in the present study practically complicated the interpretation of results regarding obtaining correct and accurate reduced glutathione level. To solve this problem, we decided to determine the FRAP level of the brain. There are many antioxidants in the brain and plasma, including vitamins, glutathione peroxidase, catalase, melatonin, and other antioxidants (de Oliveira et al., 2019). They informed the defense system mechanism of the body against free radicals and oxidative. AD's and Parkinson's disease lead to the elimination of antioxidants in brain tissue and create CNS disorder (Calabrese et al., 2008). Therefore, measurement of FRAP level is the criteria to assess the total antioxidant defense level to be able to overcome Michael's addition reaction in GSH level. Although in the AD animal model the GSH level significantly reduced compared to control rats. The study of antioxidants in the brain tissue showed that the Alzheimer group has the lowest level of antioxidants (Fig. 6). It can be considered as a verification of the above note. The data indicated a significant decrease in the FRAP level in Alzheimer's rats compared with the control or non-AD groups.

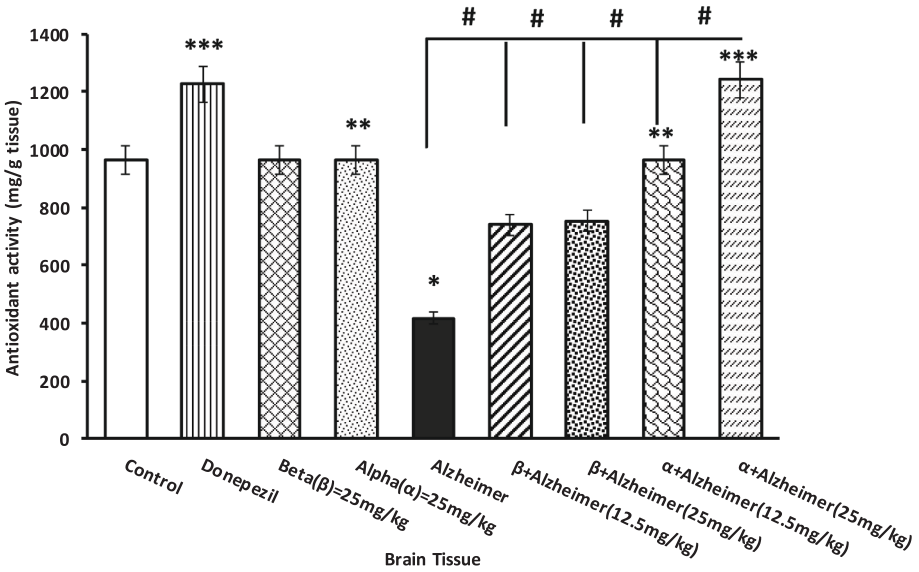


Fig. 6. Comparison of antioxidant activity in all groups such as treatment and control groups. Control, Alzheimer(ALZ), Donepezil (DON), Alzheimer + Donepezil(Alzheimer group receiving Donepezil), α and β curcumin glucosides(contain control and Alzheimer groups), Beta(control with 25 mg/kg), Alpha (control with 25 mg/kg), Alzheimer group receiving β curcumin glucosides(ALZ + β) and Alzheimer group receiving α curcumin glucosides (ALZ + α). CGs evaluated in every two doses (12.5 and 25 mg/kg). Treatment groups increased antioxidant activity in the brain in comparison Alzheimer group. There is a statistically significant difference (*P < 0.05, **P < 0.01, ***P < 0.001). Error bars represent mean ± SEM; n = 5.

