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Protection against Cholinesterase and Oxidative Stress Contributes to The Effect of Rutin ameliorating Ethanol-Induced Memory Dysfunction in Rats

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ABSTRACT

Ethanol-induced memory impairment in rats is a consequence of changes within the central nervous system that are secondary to impaired oxidative stress and cholinergic dysfunction. Treatment with antioxidants and cholinergic agonists are reported to produce beneficial effects in animal models. Rutin is reported to exhibit antioxidant effect and cholinesterase (ChE) inhibitor activity. However, no report is available on the influence of rutin on ethanol-induced memory impairment. Therefore, we tested its influence against cognitive dysfunction in ethanol-induced rats using Morris water maze test and Novel object recognition test. Lipid peroxidation and glutathione levels as parameter of oxidative stress and ChE activity as a marker of cholinergic function were assessed in the cerebral cortex and hippocampus. Forty five days after ethanol treated rats showed a severe deficit in learning and memory associated with increased lipid peroxidation, decreased glutathione, and elevated ChE activity. In contrast, chronic treatment with rutin (20-80 mg/kg, p.o., once a day for 45 days) and vitamin C (100 mg/kg, p.o.) improved cognitive performance, and lowered oxidative stress and ChE activity in ethanol treated rats. In conclusion, the present study demonstrates that treatment with rutin prevents the changes in oxidative stress and ChE activity, and consequently memory impairment in ethanol treated rats.

Keywords: Rutin; Ethanol; Memory impairment; Oxidative stress; Morris water maze.

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INTRODUCTION

Alzheimer's disease (AD) is clinically characterized by a progressive loss of cognitive abilities, which particularly affects elder population in their daily activities such as memory, speaking, and problem solving. The pathophysiology of AD is complex and involves several different biochemical pathways¹. The key symptoms of AD are primarily caused by cholinergic dysfunction². In order to treat and prevent AD, the most pharmacological investigator has focused on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors to alleviate cholinergic deficit and improve neurotransmission^{3,4}.

Ethanol consumption is the most common cause of peripheral as well as central nervous system toxicity. Ethanol induced brain damage produces some of the most insidious effects of alcoholism, including cognitive deficits such as learning and memory impairment^{5,6}. Ethanol administration also interferes with performance of spatial learning tasks via its influence on hippocampal functioning. Chronic alcohol exposure causes cell loss in hippocampal structures, decreases cerebral cholinergic activity⁷ and generates ROS which are all associated with learning and memory impairments⁸. Ethanol enhances oxidative stress directly through generation of oxy free radicals and lipid peroxidation⁹ and depletion of endogenous antioxidants such as alpha-tocopherol, glutathione, vitamin C, and vitamin E. Ethanol is oxidized to acetaldehyde by cytochrome P450, which increases reactive oxygen species, with concomitant changes in redox balance^{10,11}.

More over, increased acetylcholinesterase activity (which causes degradation of acetylcholine, a neurotransmitter associated with learning and memory) in the brain has been correlated with memory in disruption ethanol-treated rats¹²⁻¹⁴. Some studies also suggested that galantamine improves the speed of learning, short-term memory and spatial orientation of rats in conditions of prolonged alcohol intake indicating the deficits in cholinergic neurotransmission in chronic ethanol administered rats¹². Hippocampal neurogenesis is involved in hippocampal mediated learning and memory formation. It has been proposed that changes in neurogenesis in the hippocampus may be involved in some of the alterations of cognitive function observe during aging¹⁵. Studies in rodents provide clear evidence that chronic ethanol has adverse effects on synaptic function and synaptic plasticity in the hippocampus and cortex, among other areas¹⁶, and impaired plasticity correlates with defects in learning and memory, particularly spatial learning¹⁷. Recently it was reported that acute ethanol administration impaired spatial memory and cognitive flexibility in barnes maze task¹⁸.

Rutin is a flavonoid with a wide range of biological activities. Rutin is found in many plants (such as buckwheat seeds), fruits (such as citrus fruits), and vegetables^{19,20}. Rutin is a powerful antioxidant and anti-inflammatory polyphenol²¹. Moreover, rutin has been found to have several neuropharmacological effects including neuroprotective, anticonvulsant and antidepressant effects on the central nervous system²²⁻²⁶. Rutin and its analogues, such as epigallocatechin-3-gallate (EGCG) and quercetin, act as efficient radical inhibitors and are reported to rescue spatial memory impairment in rats with cerebral ischemia²⁷. Pretreatment with rutin in chronic dexamethasone administered mice attenuated cognitive deficits and brain impairment²⁵.

In addition, flavonoid derivatives have been proposed to be useful in the treatment of neurodegenerative disorders such as Alzheimer's disease²⁸⁻²⁹. Recently, it was reported that rutin supplementation was effective in suppressing memory dysfunction caused by streptozotocin in rats³⁰. There were several studies have also focused on the effects of rutin on cognition and memory in different models of memory impairment in different animals^{27,31-33}. Therefore, the present study was designed to investigate the protective role of rutin on cognitive dysfunction in ethanol-induced rats.

