Rutin attenuates the alcohol withdrawal-induced depressive like behaviour in rats

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Abstract

The presence of rutin in *Schinus molle* elicited antidepressant-like effects by enhancing the availability of serotonin and noradrenaline in the synaptic cleft. Thus, the main objective of the present study was to explore the antidepressant potential of rutin and its probable underlying mechanism(s) in alcohol withdrawal-induced depression-like behavior in rats. Depressive behaviors were induced by subjecting the rats to ethanol-dependent withdrawal syndrome. The rats were administered varying concentrations of alcohol for 21 days, and withdrawal symptoms were investigated. The animals were administered vehicle, fluoxetine, or rutin for 7 days. Animals were observed for depressive-like state via helplessness, which was reflected as an increase in immobility time in the forced swim test and tail suspension test. Various biochemical alterations, including serum corticosterone levels; endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH); and lipid peroxidation, in terms of malondialdehyde (MDA) formation in the brain, were studied. The experimental findings demonstrated that rutin elicited a significant reduction in immobility time and prevented the decrease in SOD, CAT, and GSH levels in alcohol withdrawal-induced depressive-like behaviour. Furthermore, to substantiate these findings, our histopathological studies corroborated that rutin ameliorated brain alterations due to stress mediated by alcohol withdrawal. Thus, rutin attenuated depressive-like behaviour through amelioration of oxidative stress by restoration of SOD, GSH, and CAT levels and attenuation of corticosterone, MDA, and NO levels.

1. Introduction

Alcoholism is a recurrent, chronic condition marked by compulsive intake of alcoholic beverages. Alcohol metabolism involves the formation of acetaldehyde and, subsequently, acetate via aldehyde dehydrogenase and acetaldehyde dehydrogenase, respectively (Crews et al., 1996; Enoch and Goldman, 2001; McIntosh and Chick, 2004). Alcohol cessation induces a syndrome called alcohol withdrawal symptoms and is characterized by helplessness, irritation, anxiety, stress, and sleep disturbance. These symptoms are largely found in depression; thus, they are known as depression-like behaviour. In many alcohol-dependent individuals, depression is a reactive response to chronic alcohol use and withdrawal, potentially exerting a significant influence on treatment prospects and increasing the likelihood of relapse (Greenfield et al., 1998). Furthermore, Fu et al. (2019) demonstrated that rats withdrawn from chronic alcohol administration exhibited anhedonia and helplessness in a depressive-like state, as measured by sucrose preference and forced swimming tests [Fu et al., 2019].

Rutin (3, 3', 4, ’ 5, 7-pentahydroxyflavone-3-rhamnoglucoside), a flavonol found in passion flowers, buckwheat, tea, and apples, is a key component of dietary nutrition. Buckwheat contains rutin (a glycoside of rutinose) and quercetin. It is composed of a disaccharide and a flavonolic aglycone. Ganeshpurkar and Saluja (2017) reported that rutin has antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective effects. A recent study has shown that rutin is a neuroprotective agent against ACR-induced neurotoxicity in rats. Intraperitoneal injection of rutin decreased the immobility time, a measure of depressive-like behavior, in mice in the forced swim test

(FST) compared to the vehicle. Rutin has been shown to exhibit antidepressant-like effects in tail suspension tests in animal models (TST) (Foudah et al., 2022). Furthermore, rutin has recently been shown to be involved in ethanol-induced cognitive impairment and depressive-like behavior in rats (Ebokaiwe et al., 2023) and to elicit antidepressant effects by reducing antioxidant activity and acetylcholinesterase (Foudah et al., 2022). Oxidative stress due to impairment of the oxidative-antioxidative systems has been consistently observed in depressive behavior during clinical investigations (Khanzode et al., 2003). Oxidative stress may be the underlying cause of the pathophysiology of depression or the associated behavioral changes induced by alcohol withdrawal in rats.

Although rutin modulates oxidative stress in the brain, it produces antidepressant-like activity in experimental animals, and its effect on alcohol withdrawal-induced depressive-like behavior has not been studied. Thus, the present study was undertaken to understand the potential antidepressant-like effect of rutin and its underlying mechanism of action in alcohol withdrawal-induced depressive like behaviour in rats.

2. Materials and Methods

2.1. Experimental animal

Wistar rats of either sex (180–230 grams) were procured from the National Institute of Biosciences, Pune, India. Animals were housed in polypropylene cages (28×21×14 cm) under standard laboratory conditions at a temperature of 22 ± 2°C under a 12:12 h light/dark cycle, with access to standard feed and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee with approval No. SCOP/IAEC/2019-20/02/269 complies with the guidelines in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

2.2. Drugs and chemicals

Rutin and Fluoxetine as standard antidepressant were purchased from Sigma Aldrich (USA). The corticosterone ELISA kit and GST, CAT, and SOD kits were procured from Krishgen Biosystem, India. All other reagents used were of analytical grade and procured from local suppliers.

3. Alcohol withdrawal induced depressive like behaviour in rats

Alcohol withdrawal depressive behaviour was induced in the rats as per the procedure described by Uzbay et al. (1997), with minor modifications. Briefly, rats were housed in polycarbonate cages for 7 days and with balanced liquid diet containing, low fat lactogen powder, 95.6% ethanol, and 17-gram sucrose in 1000 mL providing 1000.7 kcal/l. After 7 days, a balanced liquid diet was modified with 2.4% ethanol (v/v) and given for next 3 days and further increased to 4.8% for 4 days and 7.2% for 21 days respectively. Balanced liquid diet was prepared daily and served at 10:00 hrs. After 21 days, the various

signs and Symptoms due to alcohol withdrawal were observed and recorded as per Table 1. The withdrawal symptoms were observed after discontinuation of balanced liquid diet with alcohol. These symptoms were viz abnormal posture, gait, tail stiffness, and agitation were scored using a rating scale at various intervals. The median value represented the intensity of these parameters. Behavioural indicators were converted into scores from 1–5 based on percent incidence to calculate the total ethanol withdrawal score. The median values of each behaviour were then summed for each observation period (Uzbay et al., 1997)

