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Evaluation of Anti-Amnesic Activity of Essential Oil of *Kaempferia galanga* in Mice

	
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ABSTRACT

Background: Herbal drugs have used in India since ancient times. But its utilization got reduced due to global urbanization. The world is again moving towards herbal alternatives due to the side effects of allopathic drugs. The essential oils penetrate better through the brain cells, have fewer side effects, and thus can be used to prevent neurodegeneration effectively. **Aim:** The study investigated the neuroprotective efficacy of the essential oil of rhizomes of *Kaempferia galanga* (EORKG) in mice. **Materials and methods:** We assessed behavioural parameters using the Elevated plus maze test, Morris water maze test, and antioxidant parameters by TBARS, Glutathione, and AChE assay respectively. **Results:** The escape latency and transfer latency was significantly reduced in mice receiving the standard drug and the test drugs and spent more time in the target quadrant compared to the disease control group. **Conclusion:** The EORKG exhibited neuroprotective efficacy.



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INTRODUCTION

Any pathological condition that mainly affects neurons is known as neurodegeneration. Increasing age is the biggest threat to the development of neurodegenerative disorders. [1] Currently, 30 million people all over the world are suffering from Alzheimer's dementia and the World Health Organization projects this count will triple in the coming 30 years. It has become a very common disease since its average incidence has been projected to climb from about 5% -50% by age 70 - 90. The first symptom noted by Alzheimer patients is amnesia. [2] Amnesia is a serious loss of memory generally caused either due to brain trauma or by toxic substance ingestion. Nootropic agents such as piracetam, pramicacetam, aniracetam, and anticholinesterase such as donepezil are principally being used to treat amnesia. The adverse effects related to these drugs such as liver toxicity, drowsiness, headache, gastrointestinal disturbances, tiredness, constipation, nasal congestion, hypotension, rashes, and systemic side effects on prolonged use restricts utilization. [3] Consequently, necessity emerges for developing a new drug with adequate efficacy and fewer side effects. World Health Organization (WHO) estimates that herbal medicine is presently being used by 80% of the world's people for some aspects of principle health maintenance. [4] India has been using herbal remedies since ancient times. Ayurvedic literature has described most neurodegenerative disorders and defined many plants for treating those with plausible remedial benefits. [5, 6, 7, 8] The plant's active substance enters the body through an oral or topical route or even inhaling the aroma. [9] Aromatherapy uses essential oils and is a holistic process that uses fragrances for the healing of the body and mind. Essential oils are volatile complex secondary metabolites of plants. [10] These have become noteworthy for the treatment of various neurological disorders due to better understandings of the chemistry of EOs and the capability to penetrate through biological membranes. [11] The EORKG owing to its volatile oil constituents and fragrance is used in aromatherapy. The EORKG has been mentioned in the standard reference book of medicinal herbs to possess nerve strengthening activity. [12] So far its preclinical anti-amnesic activity has not been investigated. Thus the present work was an investigation of the anti-amnesic potential of essential oil EORKG.

MATERIALS AND METHODS

Drugs

All the doses of EORKG were made in distilled water at a concentration of 1ml/100g of body weight using a suspending agent (5% Tween 80 solution).

Chemicals and reagents

We used Analytical grade chemicals and reagents: Scopolamine hydrobromide Brand name: Buscopan (mfg. Boehringer Ingelheim Vetmedica), Reserpine (Loba Chemie); Donepezil (Ranbaxy); Imipramine (SD Fine Chemicals).

Animals

We purchased Swiss Albino mice weighing 20g -25g of either sex from Bombay Veterinary College, Parel, Mumbai, and acclimatized them for 14 days with access to water and food *ad libitum*. The animal house had a controlled temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and humidity ($50 \pm 10\%$). The Institutional Animal Ethics Committee approved the study on 11/8/18 with protocol no. PCOL/IAEC/2018/13. We followed the guidelines and ethical principles laid by CPCSEA (Committee For The Purpose of Control and Supervision of Experiments on Animals).