MATERIALS AND METHOD

Subjects

Adult male Wistar rats born and reared in the Animal House of the Agnihotri College of Pharmacy, Wardha, from a stock originally purchased from Shree Farms, Bhandara, India were used in the present study. Young, healthy male rats (150-250 g) were group housed (three per cage) and maintained at 23 ± 2 °C under 12:12 h light (08:00-20:00 h) /dark cycle with free access to rodent chow and tap water. Animals were naive to drug treatments and experimentation at the beginning of all studies. All tests were conducted between 08:00 and 13:00 h.

All experimental protocol were approved by the Institutional Animal Ethics Committee and carried out under strict compliance with ethical principles and guidelines of Committee for Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India, New Delhi, India.

Drugs and solutions

Rutin was purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA), ethanol (Changshu Yangyuan Chemical, China), vitamin C (Sisco Research Laboratories, Mumbai, India) were used in present study. All the drugs were dissolved in double distilled water (DDW). Drug solutions were prepared fresh and their doses are expressed in terms of their free bases.

Experimental induction of Alcoholic dementia

Alcoholic dementia was induced by 15% w/v ethanol (2 g/kg) in double distilled water once a day, administered intraperitoneally, over a period of 30 s for 45 days. The rats serving as controls were given 1 ml/kg double distilled water per oral (p.o.)³⁴.

Treatment schedule

Chronic treatment

Separate groups of rats (n = 6) were orally administered rutin (20, 40 and 80 mg/kg), vitamin C (100 mg/kg), and vehicle (1 ml/kg) 1 h before ethanol dosing was started from the first day of experiment once a day (20:00 h) for next 45 days (days 1-45), and at the end of treatment schedule rats were subjected to Morris water maze test or novel object recognition test. Similar treatments were given to DDW control rats. The learning and memory were evaluated (days 46-51 for Morris water maze test and days 46-47 for Novel object recognition test).

Assessment of cognitive function

Morris water maze test

Cognitive function of rats was assessed by using Morris water maze test (MWM) as described earlier³⁵. The test apparatus was a circular water tank (180 cm in diameter and 60 cm high) made up of dark gray plastic that was partially filled with water (24 ± 1 °C). Full cream milk (1.5 l) was used to render the water opaque. The pool was divided virtually into four equal quadrants, labeled A–B–C–D. A platform (12.5 cm in diameter and 38 cm high) was placed in one of the four maze quadrants (the target quadrant) and submerged 2.0 cm below the water surface. The platform remained in the same quadrant during the entire experiment. The rats were required to find the platform using only distal spatial extra-maze cues existing in the testing room. The cues were maintained constant throughout the testing. The rats received four consecutive daily training trials for 5 days (days 46–50 after alcoholic dementia 45 days), with each trial having a ceiling time of 90 s and a trial interval of approximately 30 s. The rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 20 s previous to the commencement of the next trial. The escape platform was reserved in the same position relative to the distal cues. If the rat was unsuccessful to reach the escape platform within the maximally allowed time of 90 s, it was gently located on the platform and allowed to remain there for the same amount of time. The time to reach the platform (latency in seconds) was measured.

To test possible deficits in sensorimotor processes, rats were experienced in the water maze with a visible platform on a new location on the final day of training^{35,36}. The test with the visual platform

does not require special orientation³⁷ and was used to show possible deficits in sensorimotor processes. For the test, target platform was placed inside the pool 1 cm above the water line. Rats were allowed to swim for 60 s. Time to reach the platform was recorded as escape latency. After completion of the last trial, rats were gently dried with a towel, kept warm for an hour and returned to their home cages.

Memory consolidation test

A probe trial was performed wherein the extent of memory consolidation was assessed^{35,36}. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. The probe trial was conducted on day 51, wherein the individual rat was placed into the pool as in the training trial, but that the hidden platform was removed from the pool. The time spent in the target quadrant was considered for 60 s. In probe trial, each rat was placed in a starting position directly opposite to where the platform was located. Further, the number of times crossing over the platform site of each rat was also measured and calculated.

Novel object recognition test

Forty five days after confirmation of alcoholic dementia, rats were tested in the novel object recognition test (days 46-47). This test is based on the natural propensity of animals to spend more time exploring a new rather than a formerly encountered object. Memory was evaluated at two retention intervals (30 min and 24 h) as described earlier³⁸. Rats were transported from the animal vivarium to the testing laboratory and allowed to acclimatize for at-least 30 min before behavioral testing. Testing was monitored by an overhead camera. The test was performed in the open field arena (60 cm×40 cm×28 cm) as previously described³⁹. Each rat was exposed to three experimental conditions in the open field. In the initial trial (T1), one object stimulus (O1) was placed in one corner of the open field and the rat positioned in the opposite corner of the arena, and time spent in exploring the object (touching the object with paws or exploring it by olfaction with direct contact of the snout) was measured. The session was terminated when the animal explored the object for 20 s or when 10 min had elapsed. During the second trial (T2), performed 30 min following T1, a second object (O2) was introduced in the adjacent corner to that of the reference object. The time spent exploring the familiar (O1) and the novel (O2) objects was measured for a period of 10 min. In the final trial (T3), performed 24 h following T1, O2 was replaced by a new object (O3) and the time spent by a rat in exploring the reference (O1) and novel (O3) objects was measured for 10 min. From rat to rat, the object presentation order was randomly permuted. After each trial, the objects and arena were cleaned with 70% ethanol solution in order to remove olfactory cues. The objects (plastic toys) were heavy enough to prevent displacement by rats. Raw