Table 1
Evaluation of various signs and Symptoms in alcohol withdrawal

Signs	Symptoms
Stereotyped behavior	1) One behavior
	2) Two stereotyped behaviors
	3) Three stereotyped behaviors
	4) Four stereotyped behaviors
	5) All stereotyped behaviors
Agitation	1) Mild or moderate irritability
	2) Very irritable
	3) Handling vocalization and moderately aggressive
	4) Handling vocalization and very aggressive
	5) Spontaneous vocalization and very aggressive
Tail stiffness	1) Mild tail rigidity
	2) Moderate tail rigidity
	3) Tail rigid but mildly flexible during ambulation.
	4) Tail rigid & not flexible
	5) Tail very rigid & not flexible
Posture	1) Mild head down, back hunched
	2) Moderate head down, back hunched
	3) Prominent head down, back hunched
	4) In addition, hind legs wide apart
	5) In addition, fore limbs apart
Gait	1–2) Mild difficulty ambulating & rearing normal
	3–4) Moderate difficulty ambulating & rearing
	5) Prominent difficulty ambulating & no rearing

3.1. Measurement of blood alcohol concentrations in alcohol withdrawal induced depressive like behaviour in rats

In order to ensure that, animals were drinking the alcohol, we measured blood alcohol concentration at various time intervals. Briefly, the blood (40 µl) was withdrawn from the tail vein and the concentration was measured as per method described by Bhutada et al [22]. Briefly, the perchloric acid (160 µl, 3%) was added, in above blood sample, vortexed and then centrifuged at 10,000×g for 10 minutes (Neaution, India). Supernatant 60 µl was incubated with 0.5 M Tris–Cl buffer (pH 8.8) containing 5.5 µg/ml of alcohol dehydrogenase and 1.5 mM β-nicotinamide adenine dinucleotide (β-NAD) for 40 minutes. The concentration of generated β-NADH was measured at 340 nm and we observed that all the samples exhibited alcohol concentration (Results not shown).

3.2 Determination of immobility time in forced swim test (FST) in rats

The helplessness, which is depressive behaviour was determined by measuring the immobility time in forced swim test (FST) as per the procedure described by Castagn et al (Castagn et al., 2010). Briefly, rats were placed individually in glass cylinders (height, 40 cm; diameter, 20 cm), containing 25 cm of water at 25 ± 0.5°C, 30 min after last day of treatment. Each rat was considered to be immobile when it ceased struggling and remained floating motionless in the water, elicit only those movements necessary to keep its head above water. The duration of immobility during the 6 min of the trial was measured blindly and tracked video camera. The FST was performed at tested between 8:00 am to 12:00 pm.

3. 3. Determination of immobility time in tail suspension test swim test (TST) in rats

In tail suspension test rats were suspended on the edge of a table 58 cm above the floor by the adhesive tape placed approximately 2–3 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 min and observed to the extent of immobility versus active movement. The animal was considered to be immobile when it does not show any movement of the body and remain hanging motionless (Steru et al., 1985; Castagn et al., 2010).

3.4. Biochemical investigation

After the behavioural evaluation, blood was collected between in the morning session from direct cardiac puncture and serum was separated and used for estimation of corticosterone. After blood withdrawal, rats were sacrificed by CO_2 euthanasia and brain samples were isolated, half brain sample was kept in neutral buffer solution for histopathological studies and half brain was washed with 10% cold sucrose solution and homogenized (1:10 w/v) in phosphate buffer solution (PBS). The tissue homogenates were centrifuged (NEUATION, INDIA) at 16,000 x g, at 4 ^{0}C for 15 min and resultant supernatants were used for estimation of oxidative stress parameters (Nanaware et al., 2017)

3.4.1 Measurements of serum corticosterone

Serum corticosterone was measured by ELISA method as per the procedure described by manufacturer's kit. The results were expressed as µg/ml of corticosterone level.

3.4.2. Determination of MDA (malondialdehyde) formation by lipid peroxidation process in the brain homogenate

The MDA generated in the brain due to oxidative stress as function of lipid peroxidation process was estimated as per the procedure described Grotto et al (1998). Briefly, A 75mL of supernatant was mixed with 25mL of standard and 25mL of NaOH (3N) and incubated at 60°C for 30 min in a water bath with occasional shaking. After this 125mL of H3PO4 (6%) and 125mL of thiobarbituric acid (TBA, 0.8%) were added and the mixture was heated at 90°C for 45 min. After cooling of the mixture, 50mL of sodium dodecyl sulfate (SDS, 10%) was added. By using vortex mixer, above mixture was extracted with 300mL of n- butanol and centrifuged at 3000 x g for 10 min 20mL of butanol layer was injected into HPLC with visible detector, and detection was carried out at 532 nm wavelength and using mobile phase water and methanol (50:50, v/v) with flow rate at 0.6 mL/ min. The column was used Thermosil C18 and run was 8 min. The amount of MDA was found in plasma was calculated by calibration plot of standard MDA. For calibration of MDA, six different concentrations (1–6mM) were prepared from 3 mM stock solution (Grotto et al., 2007; Nanaware et al., 2017).

3.4.3. Estimation of Superoxide dismutase (SOD) activity in brain homogenate

The SOD activity in brain was measured as per the procedure described by Mishra et al 1972. Briefly, the equal volumes of brain homogenate (supernatant) and distilled water were mixed, to which 0.25 mL of ice-cold ethanol and 0.15 mL of ice-cold chloroform added mixed and centrifuged at 600×g at 4°C for 15 min. To this 0.5 mL of Ethylenediaminetetraacetic acid (EDTA) solution was added. The reaction was initiated by the addition of 0.4 mL of epinephrine and the changes in optical density/min was measured at 480 nm against reagent blank(Misra and Fridovich, 1972)

3.4.4. Estimation of reduced glutathione (GSH) level in brain homogenate

The GSH levels was measured by addition of the equal volumes of brain tissue homogenate (supernatant) in 20% trichloroacetic acid (TCA) were mixed. The precipitated fraction was centrifuged at 600×g at 4°C for 15 min and 2 mL of DTNB reagent was added to 0.25 mL of supernatant. The final volume was adjusted up to 3.0 mL with phosphate buffer. The colour developed was read at 412 nm against reagent blank (Moron, Depierre and Mannervik, 1979; Nanaware et al., 2017).