Phytochemical Analysis

Preliminary phytochemical tests were performed on EORKG for detecting alkaloids, glycosides, carbohydrates, proteins, triterpenoids, amino acids, flavonoids, saponins, steroids, and tannins. The confirmatory test for active constituent was also performed.

Acute toxicity of EORKG ^[13]

The EORKG's acute toxicity was performed according to the OECD guideline no. 423. We administered 2000mg/kg EORKG as the limit dose and observed for any signs or symptoms of toxicity for 14 days.

Experimental group:

Group 1: Vehicle control (tween 80+Water)

Group 2: Disease control (Scopolamine 0.5mg/kg i.p.)

Group 3: Standard Control (Donepezil 4mg/kg p.o + Scopolamine 0.5mg/kg i.p)

Group 4: Test group 1 (EORKG 200mg/kgp.o +Scopolamine 0.5mg/kg i.p)

Group 5: Test group 2 (EORKG 400mg/kgp.o + Scopolamine 0.5mg/kg i.p)

Group 6: Test group 3 (100 microliters EORKG inhalation route + Scopolamine 0.5mg/kg i.p)

Behavioural assessment

Elevated plus maze (EPM) test ^[14]

Six groups of mice (n=8) were used. The respective treatments were given to the mice for 8 days for each group. We followed the procedure as described by Desai *et.al.* ^[14]

Morris water maze test ^[15]

“Six groups of mice (n=6) were used. The respective treatments were given to the mice for 10 days for each group. We followed the procedure as described by Dhingra and Kumar. ^[15]

Biochemical estimation

After behavioral assessments, we sacrificed mice using CO₂ chamber and removed the brains. We kept each brain on ice and rinsed with ice-cold isotonic saline and prepared (10% w/v) homogenate in 0.1M phosphate buffer (pH 7.4) and centrifuged the homogenate at 3000 rpm for 15 minutes and collected the aliquots of supernatant and used for biochemical estimations.

Thiobarbituric Acid Reactive Substance Assay ^[16]

“0.5 ml of phosphate buffer (pH 7.2) and 1 ml of 10% trichloroacetic acid was added to the tissue homogenate (0.5 ml). The mixture was centrifuged at 3000 rpm at 4°C for 10 min. The supernatants of the tissue homogenates were incubated with 1 ml of 0.8% w/v of the thiobarbituric acid at 100°C for 15 min. After a cooling period, TBARS concentration was determined spectrophotometrically at 532nm. The unit for expressing lipid peroxide levels was nanomoles of TBARS.”^[16]

Estimation of reduced glutathione ^[17]

“For the estimation of reduced glutathione, the 1mL of tissue homogenate was precipitated with 1mL of 10% TCA. To an aliquot of the supernatant, 4mL of phosphate solution and 0.5mL of 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent were added and absorbance was taken at 412nm. The values were expressed as nM of reduced glutathione per mg of protein.”^[17]

Estimation of acetylcholine level ^[16]

“Acetylcholinesterase, the cholinergic marker, was estimated in the whole brain employing the Ellman method. Ellman's reagent is 5, 5'-dithiobis (2-nitrobenzoate). It is also known as DTNB. Incubate 0.1 ml of brain tissue homogenate for 5min after adding 0.1 ml of DTNB and 2.7 ml of phosphate buffer. Then prepare acetylthiocholine iodide (pH 8) freshly. Add 0.1 ml of it. The absorbance was recorded at 412 nm.”^[16]

Statistical Analysis

We used Graph Pad prism software for statistical analysis. The data was analyzed by using one way ANOVA followed by Tukey's multiple comparison test. The results (values) were written as mean \pm SEM. The level of significance was chosen at $p < 0.05$.

RESULTS

Phytochemical tests

Preliminary phytochemical screening confirmed the presence of phenols, triterpenoids in the EORKG. The confirmatory test for active constituent p-methoxy ethyl cinnamate confirmed its presence.