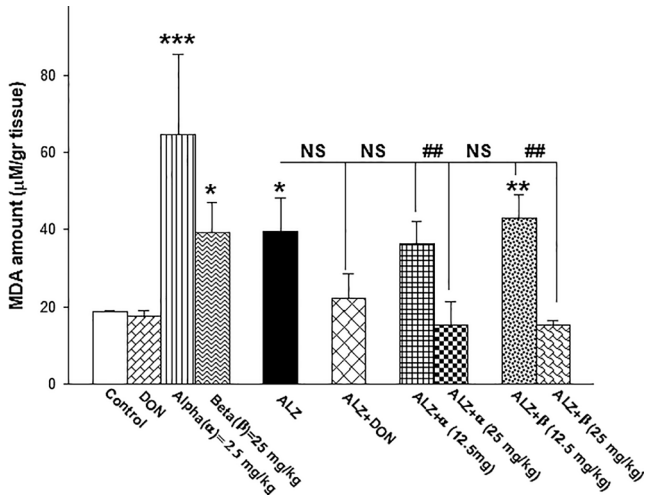


Fig. 7. Comparison of lipid peroxide level in brain tissue for all groups including Control, Alzheimer(ALZ), Donepezil (DON), Alzheimer + Donepezil (Alzheimer group receiving Donepezil), α and β curcumin glucosides(contain control and Alzheimer groups), Beta(control with 25 mg/kg), Alpha(control with 25 mg/kg), Alzheimer group receiving β curcumin glucosides(ALZ + β) and Alzheimer group receiving α curcumin glucosides (ALZ + α). CGs evaluated in every two doses (12.5 and 25 mg/kg). Lipid peroxidation occurs in the brain of rats. Brain homogenates were prepared from the cerebral cortex of rats and the extent of lipid peroxidation was evaluated by determining the production of MDA. Data, expressed as micromoles per gram of protein, are means ± SEM of the values from at least 8 animals. The differences in the mean values among the treatment groups are greater than would be expected; there is a statistically significant difference (P = <0.001).

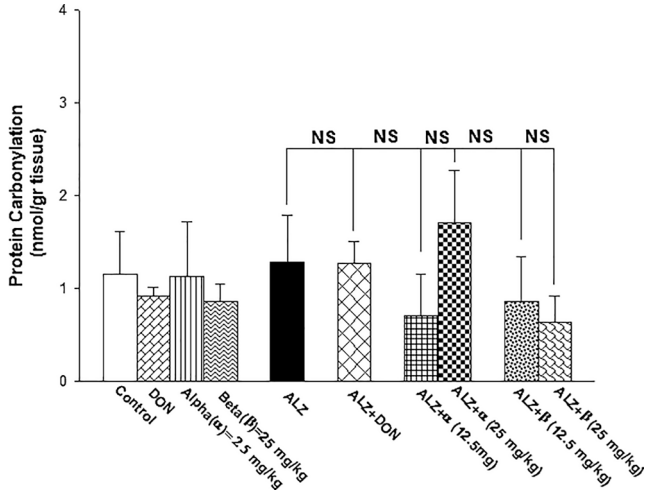


Fig. 8. Comparison of carbonyl protein level in the brain of treatment groups with Control, Alzheimer(ALZ), Donepezil (DON), Alzheimer + Donepezil(Alzheimer group receiving Donepezil), α and β curcumin glucosides(contain control and Alzheimer groups), Beta(control with 25 mg/kg), Alpha(control with 25 mg/kg), Alzheimer group receiving β curcumin glucosides(ALZ + β) and Alzheimer group receiving α curcumin glucosides (ALZ + α) . CGs evaluated in every two doses (12.5 and 25 mg/kg). decreased carbonyl protein level, The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.141). Error bars represent mean ± SEM; n = 8.

Furthermore, no significant difference was observed in FRAP in all treated groups compared with control rats. As shown in Fig. 6, FRAP levels in Donepezil and α-anomer -treated groups increased compared with AD rats. However, increase their FRAP levels of β anomer treated