3.4.5. Estimation of Catalase (CAT) activity in brain homogenate

The CAT activity was determined as per the procedure described earlier by Beers and Sizer (1952). The results are expressed as mmol H_2O_2 consumed/min/mg protein (Beers Jr. and Sizer, 1952; Nanaware et

al., 2017).

3.4.6. Estimation of nitric oxide (NO) levels brain homogenate

The NO level in the brain homogenate was measured as per the documented procedure by Sreejayan and Rao (1997) with minor modifications. Briefly, brain homogenate supernatant was incubated with 5 mM sodium nitroprusside incubation. Further, in 5.6 mL of resultant mixture was added in 0.2 mL of Griess reagent and incubated at 30°C for 10 min. After incubation 0.2 mL of Griess reagent B was added and kept for incubation 30° C for 10 min and absorbance was measured at 542 nm against blank (Sreejayan and Rao, 1997; Nanaware et al., 2017).

3.5 Brain histopathological studies

The half brain samples were subjected for histopathological studies using haematoxylin and eosin stain

3.6. Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. A value of $p < 0.05$ was considered to be statistically significant. The statistical analysis was performed by GraphPad Prism Trial Version 5 software (GraphPad Software, Inc., La Jolla, CA, USA).

4. Results

4.1 Effect of rutin or fluoxetine on various alcohol withdrawal syndrome

The development of alcohol withdrawal symptoms was assessed on day 22 by scoring withdrawal-induced physical signs of craving showing depressive like behaviour at 2, 4, 6, and 8 hours. Rats in the alcohol control group exhibited significant ($t = 4.24$; $F = 4.53$; $P < 0.0075$) higher withdrawal score compared with the normal control group (Fig. 1A). However, prior treatment of rutin 100 and 200 mg/kg did exhibit non-significant reduction of withdrawal symptoms compared to alcohol control rats (Fig. 1A). In addition, after treatment with rutin 200 mg/kg and fluoxetine induced significant ($t = 3.54$; $F = 12.61$; $P < 0.0001$) attenuation withdrawal symptoms compared to alcohol control (Fig. 1B).

4.2. Effects of rutin or fluoxetine on immobility time by FST in alcohol withdrawal induced depressive like behaviour in rats

Our experimental indicated that, immobility time was found to significantly increased in vehicle treated alcohol withdrawal rats ($t = 10.07$, $F = 48.26$, $p < 0.0001$) in FST on day 30th day. *Post hoc* analysis demonstrated that immobility time was significantly attenuated with fluoxetine and rutin treatment for 30

days in FST. The attenuation with rutin treatment was found to be in dose dependent manner as shown in Fig. 3.

4.3. Effects of rutin or fluoxetine on immobility time by TST in alcohol withdrawal induced depressive like behaviour in rats

In the present experimental findings by one way ANOVA suggested that immobility time by TST was also found to elevated significantly (t = 18.04, F = 116.6, $p < 0.0001$) due alcohol withdrawal on 30th day. *Post hoc* analysis indicated that, fluoxetine or rutin significantly attenuated immobility time in TST which was similar to that of TST. The attenuation of immobility time with rutin treatment was found to be dose dependent manner (Fig. 4).

4.4. Effects of rutin or fluoxetine on serum corticosterone levels in alcohol withdrawal induced depressive like behaviour in rats

One way ANOVA analysis demonstrated that, serum corticosterone levels was found to increased significantly (t = 5.642, F = 25.59, $p < 0.0001$) in alcohol withdrawal group. *Post hoc* results showed that rats in vehicle treated alcohol withdrawal group elicited increased serum corticosterone which were subsequently attenuated significantly with rutin 200 mg/kg (Fig. 4).

4.5. Effects of rutin or fluoxetine on MDA formation in alcohol withdrawal induced depressive like behaviour in rats

In the present experimental findings by one way ANOVA revealed elevation of MDA formation in significant (t = 5.97, F = 13.29, $p < 0.0001$) manner in alcohol withdrawal rats on 30th day. *Post hoc* analysis suggested that, fluoxetine or rutin significantly prevented MDA formation due to alcohol withdrawal rats. The prevention of MDA formation with rutin treatment was observed at 200 mg/kg dose level per se (Fig. 5).

4.6. Effects of rutin or fluoxetine on NO levels in alcohol withdrawal induced depressive like behaviour in rats

Our experimental findings by one way ANOVA suggested that NO levels in brain homogenate was increased significantly (t = 18.40, F = 160.62, $p < 0.0001$) in alcohol withdrawal rats on 30th day. *Post hoc* analysis revealed that, rutin treatment elicited significant attenuation of elevated NO levels in brain homogenate. The degree of attenuation was found to highest and significant with rutin 200 mg/kg and fluoxetine treatment. Rutin 50 and 100 mg/kg exhibited non-significant attenuation effects on NO levels in alcohol withdrawal induce depressive like behaviour in rats (Fig. 6).

4.7. Effects of rutin or fluoxetine on SOD activity in alcohol withdrawal induced depressive like behaviour in rats

Our findings by one way ANOVA suggested that SOD activity was declined significantly (t = 16.90, F = 104.30, $p < 0.0001$) alcohol withdrawal group. *Post hoc* analysis revealed that, rutin or fluoxetine treatment elicited significant amelioration of SOD activity. The rutin treatment showed improvement in SOD activity in dose dependent manner due to alcohol withdrawal (Fig. 7).