data obtained in the object recognition test were transformed into a ratio, reflecting the preference of the animals for the novel versus the familiar object. The ratio formula was $[t_{\text{novel}}/(t_{\text{novel}}+t_{\text{familiar}})]$, where t_{familiar} is the time spent exploring the familiar object and t_{novel} is the time spent exploring the new object in seconds. The closer this ratio to unity, the more the animal spent time exploring novel objects.

Biochemical estimation

Post-mitochondrial supernatant preparation

After behavioral tests, the animals were submitted to euthanasia being previously anesthetized with ethyl ether and brain structures were removed and separated into the cerebral cortex and hippocampus. Cerebral cortex and hippocampus were rinsed with ice cold saline (0.9% sodium chloride) and homogenized in chilled phosphate buffer (pH 7.4). The homogenates were centrifuged at $800 \times g$ for 5 min at 4 °C to separate the nuclear debris. The supernatant thus obtained was centrifuged at $10,500 \times g$ for 20 min at 4 °C to get the post-mitochondrial supernatant, which was used to assay cholinesterase activity.

Cholinesterase activity (ChE)

Cholinergic dysfunction was assessed by measuring ChE levels in the cerebral cortex and hippocampus according to the method described previously by Ellman et al. (1961) with slight modifications. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01M sodium phosphate buffer (pH 8), 0.10 ml of 0.75 mM acetylthiocholine iodide (AcSCh) and 0.10 ml Ellman reagent (5'5 dithiobis [2-nitrobenzoic acid] 10 mM, NaHCO₃ 15 mM). The change in absorbance was measured at 412 nm for 5 min. The results were calculated using molar extinction coefficient of chromophore ($1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$). All samples were run in duplicate or triplicate and the enzyme activity were expressed in $\mu\text{mol AcSCh}/\text{min}/\text{g}$ of protein⁴⁰.

Estimation of lipid peroxidation (LPO)

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARSs) by the method of Wills (1965). Briefly, 0.5 ml of post-mitochondrial supernatant and 0.5 ml of Tris HCl were incubated at 37 °C for 2 h. After incubation 1 ml of 10% trichloroacetic acid was added and centrifuged at $1000 \times g$ for 10 min. To 1 ml of supernatant, 1 ml of 0.67% thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling 1 ml double distilled water was added and absorbance was measured at 532 nm. Thiobarbituric acid reactive substances were quantified using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as nmol of malondialdehyde per mg protein.

Tissue protein was estimated using the Biuret method and the brain malondialdehyde content expressed as nmol of malondialdehyde per mg of protein⁴¹.

Estimation of glutathione

Glutathione (GSH) estimation was done according to the method of Ellman (1959). Briefly, 160 μ l of supernatant was added to 2 ml of Ellman's reagent (5'5 dithiobis [2-nitrobenzoic acid] 10 mM, NaHCO₃ 15 mM) and the mixture was incubated at room temperature for 5 min and absorbance was read at 412 nm⁴².

Statistical analysis

Results were expressed as mean \pm S.E.M. The data were analyzed by two-way or one-way analysis of variance (ANOVA) followed by Bonferroni and Tukey's multiple comparison tests, respectively. Statistical significance was considered at $P < 0.05$ in all the cases.

RESULTS AND DISCUSSION

Effects of rutin on ethanol-induced cognitive dysfunction

Effects of rutin treatment on performance of Morris water maze test

The cognitive function was assessed in the Morris water maze test. The mean escape latency for the trained rats decreased over the course of the 20 learning trials in all groups. Ethanol control rats exhibited significantly higher escape latency on day 2, 3, 4, and 5 during training trials compared to DDW control rats ($P < 0.05$). Two way repeat measure ANOVA revealed that chronic rutin treatment significantly influenced escape latency in ethanol control rats; treatment effect [$F(6, 170) = 14.86, P < 0.0001$] and time effect [$F(4, 170) = 256.1, P < 0.0001$]. Further, post hoc test revealed that rutin treatment (20, 40 and 80 mg/kg) significantly decreased escape latency compared to ethanol control rats on day 2, 3, 4 and 5 ($P < 0.05$). Chronic treatment with vitamin C (100 mg/kg) in ethanol treated rats showed similar results. Treatment with rutin (80 mg/kg) in vehicle treated rats had no significant influence on escape latency ($P > 0.05$) compared to DDW control rats (Figure 1).