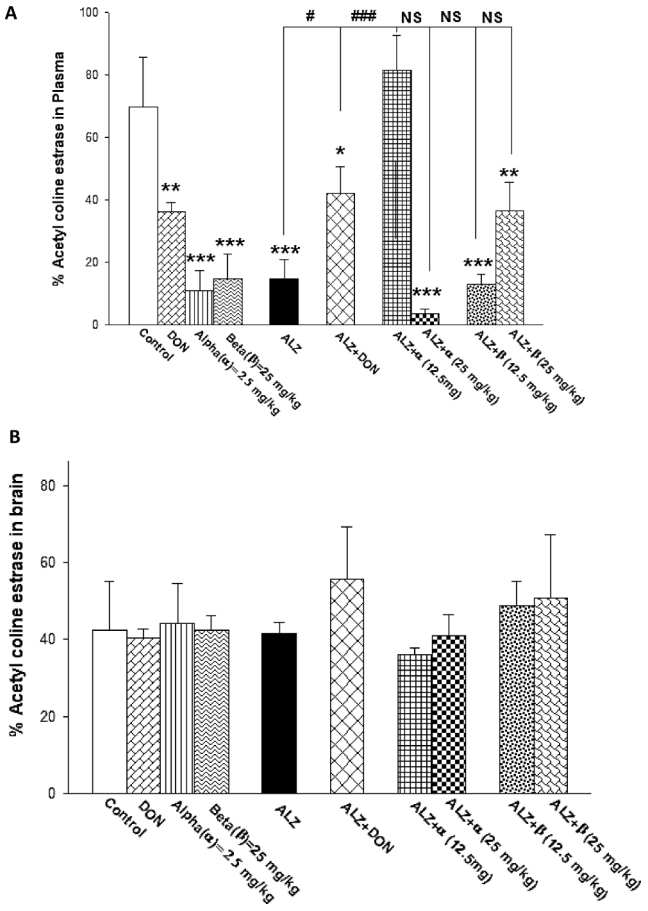


Fig. 9. Comparison of acetylcholine level (A) in plasma, the differences in the mean values among the treatment groups are greater than would be expected; there is a statistically significant difference (P = <0.001). (B) In brain tissue, The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.332). Control and treatment groups consist Control, Alzheimer(ALZ), Donepezil (DON), Alzheimer + Donepezil(Alzheimer group receiving Donepezil), α and β curcumin glucosides(contain control and Alzheimer groups), Beta(control with 25 mg/kg), Alpha(control with 25 mg/kg), Alzheimer group receiving β curcumin glucosides(ALZ + β) and Alzheimer group receiving α curcumin glucosides (ALZ + α). CGs evaluated in every two doses (12.5 and 25 mg/kg).All treatment groups have a positive result. Error bars represent mean ± SEM; n = 8.

rats were groups significantly compared with AD rats, but the rise in FRAP level was at the lowest level compared to other treatments (P > 0.05).

The studies clearly show that involved regions of the brain from patients with advanced AD have increased levels of lipid peroxidation products compared to the controls (Montine et al., 2002; Manczak et al., 2016). According to Fig. 7, the data showed that control and AD rats were significantly different in MDA level; while in the presence of the high dose of CGs (α and β anomers) lipid peroxide levels were low. However, none of the treatments were able to reduce the augmented level of lipid peroxide induced by scopolamine. It is because, according to the literature, the extent of the lipid peroxidation process cannot be appraised because the reference values for these parameters are not known (Jeandel et al., 1989) and Carbonyl (CO) groups (aldehydes and ketones) are produced in protein side chains (especially of Pro, Arg, Lys, and Thr) when they are oxidized.

Protein carbonyl derivatives can also be generated through oxidative cleavage of proteins by either α-amidation pathway or by oxidation of glutamyl side chains (Berlett and Stadtman, 1997). Interestingly,