4.8. Effects of rutin or fluoxetine on CAT activity in alcohol withdrawal induced depressive like behaviour in rats

One way ANOVA indicated that CAT activity was found to declined significantly (t = 11.51, F = 52.14, $p < 0.0001$) alcohol withdrawal group. *Post hoc* analysis suggested that rutin or fluoxetine treatment significantly prevented decrement in CAT activity due to alcohol withdrawal. Rutin treatment exhibited dose dependent decrement in CAT activity due to alcohol withdrawal (Fig. 8).

4.9 Effects of rutin or fluoxetine on GSH levels alcohol withdrawal induced depressive like behaviour in rats

Figure 9 presented NPSH contents were estimated as GSH levels and one way ANOVA showed a significant reduction(t = 10.19, F = 5.16, $p < 0.0001$) in the level of NPSH contents in the alcohol withdrawal group. Rutin treatment significantly ameliorated the NPSH contents in dose dependent manner due to alcohol withdrawal. Furthermore, fluoxetine a standard antidepressant exhibited non-significant effects on NPSH contents (Fig. 9)

4.10. Histopathological studies

Histopathological findings showed that, alcohol withdrawal group elicited oedematous and inflammatory neuronal infiltration and impaired structural alterations. Treatment with of rutin 200 mg/kg exhibited normal neuronal with minor swelling and inflammatory changes due to alcohol withdrawal (Fig. 10).

5. Discussion

In the present experiment we demonstrated potential antidepressant like activity of rutin in alcohol withdrawal induced depressive like behaviour in rats. In order to understand the underlying mechanism of stressed-induced depressive biological changes. Stress induced related depressive behaviour models are widely used and documented (Thakare et al., 2017; Wang et al., 2018; Mao et al., 2014b, 2014a). In the present study, we found that alcohol withdrawal in alcohol dependence developed rats elicited severe rigidity, posture alterations, Staub tail and altered locomotor activity. Our findings are in agreement with the reports Sachdeva et al (2015) and Majchrowicz (1975) wherein they demonstrated that cessation or reduction of ethanol use following periods of extended ethanol consumption causes an ethanol withdrawal syndrome (Majchrowicz, 1975; Sachdeva, Choudhary and Chandra, 2015). Treatment with rutin significantly prevented alcohol withdrawal symptoms in dose dependent manner. Further, the alcohol withdrawal known to exhibited depression and anxiety like behaviour.

A significant comorbid expression of alcoholism and affective disorders (major depression, dysthymia) has been shown by clinical evidence (Greenfield et al., 1998). The stressful life events have been documented to participate in the development of depressive like symptoms in clinical practice(Calabrese, Molteni and Riva, 2011). Depressive like state in experimental animals were studied and documented in terms of increased immobility time in FST and consequently its reduction or prevention are considered to induce antidepressant-like activity (Kumar and Goyal, 2008; Mao et al., 2014b, 2014a). Further, the documented findings showed that animals withdrawn from alcohol showed an increased immobility time in the forced swim test (FST) and thereby exhibiting depressive (Smith and Lynch, 2012; Pang et al., 2013) like behaviour. In the present findings too, we observed depressive like behaviour as helplessness as indicated by increased immobility time which is reflection of helplessness in FST and TST significantly attenuated with repeated treatment of rutin for 30 days. Our findings are also in agreement with documented report of Kim et al., (2017) wherein they demonstrated that, immobility time was significantly reduced with Gintonin treatment in FST in mice(Kim et al., 2017).

In addition, to demonstrate the role of stress in development of depressive like symptoms, since the abrupt withdrawal of alcohol from rats who developed the dependence to alcohol exhibited severe stress related paradigms reflected as rigidity, posture alterations, Staub tail. Thus, we have studied the serum corticosterone level in alcohol withdrawal rats to understand the effects of rutin on stress induced corticosterone levels. We observed that rutin significantly prevented the elevation of serum corticosterone levels due to alcohol withdrawal.

It was further demonstrated by Freitas et al., (2014) and Thakare and Patel (2017) that lipid peroxidation process in response to oxidative stress developed resulted into impaired antioxidant enzymatic activity in the brain which is reflected in terms of increased MDA formation, an index of lipid peroxidation process. As lipid peroxidation process is responsible for Reactive Oxygen Species (ROS) and free radical production, this in turn activate endogenous antioxidant system to neutralize or scavenge these radicals, while doing this the activity of antioxidants reduced drastically(Freitas et al., 2014; Thakare et al., 2017; Thakare, Dhakane and Patel, 2017) which is largely accountable for development of depressive state. In addition, Impairment in GSH, CAT and SOD levels are implicated in the induction of depressive behaviour due to excessive scavenging of formed ROS in response to stress. The brain oxidative stress might occurs due to various ways as either (a) presence of iron in brain which is involved in the production of free radicals, (b) lower endogenous antioxidant enzymes and (c) high amounts of lipids and fatty acids in the brain, making it more vulnerable for peroxidation process and consequently increased free radicals formation(Muley et al., 2012, 2013). Our present experimental findings demonstrated that rutin significantly attenuated the elevation of MDA formation and NO levels in rat brain due to its capability to prevent the lipid peroxidation process and reducing the oxidative stress. Our current results are in complete agreement with recent findings of Ebokaiwe et al., (2023) wherein they showed that, MDA production, was significantly ($p < 0.0001$) prevented with rutin treatment compared with control group.