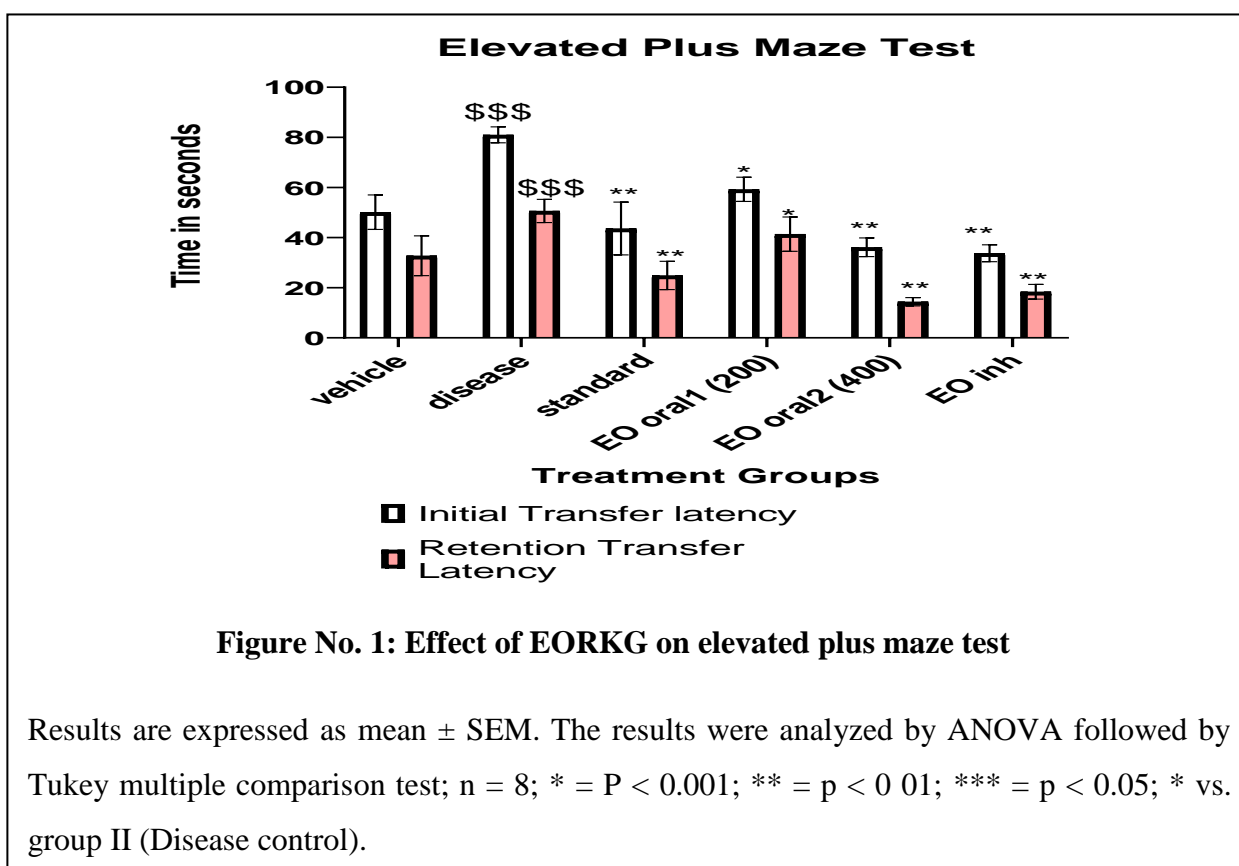
Acute toxicity of EORKG

None of the mice were found dead at this dose. There were no changes in a behavioral pattern throughout the period of 14 days. The EORKG was observed to be safe at the maximum dose of 2000mg/kg bodyweight.