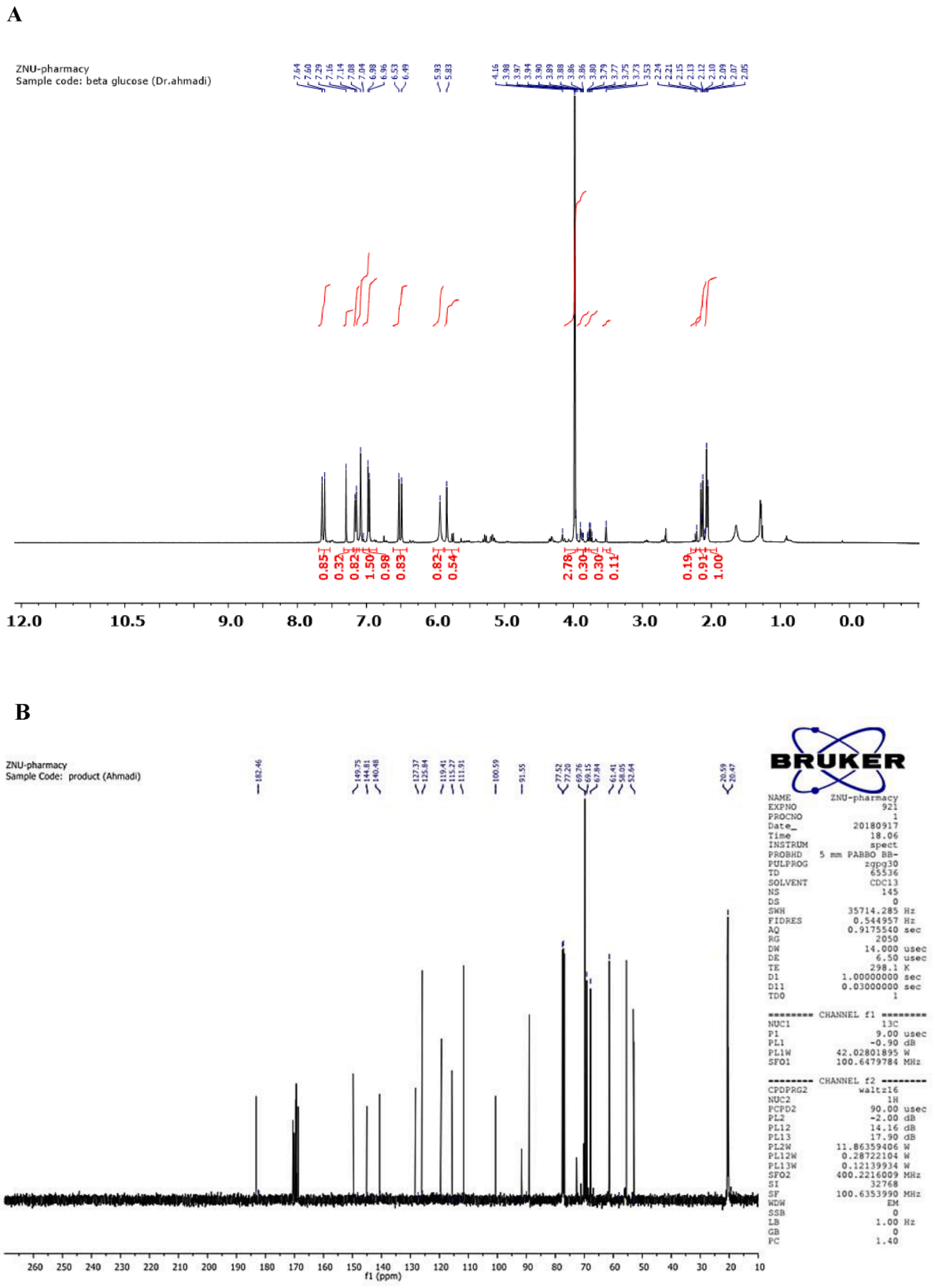


Fig. 10. A) ^{1}H NMR (400 MHZ, $CDCl_3$) spectra of Curcumin tetrahydro β-D-glucoside. B) ^{13}C NMR (100MHZ, $CDCl_3$) spectra of Curcumin tetrahydro β-D-glucoside.

increased concentrations of protein carbonyls have been documented in normal aging as well as in AD and Parkinson's disease. That is why we measured the level of carbonyl protein in all groups (Fig. 8). The Alzheimer group has a high level of carbonyl protein while the rest of the group reduced its level. Although β anomer doses decreased CO group level more than the control group, and Donepezil and doses of α-anomer did it as less as the control group.

