

Anticataleptic activity of Zamzam water in chlorpromazine induced animal model of Parkinson disease

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Abstract: Parkinsonism is characterized by rest tremor, inflexibility, balance debilitation, slow motion and dementia. It is known to be caused by the deficiency of dopaminergic neurons in nigrostriatal pathway. Different studies propose that oxidative burden may be included in the apoptotic process in parkinsons disease. Zamzam water being alkaline in composition may diminish the oxidative stress and hence relieve the symptoms. Therefore, the purpose of this study was to explore the neuroprotective effect of zamzam water in chlorpromazine induced animal model of Parkinsonism. Results revealed that zamzam water did not show significant anticataleptic effect after 21 days as compared to chlorpromazine treated group. However, after 30 days of giving zamzam water showed highly significant decrease ($p<0.001$) in cataleptic score as compared to chlorpromazine treated group that is negative control. After 30 days of dosing, cataleptic scores by zamzam water were closer to standard drug but standard drug (levodopa/carbidopa) still showed better results than zamzam water. Results from histopathological study of rat's brain also revealed regenerative changes by zamzam treated water when compared with negative control. This regenerative change after zamzam water treatment might play a positive role in future if administered continuously. These results also suggest that zamzam water can be used in combination with standard drug to produce synergistic effect in the management of parkinsons disease.

Keywords: Catalepsy, chlorpromazine, Parkinson disease, Zamzam water.

INTRODUCTION

Parkinson disease (PD) is a common neurodegenerative disease that effects no less than 1% of individuals by age 70 (Nutt and Wooten, 2005; Elbaz *et al.*, 2002; De Rijk *et al.*, 2000, Hindle, 2010). Goetz (2002) concisely expressed that Parkinson's disease (PD) is due to loss of nerve cells in *Substantia nigra* that mediates dopamine neurotransmitter. Parkinsons is characterized by rest tremor, inflexibility, balance debilitation and slow motion. Anomalies in the *Substantia nigra* were also considered to be the pathological markers of the disease (Greenfield and Bosanquet, 1953). Different elements might play role in this specific disorder, among which oxygen free radical biotransformation seems to be considered as particularly essential part (Hirsch, 1993). Morphological studies on autopsical brain, have demonstrated that mesencephalic dopaminergic neurons eventually deteriorate by apoptosis in patients with Parkinson's disease. Different studies propose that oxidative burden may be included in the apoptotic process (Hirsch, 1993). At a later phase of the ailment, *Substantia nigra* shows elevated lipid per oxidation (Dexter *et al.*, 1989), raised manganese-dependent super oxide dismutase action (Saggu *et al.*, 1989), diminished glutathione levels (Sian *et al.*, 1994; Riederer *et al.*, 1992), and high iron level, which might support production of hydroxyl radicals (Hirsch *et al.*, 1991).

As therapeutic solutions, the interest for complementary and alternative medicine (CAM) has additionally expanded over the previous decade. Kessler (2001) expressed that lifetime pervasiveness of CAM treatment use in the United States has expanded consistently since 1950. In fact, current studies have demonstrated that CAM has progressively been adopted in the US (Eisenberg *et al.*, 1993; Barnes *et al.*, 2014) and throughout the world (Haertel and Volger, 2004; MacLennan *et al.*, 2002).

Hydrotherapy, in the past called hydropathy, is included in traditional and alternative medicine, specifically of naturopathy. This includes the use of water for pain alleviation and treatment. The term envelops a wide scope of methodologies and remedial systems that utilizes the physical properties of water, for beneficial effects. Zamzam water is used by Muslims either medicinally or religiously. Muslims refer to water of Zamzam as something revered and unique (Shomar, 2012). Being alkaline in nature, Zamzam water ameliorates oxidative stress and reduces the chance of disease progression. According to Bamosa *et al* (2013), Zamzam water decreases hemoglobin A_{1c} in diabetic TYPE 2 patients. Pathogenesis and complication of different diseases may be prevented by Zamzam water due to its antioxidant potential. Based on the aforementioned facts therapeutic actions of Zamzam water were explored on Parkinson's disease using animal model of Parkinsonism.

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MATERIAL AND METHODS

Procurement of Zamzam water

Zamzam water was collected from Mecca, Saudi Arabia in 10litre bottles provided by Saudi authority.

Selection of animals

Albino rats weighing 200-250g were bred in the animal house of Department of Pharmacology, University of Karachi. Rats were housed in transparent cages with saw dust covered floor in a quiet room. The selected animals were maintained under constant environmental conditions $23\pm 2^{\circ}\text{C}$. They were given standard diet and water *ad libitum* for 30 days. Animals were acclimatized at least a week before the beginning of experiments.

Screening of anti-Parkinson activity

Randomly rats were categorized into 4 groups, each group contained 10 rats. Group 4 was administered levodopa/carbidopa (1:10 ratio) (10mg/kg, orally) with chlorpromazine and was set as standard group. Group 3 was administered Zamzam water *ad libitum* with chlorpromazine and was set as treated group. Group 2 was set as negative control and was administered chlorpromazine only. Group 1 was administered distilled water and was set as normal control group. Group 2,3 and 4 were administered chlorpromazine 5mg/kg i.p daily for 21 days for the induction of parkinsonism (Bais *et al.*,1995). After 30min of the administration of chlorpromazine catalepsy was noted in all groups.