Behavioral Assessment

Effect of EORKG on elevated plus maze test

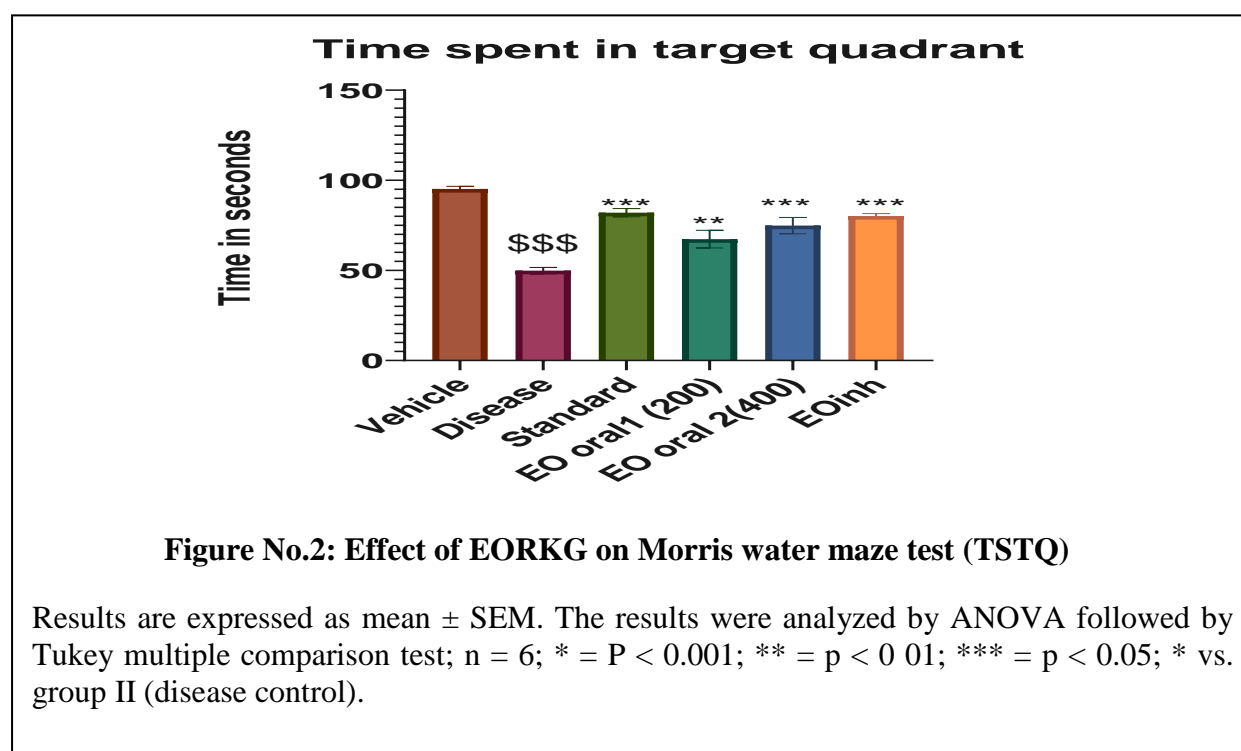
Administration of scopolamine (0.5mg/kg) intraperitoneally presented a significant ($p < 0.001$) raise in transfer latency on comparing with the vehicle control group, demonstrating the deterioration of memory in mice. The evaluation parameter transfer latency was significantly reduced ($p < 0.01$) for mice treated with donepezil (4mg/kg) through oral route on comparing with the disease control group, demonstrating reversal of scopolamine-induced memory deterioration. Treatment with EORKG by oral accompanied by inhalation route with $p < 0.05$, $p < 0.001$ respectively had significantly annulled scopolamine-induced amnesia.