Acetylcholine esterase (AChE) level in the brain tissue decreased in

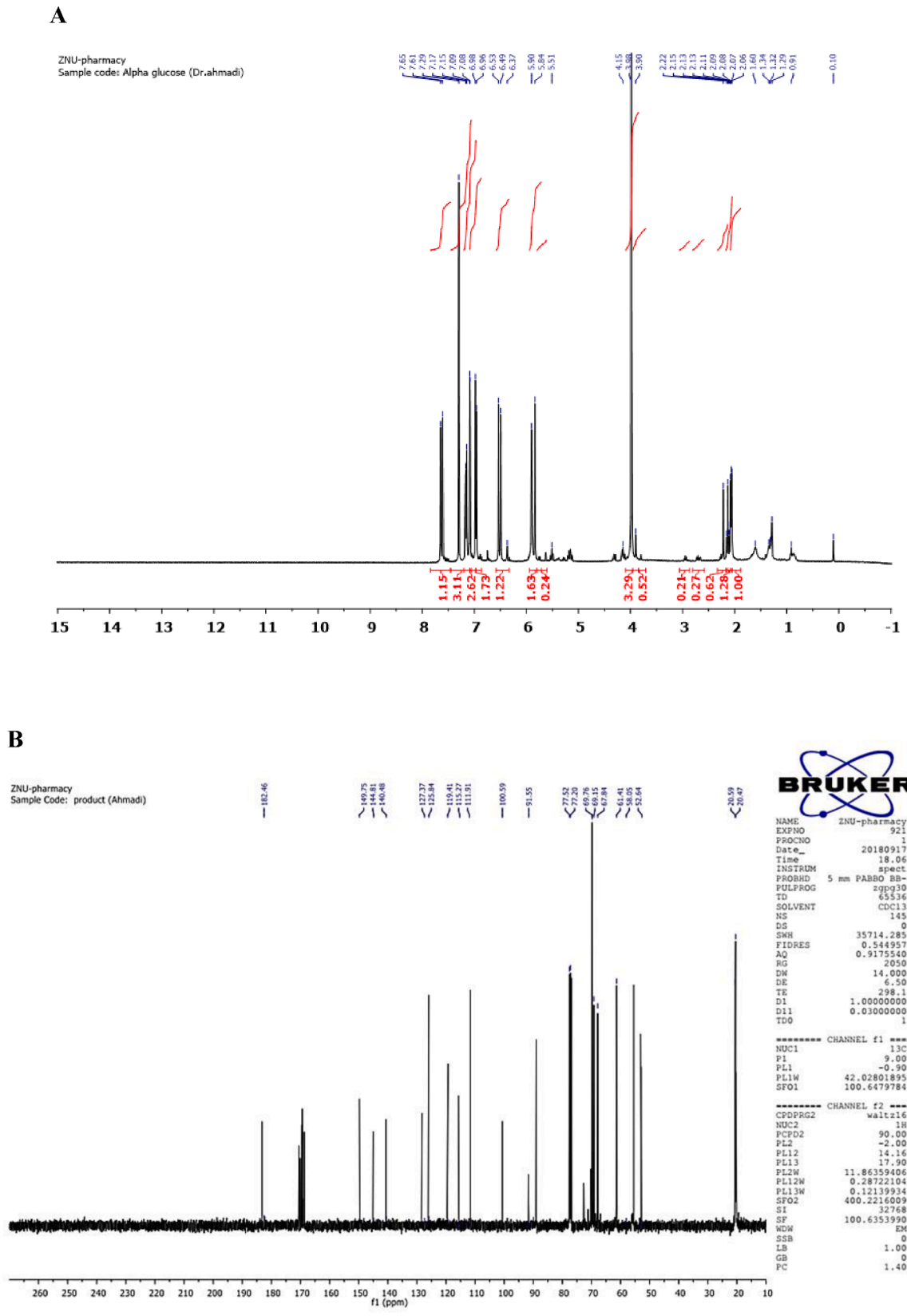


Fig. 11. A) ^{1}HNMR (400 MHZ, $CDCl_3$) spectra of Curcumin tetrahydro α-D-glucoside. B) ^{13}CNMR (100 MHZ, $CDCl_3$) spectra of Curcumin tetrahydro α-D-glucoside.