The probe trial measures how well the rats had learned and consolidated the platform location during the 5 days of training. One-way ANOVA revealed that ethanol control rats spent less time in the target quadrant as compared to the DDW control rats, and treatment with rutin, and vitamin C significantly influenced the same [$F(6, 40) = 17.00, P < 0.0001$]. Further, post hoc test revealed that rutin (20, 40 and 80 mg/kg), and vitamin C (100 mg/kg) significantly increased time spent in the target quadrant compared to ethanol control rats ($P < 0.05$). Treatment with rutin (80 mg/kg) in

DDW control rats had no significant influence on time spent in the target quadrant ($P > 0.05$) compared to DDW control rats (Figure 2).

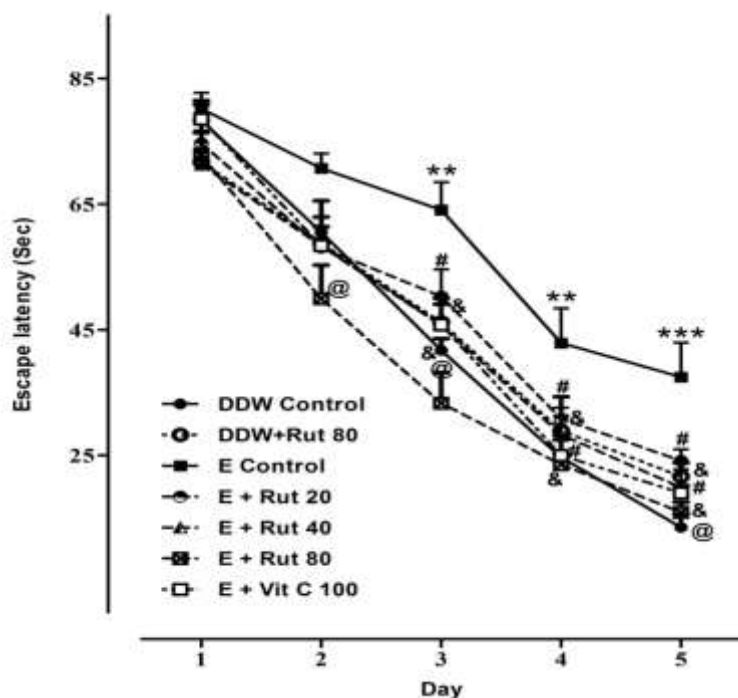


Figure. 1: Effect of rutin treatment (45days) on the performance of spatial memory acquisition phase in Morris water maze test. Each value represents mean \pm S.E.M. of 5-6 observations. $**P < 0.01$ and $***P < 0.001$ vs. DDW control group. $\#P < 0.05$, $\&P < 0.01$ and $@P < 0.001$ vs. ethanol control group (Two-way repeat measure ANOVA followed by Bonferroni multiple comparison test). DDW control: Double Distilled Water control; DDW + Rut 80: Double Distilled Water rutin (80 mg/kg) treated; E control: ethanol control; E + Rut 20: ethanol rutin (20 mg/kg) treated; E + Rut 40: ethanol rutin (40 mg/kg) treated; E + Rut 80: ethanol rutin (80 mg/kg) treated; E+ Vit C 100: ethanol vitamin C (100 mg/kg) treated.

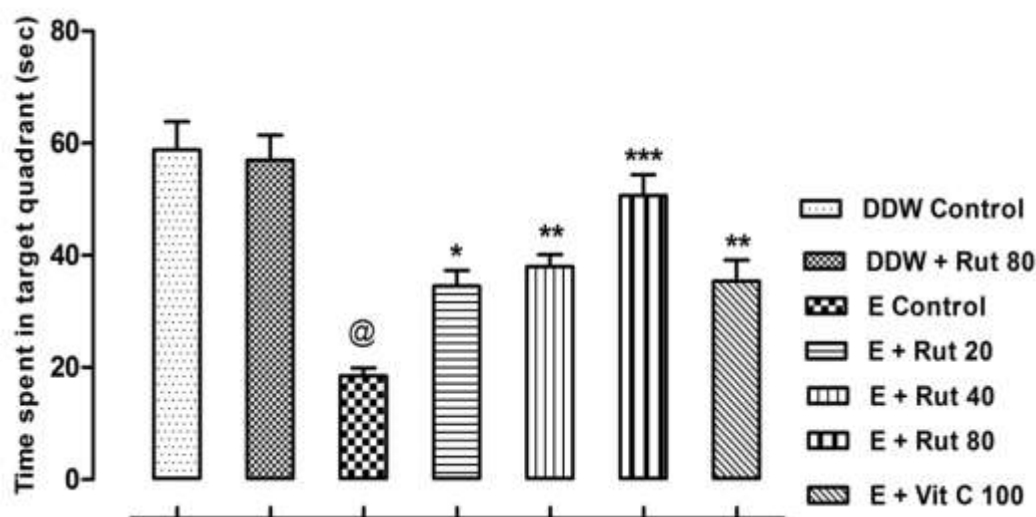


Figure. 2: Effect of rutin treatment (51 days) on time spent in target quadrant during probe trial in Morris water maze test. Each bar represents mean \pm S.E.M. of 5-6 observations. @ $P < 0.001$ vs. DDW control group, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Rut 80: Double Distilled Water rutin (80 mg/kg) treated; E control: ethanol control; E + Rut 20: ethanol rutin (20 mg/kg) treated; E + Rut 40: ethanol rutin (40 mg/kg) treated; E + Rut 80: ethanol rutin (80 mg/kg) treated; E+ Vit C 100: ethanol vitamin C (100 mg/kg) treated.