Effect of EORKG on Morris water maze test

From day 1-4, the administration of scopolamine (0.5mg/kg) intraperitoneally presented a significant ($p < 0.001$) raise in escape latency on comparing with the vehicle control group, demonstrating the deterioration of memory in mice. The evaluation parameter escape latency

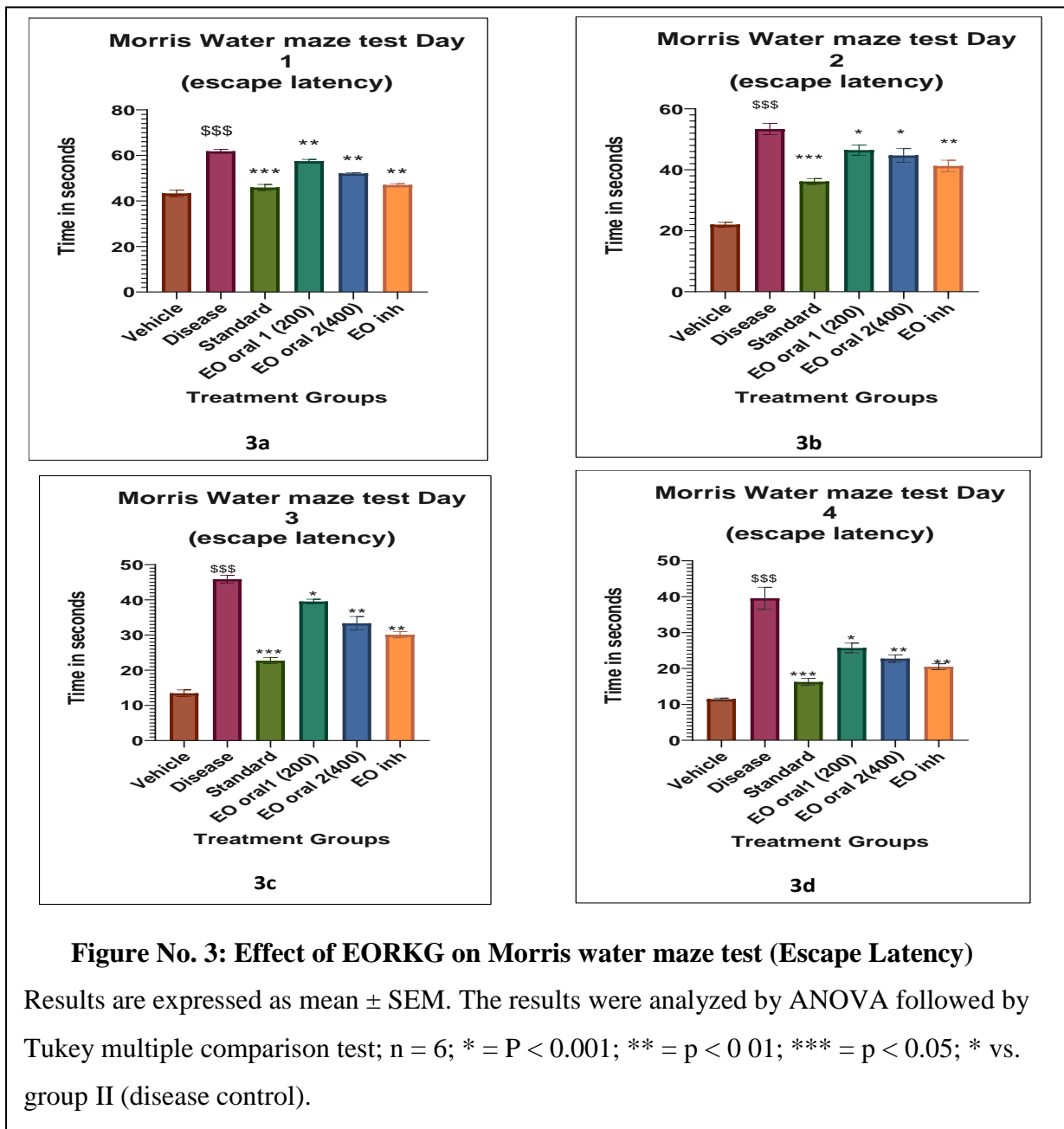
was significantly reduced ($p < 0.01$) for mice treated with donepezil (4mg/kg) through oral route on comparing with the disease control group, demonstrating reversal of scopolamine-induced memory deterioration. Similarly, treatment with EORKG by oral and inhalation route with $p < 0.05$, $p < 0.001$ respectively had significantly annulled scopolamine-induced amnesia. Administration of Scopolamine (0.5mg/kg) intraperitoneally with $p < 0.001$ presented a significant drop of time the mice spent in the target quadrant while comparing with the vehicle control group, demonstrating the deterioration of memory in mice. The evaluation parameter time spent in the target quadrant was significantly reduced with $p < 0.001$ for mice treated with donepezil (4mg/kg) through oral route on comparing with the disease control group, demonstrating reversal of scopolamine-induced memory deterioration. Likewise, treatment with EORKG by oral 200mg/kg orally and 400mg/kg orally besides with inhalation route with $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively had significantly annulled scopolamine-induced amnesia.