Alzheimer's Disease (Lombardo and Maskos, 2015). There is a correlation between the loss of ACh and decline in mental status which leads to the cholinergic hypothesis of cognitive impairment in AD. As shown in Fig. 9, the results confirmed the ACh level of the control group is more than the Alzheimer group either at plasma or the brain. All of the treatments increased ACh levels in plasma and brain tissue except α anomer in the case of brain tissue. In plasma, donepezil, the low and high doses of α anomer augmented significantly ACh level into the low

dose of β anomer. It was able to increase ACh level in the Alzheimer group. None of the groups expanded on the ACh level to the control group level. In the brain tissue, donepezil and both doses of β anomer increased ACh level, despite α anomer which even declined ACh level. So donepezil and β anomer performed successfully at plasma and brain tissue.

In conclusion, the present study confirms a novel synthesis method of CGs in good yields with shorter reaction time and in simple and eco-friendly reaction conditions. The results supported the increased hydrophilicity and biological activity of CGs. The data also reveal the compounds CGs showing enhanced antioxidant and activities against AD. It was observed that the CGs containing both anomer α and β were significantly increased glutathione and ACh level in vivo and decreased LPO and carbonyl protein in the animal models. These results uphold the fact that CGs in comparison with donepezil as a commercial drug have potential in reducing the progress of AD with less side effect. Our findings also support other results that have been reported about the protective and therapeutic effect of curcumin and its derivatives in AD in the literature (Lim et al., 2001; Ahmed and Gilani, 2009; Brondino et al., 2014; Aggarwal and Harikumar, 2009).

4. Materials and methods

4.1. Drugs and reagents

Curcumin, glucose, and scopolamine were purchased from Out Co. (Germany), Merck Co. (Germany), and Sigma- Aldrich Co. (America) respectively. Donepezil was a gift of Sobhan Daro, Rasht, Iran. All other chemicals in this study, which are analytical-reagent grade, were purchased locally from the Amertat Shimi pharmaceutical Co. (Mamuniyeh, Iran) and Ezmiran Co. (Tehran, Iran).

4.2. Synthesis of curcumin glucosides (CGs)

4.2.1. Synthesis of curcumin tetrahydro β-D-glucoside and α-D-glucoside

A solution of zinc chloride in anhydride acetic (14 ml) was stirred in an oil bath at 130℃ while Penta acetyl −β-D-glucopyranose (1.12 g, 2.9 mmol) and curcumin (0.48 g, 1.3 mmol) were added slowly. After an hour, the solution was washed with cold water and cold saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated to leave the tetra acetyl-β-D-glucoside curcuminas an orange-red solid (Fig. 1). NaOH: MeOH (10 ml) was added after cooling to room temperature, and the mixture was stirred for about 1hr (until the solid dissolved) and ester hydrolyzed to form β-D-glucoside curcumin. The same method was employed for the synthesis of α-D-glucoside curcumin.

4.3. Animals

100 Wistar male rats weighed 180–200 g were bought from Razi Institute (Tehran, Iran). They were divided into 11groups. Animals housed in the social condition under a 12hr light: dark cycle at 21–23 °C and were allowed access to food and water ad libitum. All procedures were in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. It was also approved by the Animal Ethics Committee of Zanjan University of Medical Sciences. The ethical approval code was: ZUMS. REC.1396.228. All the handlings were performed during the light phase and between 10 a.m. to 16p.m.