Effects of rutin on performance in object recognition task

Fig. 3A and B shows the investigation ratios for the two retention intervals (30 min and 24 h, respectively), for the object recognition test. One-way ANOVA revealed a significant effect of amnesia induction and rutin treatment at the short-term 30 min retention interval [$F(6, 41) = 7.470$, $P < 0.0001$] as well as at the 24 h retention trial [$F(6, 41) = 12.11$, $P < 0.0001$]. Post hoc test revealed that ethanol control rats explored less to the novel object compared to DDW control rats during both retention trials ($P < 0.001$). Further, chronic treatment with rutin rats explored more to novel objects as compared to the vehicle treated group at both retention trials ($P < 0.001$). (Figure 3A and 3B).

Effect of rutin on ethanol-induced changes in cholinesterase activity

Cholinesterase (ChE) activity was expressed as AcSCh formed. The changes in ChE activity in cerebral cortex and hippocampus after chronic administration of rutin are presented in Figure 4. As can be observed, ChE activity was significantly increased in the cortex [$F(6, 41) = 7.819$, $P = 0.0001$] and hippocampus [$F(6, 41) = 12.43$, $P < 0.0001$] of ethanol control group compared to the DDW control group. Chronic treatment with rutin (20, 40 and 80 mg/kg) significantly decreased

the ChE activity in cortex compared to ethanol control rats ($P < 0.05$, 0.01, and 0.001). Similarly, chronic treatment with rutin (40 and 80 mg/kg) significantly decreased the ChE activity in hippocampus compared to ethanol control rats ($P < 0.01$ and 0.001). These effects were comparable to that of vitamin C. Chronic rutin treatment in vehicle treated rats did not influence the ChE activity as compared to DDW control rats ($P > 0.05$) (Figure 4).