Furthermore, rutin at highest dose 200 mg/kg repeatedly for 29 days restored the declined SOD, CAT activities and GSH contents due to alcohol withdrawal. The beneficial effects of rutin on these

endogenous antioxidants are largely due to its ability to scavenge or neutralize the formed free radicals in brain, thereby reduce the oxidative stress and subsequently ameliorated endogenous defence system by enhancing SOD, CAT activity and GSH contents. Thus, due to antioxidant capability of rutin attenuated the oxidative stress by scavenge or neutralize free radicals' generation and thereby reduce the depressive like behaviour reflected in terms of attenuation of immobility time in FST and TST methods. To corroborate our behavioural and biochemical findings, histopathological studies also suggested that, rutin administered rats prevented edema and inflammatory alterations due to alcohol withdrawal and comparatively was found to much higher in vehicle treated group

Conclusion

In conclusion, from aforementioned findings it can be concluded that, rutin improved behavioural, biochemical changes (reduction in serum corticosterone level, MDA formation, restoration of antioxidant enzymes, SOD, CAT in brain) due to alcohol withdrawal in rats. Thus, rutin elicited potential antidepressant like activity in alcohol withdrawal induced depressive state by modulation of corticosterone response and restoration of antioxidant enzymes in brain.

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Conflicts of interest/Competing interests (include appropriate disclosures)

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.

Data availability

Data will be made available on request.

Code availability (software application or custom code): NA

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Table 1

Table 1: Evaluation of various signs and Symptoms in alcohol withdrawal

Signs	Symptoms
Stereotyped behavior	1) One behavior
	2) Two stereotyped behaviors
	3) Three stereotyped behaviors
	4) Four stereotyped behaviors
	5) All stereotyped behaviors
Agitation	1) Mild or moderate irritability
	2) Very irritable
	3) Handling vocalization and moderately aggressive
	4) Handling vocalization and very aggressive
	5) Spontaneous vocalization and very aggressive
Tail stiffness	1) Mild tail rigidity
	2) Moderate tail rigidity
	3) Tail rigid but mildly flexible during ambulation.
	4) Tail rigid & not flexible
	5) Tail very rigid & not flexible
Posture	1) Mild head down, back hunched
	2) Moderate head down, back hunched
	3) Prominent head down, back hunched
	4) In addition, hind legs wide apart
	5) In addition, fore limbs apart
Gait	1-2) Mild difficulty ambulating & rearing normal
	3-4) Moderate difficulty ambulating & rearing
	5) Prominent difficulty ambulating & no rearing

Figures

Figure 1 A

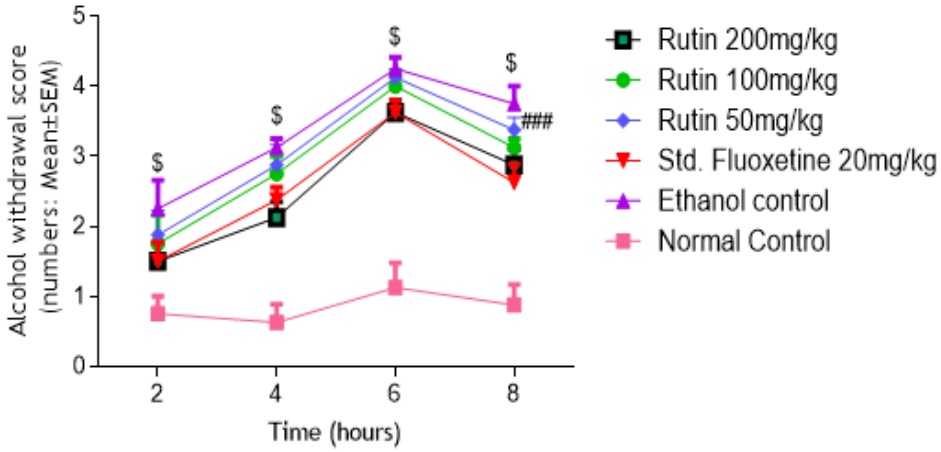

Figure 1 B

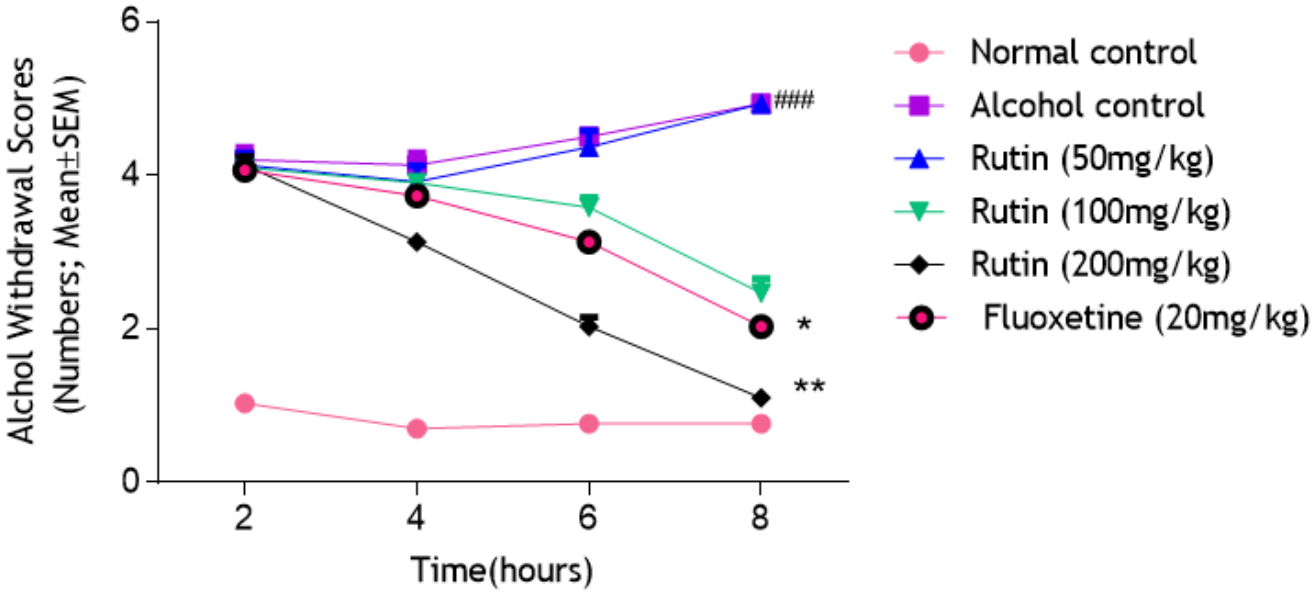

Figure 1

A: Effect of rutin or fluoxetine on various alcohol withdrawal syndrome in rats at various time intervals (Prior treatment). Data are expressed as mean ± SEM (n=8) followed by one-way ANOVA and Bonferroni post-test, where ($^{\$}P$< 0.001 and $^{***}P$ < 0.001) vs. the normal control group.