4.3.1. Behavioral assessment

Morris water maze (MWM) behavioral test is used to evaluate the spatial learning memory as described fully by Anoush, et al, in 2020, and in literature (Kandimalla et al., 2018; Manczak et al., 2018). Animals were trained for 3 consecutive days in the initial learning phase, started from day 7 of drug administration, in order to learn the place of the platform and try to find it. For each trial, rats were placed in the pool at one of four quadrants (chosen randomly by the software) and allowed to swim and find the platform, during which the time to find the hidden platform (escape latency) was measured. At the end of each run, rats were wiped and put under heather. Spatial memory retention on the test day, can be evaluated by the software on the basis of the time, which animals spent within the “target” quadrant which contains the hidden platform during training days.

4.3.2. Plasma and brain collection

On the last day (10th day), all rats were anesthetized using the CO2 chamber, blood samples were taken and their plasma was separated. Simultaneously, the brain tissues were quickly isolated in an ice - bath and prepared for biochemical examinations.

4.3.3. Antioxidant assay

100 mg of each brain tissue sample was homogenized in 1 ml of Trichloroacetic acid (TCA, 6%) and centrifuged at 10000 rpm. Then, 1.5 ml of ferric reducing Antioxidant power (FRAP) reagent was added into the supernatant and the absorbance was recorded at 593 nm.

4.3.4. Assay of lipid peroxidation level

Lipid peroxidation (LPO) was measured by determining malonyldialdehyde (MDA) and using thiobarbituric acid (TBA) which has been used as an indicator of lipid peroxidation (Ohkawa et al., 1979; Kandimalla et al., 2016). 100 mg of each brain tissue sample was homogenized in 1 ml of KCl (0.156 M) and centrifuged at 3500 rpm, the supernatant was removed and 2.5 ml sulfuric acid (0.05 M), and 2 ml TBA (0.2% w/w) was added to precipitate. It was put in a boiling water bath for 30 m, cooled, and then 4 ml of n-butanol was added to the mixture. It was centrifuged at 3500 rpm and the absorbance at 532 nm (Mahadik et al., 1995) was recorded.

4.3.5. Assay of glutathione level.

Reduced glutathione (GSH) as a non-enzymatic intracellular antioxidant defense protects cells from the damaging effects of ROS level. It also maintains the thiol-containing proteins in their active form. Briefly,100 mg of each brain tissue sample was homogenized in 1 ml of EDTA (0.02 M), then 1.5 ml of TCA (10% w/w) was added and centrifuged. 2.5 ml of Tris buffer (pH = 8.9) and 0.5 ml of DTNB (5, 5′-dithiobis-2-nitrobenzoic acid) as GSH reagent was added to 1 ml of supernatant. The change in the absorbance at 412 nm was followed and the GSH level was represented as μg mg^{-1} protein.

4.3.6. Assay of acetylcholine esterase activity

Inhibition of AChE activity was determined by a modified micro assay method of Ellman (Zhang et al., 2014). The enzyme hydrolyzes the substrate acetylthiocholine and results in the product thiocholine which reacts with Elman’s reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. According to the procedure, 40 μL of AChE (75 mm in buffer with pH 8) and 10 μL of the sample solution were added to 3.0 ml of pH 8 buffer and pre-incubated on ice (4℃) for 30 min. Duplicate tubes (blank control and test groups of plasma) were also treated in the same way. The reaction was triggered by adding 10 μL of 0.25 mM DTNB and 10 μL of 3 mM ACh, and the solution was incubated at 37℃ for 20 min. Optical density was measured at 412 nm immediately and inhibition percentage was calculated. We measured AChE activity in the brain tissue samples according to the same method just to add 1 ml of Hyamine after step incubated at 37℃. All the measurements were carried out in triplicate.

4.4. Statistical analysis

All data are presented as mean ± SEM values. All data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test. A probability (P) value of <0.05 was considered significant. Statistical analysis was performed using SPSS 23.0 software package for

Windows.

CRediT authorship contribution statement

Nahid Ahmadi: Conceptualization, Writing - original draft, Software. **Mir-Jamal Hosseini:** Visualization, Data curation. **Kobra Rostamizadeh:** Supervision. **Mahdieh Anoush:** Visualization, Data curation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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