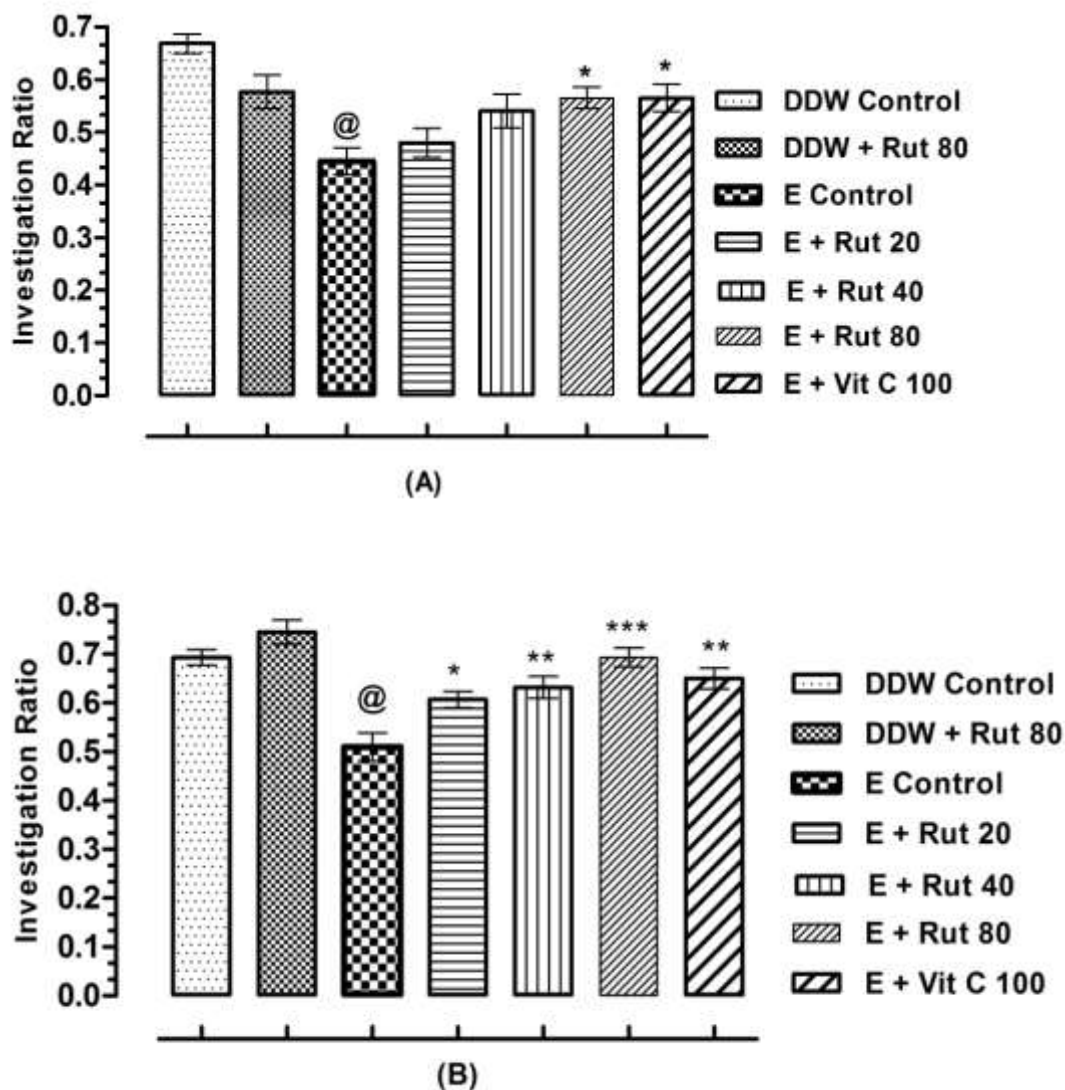


Figure. 3: Effects of rutin treatment (46-47 days) on mean investigation ratio in the object recognition test at the 30 min and 24 h retention intervals. (3A). Object recognition test at a 30 min retention interval. (3B). Object recognition test at 24 h retention interval. Each value represents mean \pm S.E.M. of 5-6 observations. @ $P < 0.001$ vs. DDW control group, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Rut 80: Double Distilled Water

rutin (80 mg/kg) treated; E control: ethanol control; E + Rut 20: ethanol rutin (20 mg/kg) treated; E + Rut 40: ethanol rutin (40 mg/kg) treated; E + Rut 80: ethanol rutin (80 mg/kg) treated; E+ Vit C 100: ethanol vitamin C (100 mg/kg) treated.

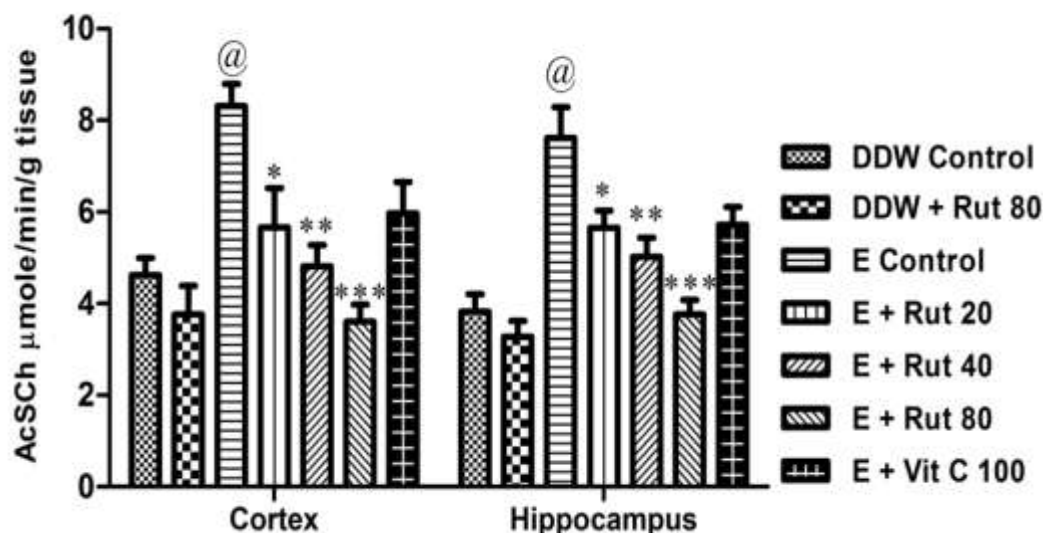


Figure. 4: Effect of rutin treatment (45 days) on cholinesterase activity in cerebral cortex and hippocampus of rat brain. Each value represents mean \pm S.E.M. of 5-6 observations. [@] $P < 0.001$ vs. DDW control group, ^{*} $P < 0.05$, ^{**} $P < 0.01$ and ^{***} $P < 0.001$ vs. ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Rut 80: Double Distilled Water rutin (80 mg/kg) treated; E control: ethanol control; E + Rut 20: ethanol rutin (20 mg/kg) treated; E + Rut 40: ethanol rutin (40 mg/kg) treated; E + Rut 80: ethanol rutin (80 mg/kg) treated; E+ Vit C 100: ethanol vitamin C (100 mg/kg) treated.

Effect of rutin on parameters of oxidative stress in brain

Effect of rutin on ethanol-induced changes in lipid peroxidation

Effects of chronic administration of rutin on lipid peroxidation (LPO) are depicted in Figure 5. There was a significant rise in MDA levels in cortical [$F(6, 41) = 7.222$, $P < 0.0001$] and hippocampal [$F(6, 41) = 8.227$, $P < 0.0001$] tissue of rat brain in ethanol control rats as compared to DDW control rats. Rutin (40 and 80 mg/kg), and vitaminC (100 mg/kg) significantly reduced MDA levels as compared to ethanol control rats in cortex and hippocampus ($P < 0.05$). Rutin per se did not influence the MDA levels (Figure 5).