B: Effect of rutin or fluoxetine on various alcohol withdrawal syndrome in rats at various time intervals (After treatment). Data are expressed as mean ± SEM (n=8) followed by one-way ANOVA and Bonferroni post-test, where ($^{###}P$< 0.001 and $^{***}P$ < 0.001) vs. the normal control group.

Figure 2

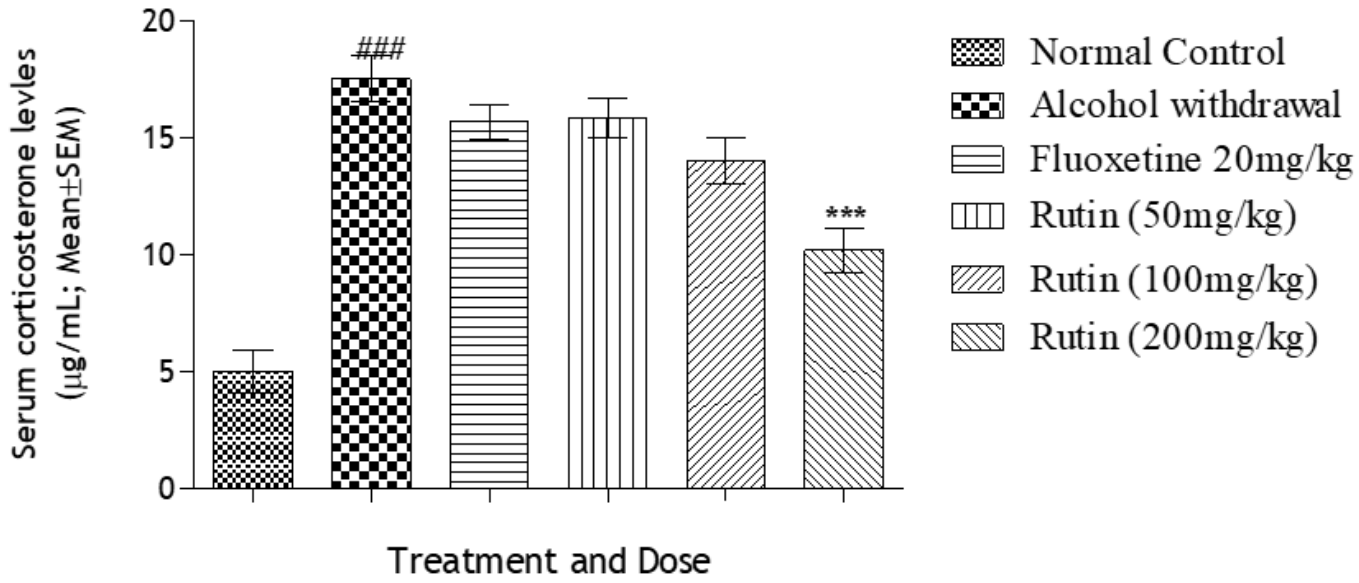

Figure 2

Effect of rutin or fluoxetine on serum corticosterone levels in alcohol withdrawal-induced depression in rats. Data are expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p$< 0.001 compared to normal control group, $^{***}p$< 0.001 compared to alcohol withdrawal group

Fig 3.

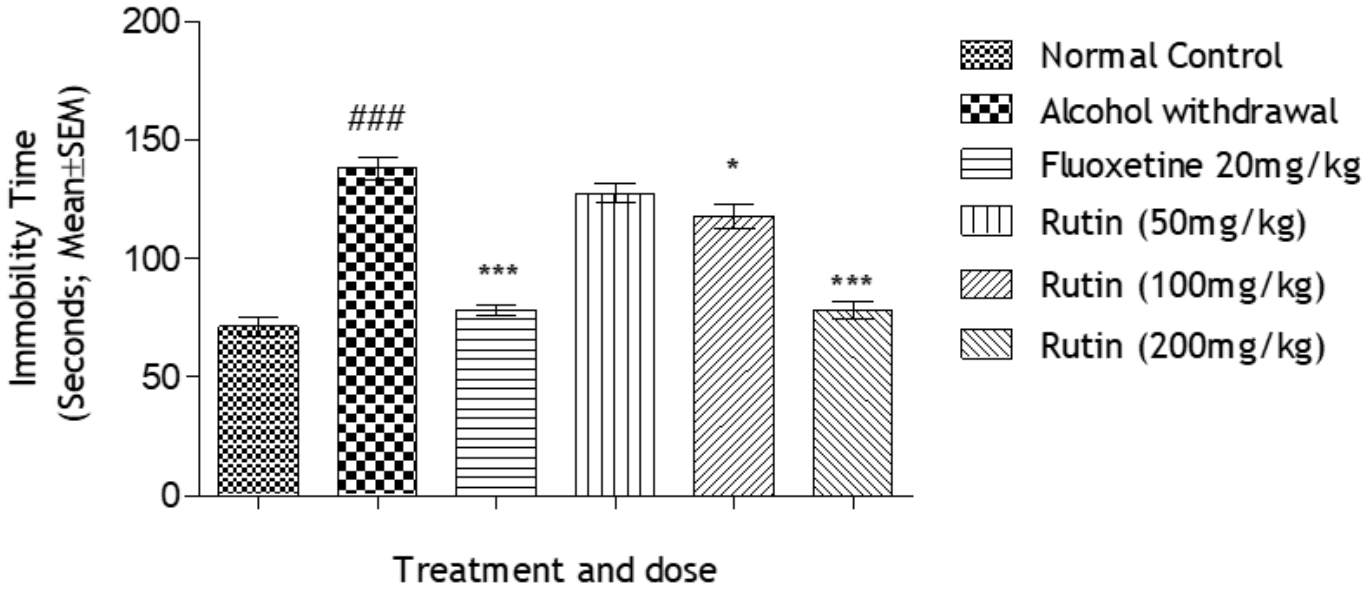

Figure 3

Effect of rutin or fluoxetine on immobility time by FST in alcohol withdrawal-induced depressive behavior in rats. Data are expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p< 0.001$ compared to normal control group, $^{***}p< 0.001$ compared to alcohol withdrawal group