Bar test

Bar test was used to observe catalepsy in the animals. The test involved placing forepaws of the rat on a 10 cm bar placed on the table top. The time taken by the rat to move its forepaws from the bar was observed. The degree of catalepsy (cataleptic score) was measured as the time the animal maintained an imposed posture (Hoffman and Donovan, 1995). Catalepsy score was analyzed on 21st and 30th day of dosing. Animals were sacrificed after 30days and the brains were analyzed after H & E staining (Kulkarni *et al.*, 2009). We selected whole brain for this histopathological analysis to present/correlate at least some basic changes in the entire brain.

Hematoxylin & eosin staining for histopathological analysis

Collected brains were preserved in buffered formalin (10%) and were sent to Dow University of Health Sciences for H & E staining and histopathological examination. H & E staining procedure was in accordance to Khatoon *et al* (2016) and was conducted under the supervision of Dr. Bushra Sikandar.

STATISTICAL ANALYSIS

One way ANOVA followed by post hoc analysis by Scheffe' test was used to evaluate the differences between

various means. P-value indicate levels of significance i.e. $P<0.05$ is considered as significant, $P<0.01$ as very significant and $P<0.001$ as highly significant.

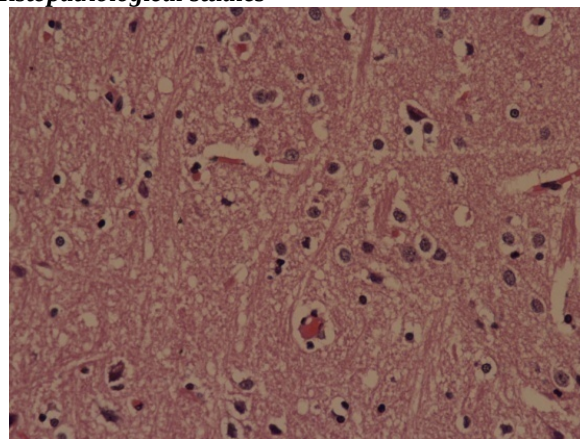
RESULTS

Result showed in test group (Zamzam water) after 21 days of dosing a considerable increase in cataleptic score time as compared to normal control while it showed insignificant effect as compared to negative control as shown in table 1. However, after 30 days of dosing, Zamzam water showed highly significant decrease in cataleptic score as compared to negative control.

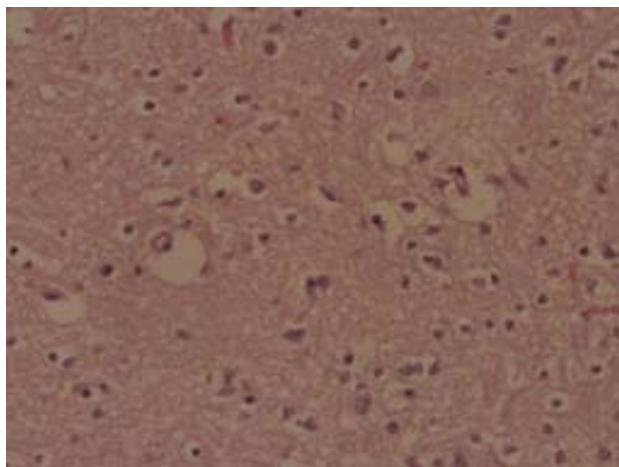


Fig. 1: Zamzam water

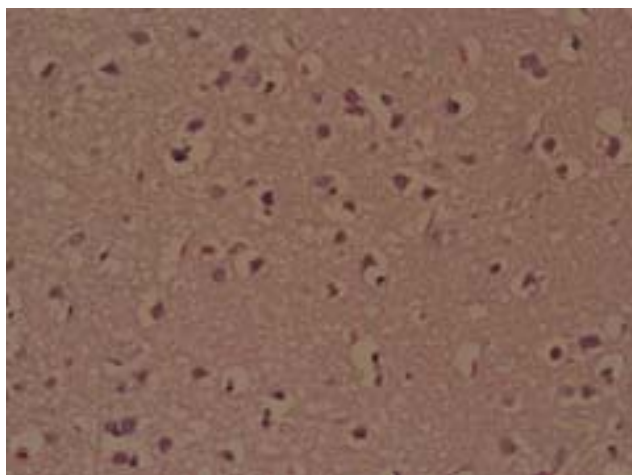
Histopathological studies



Slide 1: Histopathological slide of control brain: Microphotographs of Haematoxylin &Eosin at $\times 400$ representative section of control, shows a normal neuron lying in fibrillary background composed of neuroglial cells.



Slide 2: Histopathological slide of chlorpromazine treated brain: Microphotograph of Haematoxylin & Eosin at x400. There is presence of large to medium sized neurons with fibrillary background showing eosinophilic necrosis of neurons in the background of edema along with proliferative vessel.