3.3.2. Effect of rutin on ethanol-induced changes in glutathione levels

Effects of chronic administration of rutin on GSH levels are depicted in Figure 6. There was a significant fall in GSH levels in cortical [$F(6, 41) = 7.476$, $P < 0.0001$] and hippocampal [$F(6, 41)$]

= 7.827, $P < 0.0001$] tissue of rat brain in ethanol control rats as compared to DDW control rats. Rutin (40 and 80 mg/kg) and vitamin C (100 mg/kg) treatment significantly increased GSH levels as compared to ethanol control rats ($P < 0.05$). Rutin per se did not influence the GSH levels (Figure 6).

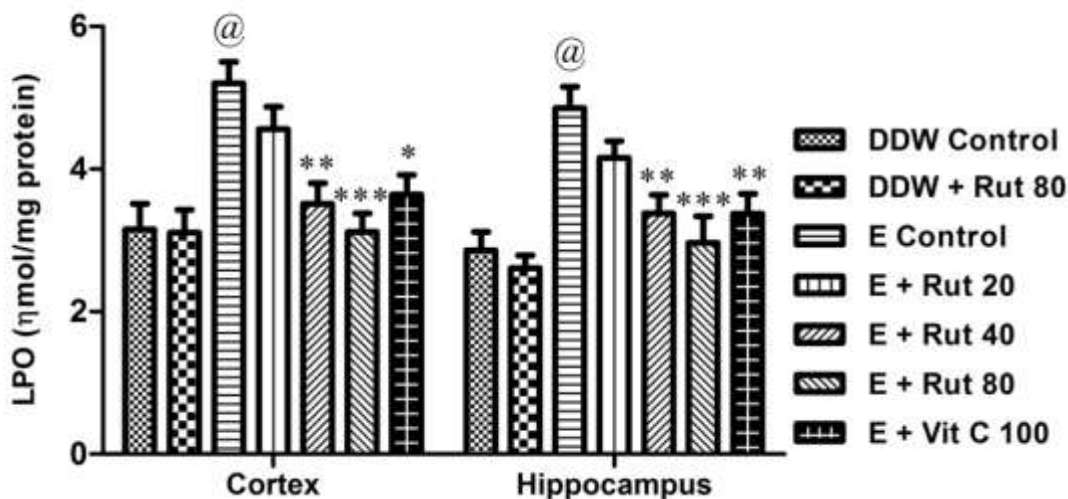


Figure. 5: Effect of rutin treatment (45 days) on lipid peroxidation levels in cerebral cortex and hippocampus of rat brain. Each value represents mean \pm S.E.M. of 5-6 observations. [@] $P < 0.001$ vs. DDW control group, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ vs. ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Rut 80: Double Distilled Water rutin (80 mg/kg) treated; E control: ethanol control; E + Rut 20: ethanol rutin (20 mg/kg) treated; E + Rut 40: ethanol rutin (40 mg/kg) treated; E + Rut 80: ethanol rutin (80 mg/kg) treated; E+ Vit C 100: ethanol vitamin C (100 mg/kg) treated.

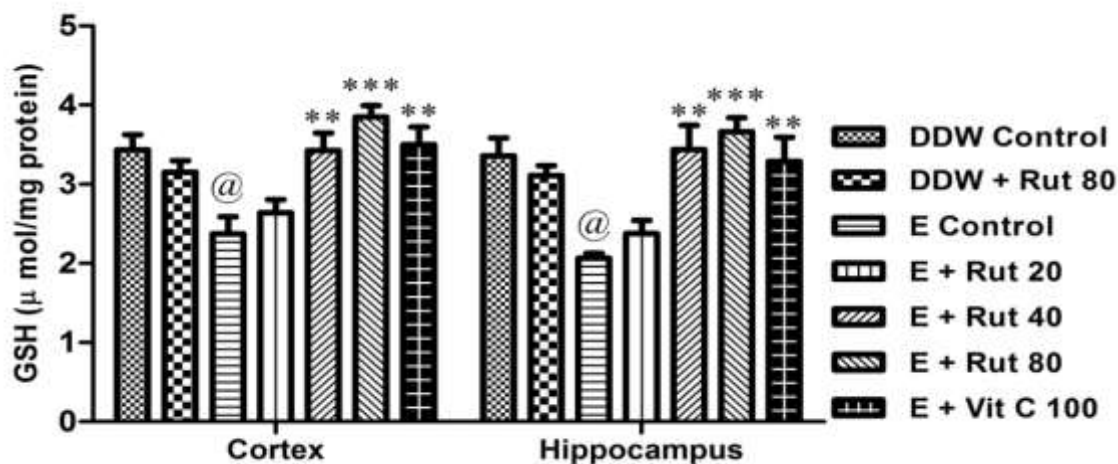


Figure. 6: Effect of rutin treatment (45 days) on glutathione levels in cerebral cortex and hippocampus of rat brain. Each value represents mean \pm S.E.M. of 5-6 observations. $^@P < 0.001$ vs. DDW control group, $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Rut 80: Double Distilled Water rutin (80 mg/kg) treated; E control: ethanol control; E + Rut 20: ethanol rutin (20 mg/kg) treated; E + Rut 40: ethanol rutin (40 mg/kg) treated; E + Rut 80: ethanol rutin (80 mg/kg) treated; E+ Vit C 100: ethanol vitamin C (100 mg/kg) treated.