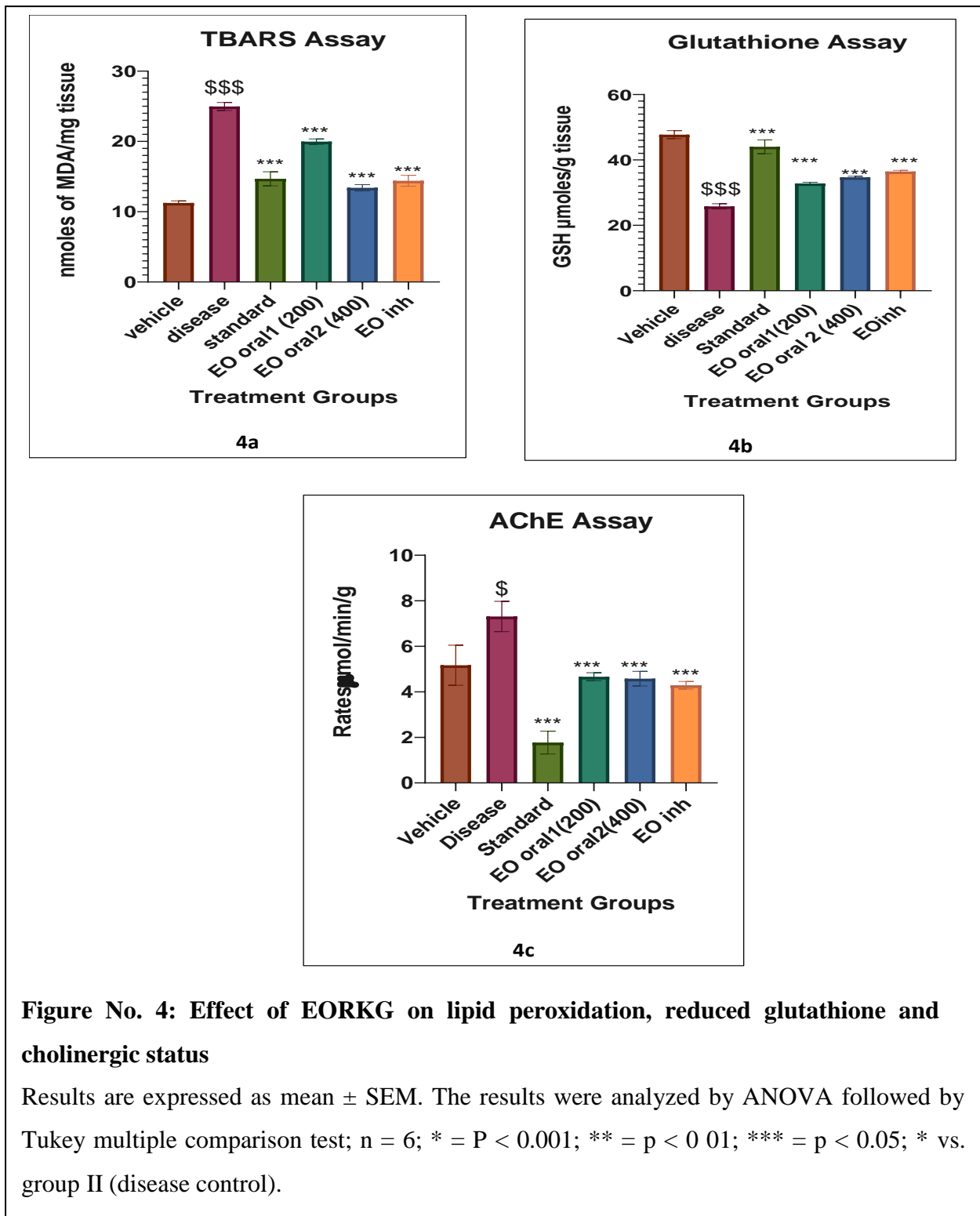


Effect of EORKG on lipid peroxidation, reduced glutathione, and cholinergic status

Administration of Scopolamine (0.5mg/kg) intraperitoneally with $p < 0.05$ presented a significant increase in AChE level and malondialdehyde level and a decrease in glutathione level on comparing with the vehicle control group demonstrating oxidative neuronal damage resulting in impairment of memory in mice. The parameters AChE level and malondialdehyde

level was significantly ($p < 0.01$) reduced and glutathione level had significantly ($p < 0.01$) increased for mice treated with Donepezil (4mg/kg) orally while comparing with the disease control group, indicative of reversal of oxidative damage and thus annulled scopolamine-induced memory impairment. Likewise, treatment with EORKG by oral 200mg/kg orally and 400mg/kg orally besides with inhalation route with $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively had significantly annulled scopolamine-induced amnesia.





DISCUSSION

The present study's objective was at demonstrating the efficacy of EORKG against the amnesic activity. To assess the efficacy, scopolamine, an anti-muscarinic agent was employed for the induction of memory deterioration in mice. Scopolamine influenced amnesic mouse has been utilized as an exact model for Alzheimer's disease (AD) because the memory weakening seen is like the memory deterioration found in AD. Scopolamine-influenced deterioration of memory is related to the altered status of oxidative pressure in the brain. The EPM and MWM test was used for evaluating the deterioration. The EPM model is generally perceived in rodents for training, learning, and memory traits. In the EPM, learning can be considered as transfer latency (TL) noted on the day 7 training/trial, and the maintenance/retention of memory is indicated by TL investigated after 