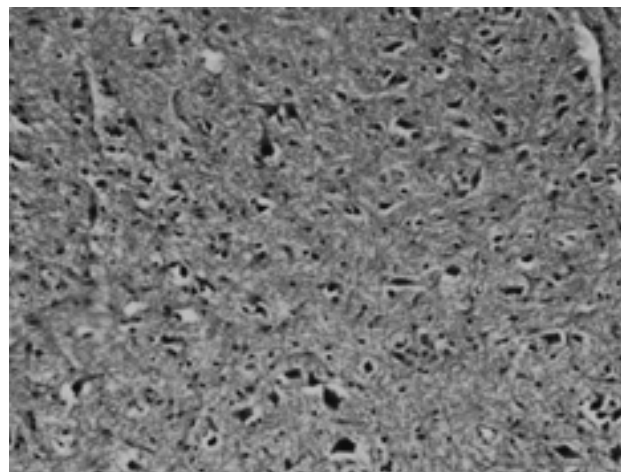


Slide 3: Histopathological slide of Zamzam water treated brain: regenerative activity, mild reactive changes with lots of proliferative blood vessels.

DISCUSSION

PD occurs due to the death of the nigral dopaminergic neuron leading to the depletion of the monoaminergic neurotransmitter, dopamine in the nigrostriatal tract. Oxidative stress is behind the apoptotic process involved in dopaminergic neuron deterioration. Oxidative stress increases as body becomes acidic which add toxicity to the body as a result of metabolic exhaust coupled with free radical production. Zamzam water serves as alkaline water which is naturally rich in minerals and counts for its pH above 7. Alkaline water contains antioxidant properties and achieves better overall health (Foss, 2015; Buchanan, 2010). Zamzam water being alkaline in nature may diminish the oxidative stress and hence relieve the symptoms. This study also revealed anti-Parkinson effect

of Zamzam water in animal model of Parkinsonism but only after 30 days of dosing. This result is also in accordance with histopathological study which revealed regenerative changes.



Slide 4: Histopathological slide of levodopa treated brain: Microphotograph of H& E x400. Representative section of levodopa/carbidopa treated animal showing relatively normal looking medium sized neuron along with proliferative blood vessels, increase cellularity and reactive glial cells. Few neurons exhibiting hyperchromatic nuclei and few show pyknotic nucleus in background of reactive astrocytes. Few eosinophilic necrosis are also present.

Levodopa/carbidopa showed remarkable anti-Parkinson effect throughout the study. It is a precursor of dopamine hence can show the regenerative changes earlier but Zamzam water has to stimulate the release of endogenous dopamine which might take some time to show complete regenerative changes as compared to standard drug. Initial regenerative changes by Zamzam water treatment are indication that it might play a positive role in future studies if given for a longer period of time. Also, Zamzam water may be given in combination with levodopa/carbidopa to observe synergistic effects. This may also allow us to probably reduce the dose of levodopa/carbidopa in patients treated with this drug. However, this needs to be evaluated and confirmed by clinical studies.

Positive effect of Zamzam water against PD may be due to its alkaline nature as it may reduce the oxidative stress which is major factor contributing to the progression of PD. The neuroprotection shown by Zamzam water may be attributed to the presence of zinc (Khalid *et al.*, 2014). Zinc is considered as an important trace element and is known to play major role in proper functioning of central nervous system. According to Singla and Dhawan (2016), zinc causes decrement in the protein expressions of caspase 8, caspase 9, caspase 3, cytochrome-c, caspase 6, Bax, Apaf-1 and caspase 7. Zinc also provides protection against Aluminium mediated neurotoxicity by stimulating anti-apoptotic pathways. Researchers also mentioned that

zinc has multiple roles in the brain and can be recommended as a prophylactic treatment in preventing the onset of neurodegenerative changes (Singla and Dhawan, 2016). Different studies propose that oxidative burden may be included in the apoptotic process (Elmore, 2007). Zinc has capability of reducing oxidative burden (Prasad, *et al*, 2007). Zn reduced the protein expressions of proapoptotic cytochrome c, Bax, Apaf-1, caspase 9, caspase 3, caspase 8, caspase 6 and caspase 7 whereas it elevated the antiapoptotic Bcl-2 in Aluminium mediated neurotoxicity. Thus it regulates apoptotic machinery (Singla and Dhawan, 2016). Researchers also suggest that zinc has multiple roles in the brain and can be recommended as a prophylactic treatment in preventing the onset of neurodegenerative diseases (Singla and Dhawan, 2016). Thus zinc can be proved beneficial in the reversal of symptoms of Parkinson disease by its anti apoptotic action.

Future prospect

This study can be further extended to confirm the exact molecular mechanism of Zamzam water in amelioration of the symptoms of Parkinson disease.

Compliance with ethical standards

The study was approved by University Board of Advanced Studies and Research. All procedures performed in studies involving animals were in accordance with the ethical standards of the university. Animals were handled according to Helsinki resolution 1964.

CONCLUSION

The results suggest that probably Zamzam water can be used in combination with standard drug to produce synergistic effect in the management of Parkinson's disease. This effect need to be further studied in clinical studies.

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