Fig. 4

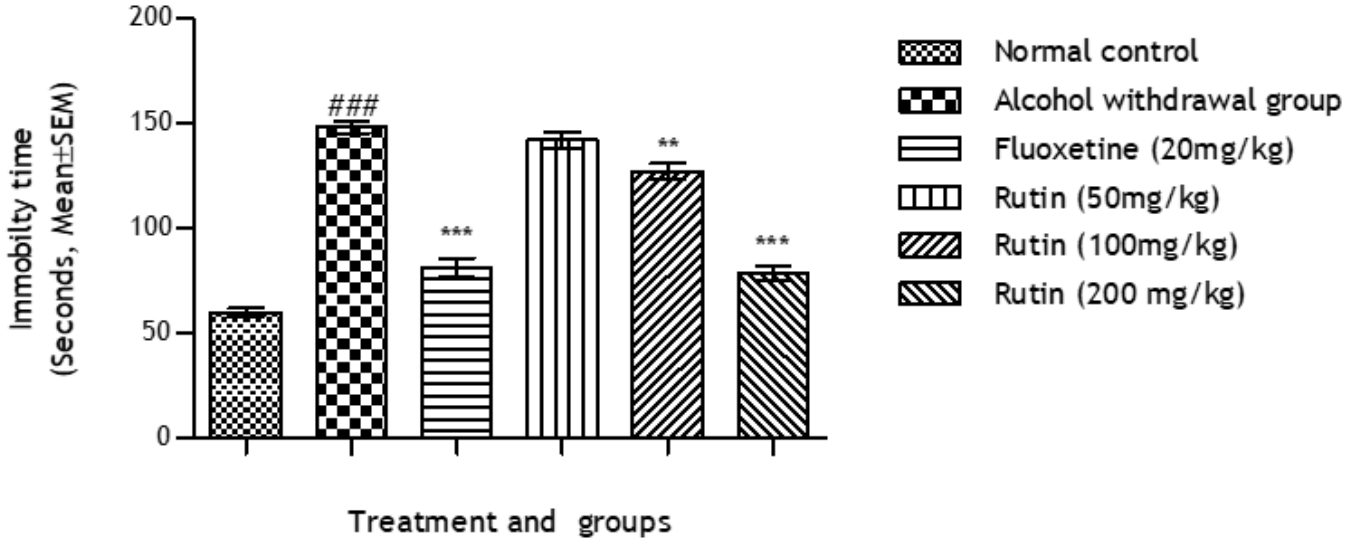

Figure 4

Effect of rutin or fluoxetine on immobility time by TST in alcohol withdrawal-induced depressive behavior in rats. Data are expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p$< 0.0001 compared to normal control group, $^{**}p$< 0.001 $^{***}p$< 0.0001 compared to alcohol withdrawal group

Fig 5

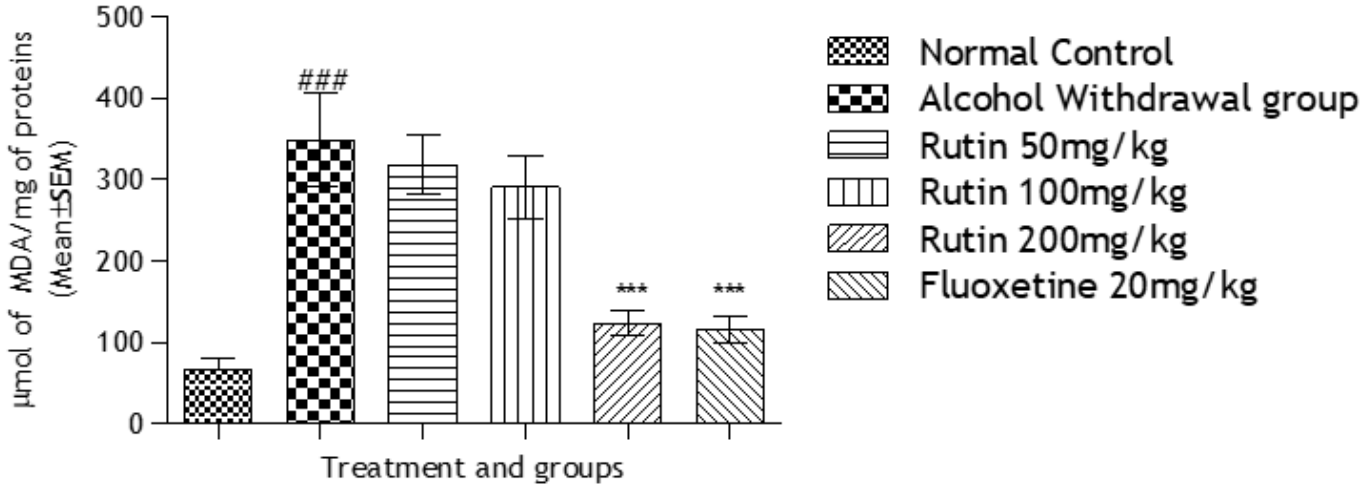

Figure 5

Effect of rutin or fluoxetine on MDA formation in alcohol withdrawal-induced depressive behavior in rats. Data was expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p$<0.0001 compared to normal control group, $^{***}p$< 0.0001 compared to alcohol withdrawal group