24hours. In our study, the administration of EORKG for 7 days to mice promoted learning and protected from memory deterioration influenced by scopolamine. A decrease in transfer latency after 24 hrs (Day 8) demonstrated improved maintenance of the learned task on day 7. Moreover, the EORKG was also assessed to show its cognitive upgrading consequences for spatial, memory, and learning capacity of mice against scopolamine influenced amnesia employing the MWM test. In this test, EORKG treatment on the scopolamine influenced amnesic mice exhibited a noteworthy reduction in escape latencies (EL) in daily trials during 4 continuous days training/trials as compared to scopolamine administered groups. Also, the EORKG treated mice spent more time in the target quadrant on the fifth day. These results of these two parameters proposed that extract improved the disabled reference memory (long-term memory) influenced by scopolamine. Moreover, the EL was significantly different in intraday trials (increased every day) on training days in treatment groups which revealed the development of working memory which is a type of short-term memory. The results of biochemical estimation also proved the efficacy against oxidative damage. The results demonstrate that EORKG enhances memory function and spatial learning against scopolamine-induced amnesia.

CONCLUSION

The behavioral test results suggest that the administration of the EORKG demonstrated significant anti-amnesic activity. From the results of biochemical estimation, it was evident that the EORKG has antioxidant potential and has neuroprotective efficacy. The synergistic effect of the drug by decreasing the AChE level, and antioxidant action in mice brain could


be the rationale of observed neuroprotection; hence, this may prove beneficial for the treatment of amnesia.

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