DISCUSSION

This study evaluated the influence of rutin, a flavonoid on the ethanol-induced cognitive and biochemical changes in rats. Ethanol-induced alcohol dependent rats produced a marked impairment in cognitive function, which was associated with significant increase in cholinesterase activity and oxidative stress in the rat brain. Chronic treatment with rutin significantly prevented the cognitive deficits, reduced cholinergic dysfunction and oxidative stress markers in ethanol treated rats.

In the present study, the Morris water maze test and Novel object recognition test were used for the assessment of learning and memory. Decreased escape latency in Morris water maze task in repeated trials demonstrates intact learning and memory function. Chronic ethanol treated rats did not show a significant decrease in the escape latency as compared to control and per se group, whereas rutin treatment of ethanol treated rats decreased the time to reach the hidden platform. In probe trial also, the time spent in target quadrant is significantly decreased in ethanol treated rats as compared to control and per se group, which was significantly reversed dose dependently on treatment with both the rutin (20, 40 and 80 mg/kg) and vitamin C (100 mg/kg). Chronic ethanol exposure significantly impaired performance during the acquisition phase (spatial learning) of the MWM task, as evidenced by increases in escape latency. Ethanol treatment also caused significant deficits in spatial memory retention. Ethanol treatment decreased the time spent in the target quadrant during the probe trial. Our results agree with previous studies that demonstrated impairment of spatial memory by ethanol in rats⁴³⁻⁴⁶. In present study the novel objective recognition test revealed that ethanol treated rats exhibited a reduced investigation ratio as compare to DDW control group and per se group, which was significantly reversed dose dependently on treatment with both the rutin (20, 40 and 80 mg/kg) and vitamin C (100 mg/kg). The results of the novel object recognition task revealed that diabetic rats exhibited a reduced investigation ratio that is well in accordance with earlier reports^{47,48}. This excludes the possibility

that the activity *per se* may have contributed to the changes in Morris water maze and Novel object recognition test in vehicle treated alcohol dependent rats and the rutin and vitamin C treated alcohol dependent rats.

The biochemical estimations indicated a significant increase in lipid peroxidation levels and marked decrease in the activity of reduced glutathione levels in the cerebral cortex and hippocampus of ethanol-treated rats. Treatment with rutin returned the levels of lipid peroxidation and reduced glutathione towards their control values. Treatment with vitamin C also showed rutin like effects on these parameters. Bhutada et al. showed that chronic treatment with vitamin C protected the cognitive dysfunction in diabetic rats. Studies reporting ethanol induced decreases in endogenous antioxidant levels in brain further support ethanol-induced oxidative stress in the brain⁴⁹. Interestingly, it was suggested that the reduction of ROS production of rutin is related to the enhancement of superoxide dismutase, glutathione per-oxidase or catalase activity and its inhibitory activity on xanthine oxidase which is an important enzyme in the oxidative injury to tissue⁵⁰.

Cholinergic neurotransmission is a central process underlying memory and cognitive function. Cholinergic basal forebrain neurons in the nucleus basalis magnocellularis innervate the cerebral cortex, amygdaloid complex and hippocampus, and are essential for learning and memory formation^{51,52}. One of the most important mechanisms responsible for correct cholinergic function is performed by cholinesterase (ChE) enzyme⁵³. In the present study, we have also observed increased cholinesterase levels in both brain regions (cerebral cortex and hippocampus) of ethanol treated rats suggesting the involvement of enhanced cholinesterase activity in chronic ethanol-induced cognitive dysfunction. The rutin (20, 40 and 80 mg/kg) treatment showed a significant decrease in elevated cholinesterase activity in cortex and hippocampus of ethanol treated rats in a dose-dependent manner. Some studies revealed presence of compounds like rutin and scopoletin in *Noni* which are reported to inhibit cholinesterase activity⁵⁴⁻⁵⁶. Oxidative damage to the rat synapse in the cerebral cortex and hippocampus has been previously reported to contribute to the deficit of cognitive functions⁵⁷⁻⁵⁹. The results of the present study revealed that treatment with rutin dose dependently prevented the learning and memory deficits in ethanol-induced rats and these effects were similar to vitamin C treatment.

Therefore, in the present study, rutin might have protected ethanol-associated memory dysfunction by reducing oxidative stress in ethanol-induced rats.

In conclusion, the findings of the present investigation suggest that rutin exerts its beneficial effects against ethanol-induced memory dysfunction and it may be attributed to its antioxidant and

cholinesterase(ChE) inhibitory activity. Thus rutin may be projected in the treatment of cognitive and neural dysfunction associated with chronic alcoholism.

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