Fig.6

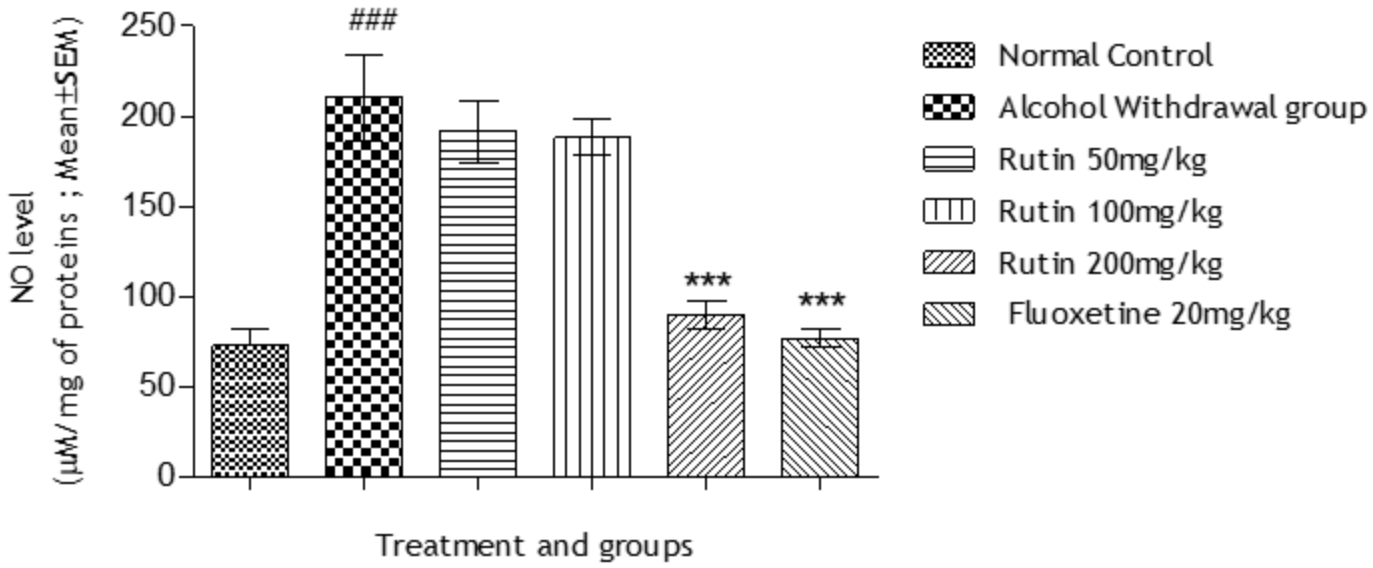

Figure 6

Effect of rutin or fluoxetine on NO levels in alcohol withdrawal-induced depressive behavior in rats. Data was expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p$<0.0001 compared to normal control group, $^{***}p$< 0.0001 compared to alcohol withdrawal group

Fig. 7

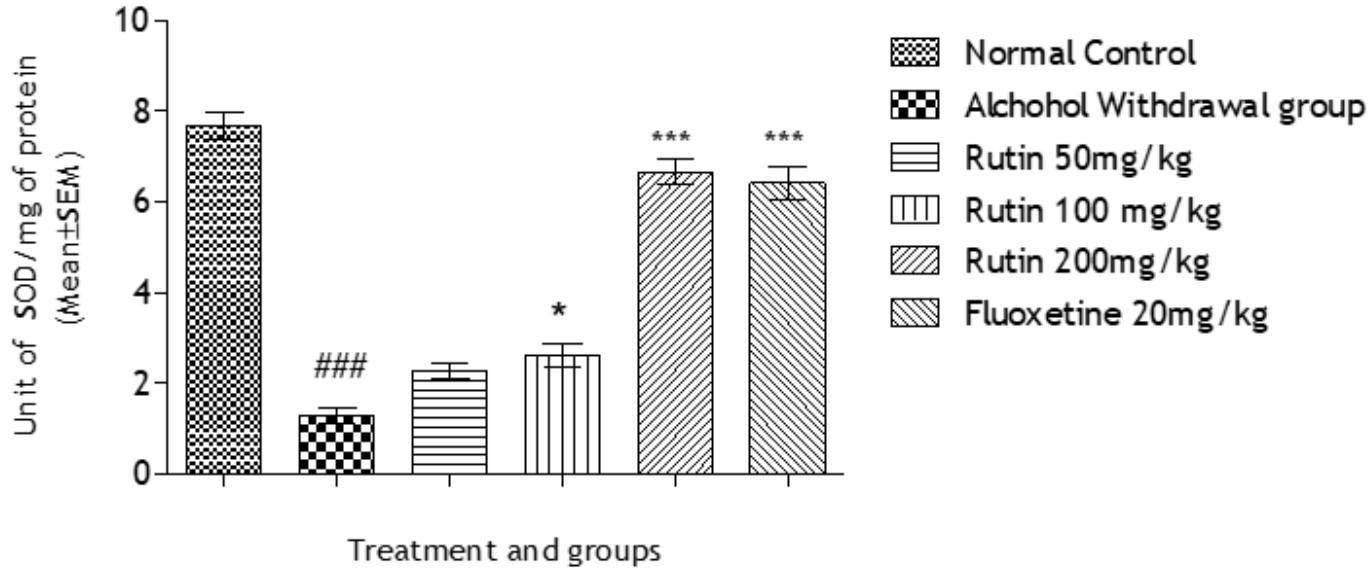

Figure 7

Effect of rutin or fluoxetine on SOD activity in alcohol withdrawal-induced depressive behavior in rats. Data was expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p$<0.0001 compared to normal control group, $^{*}p$< 0.01 $^{***}p$< 0.0001 compared to alcohol withdrawal group

Fig 8

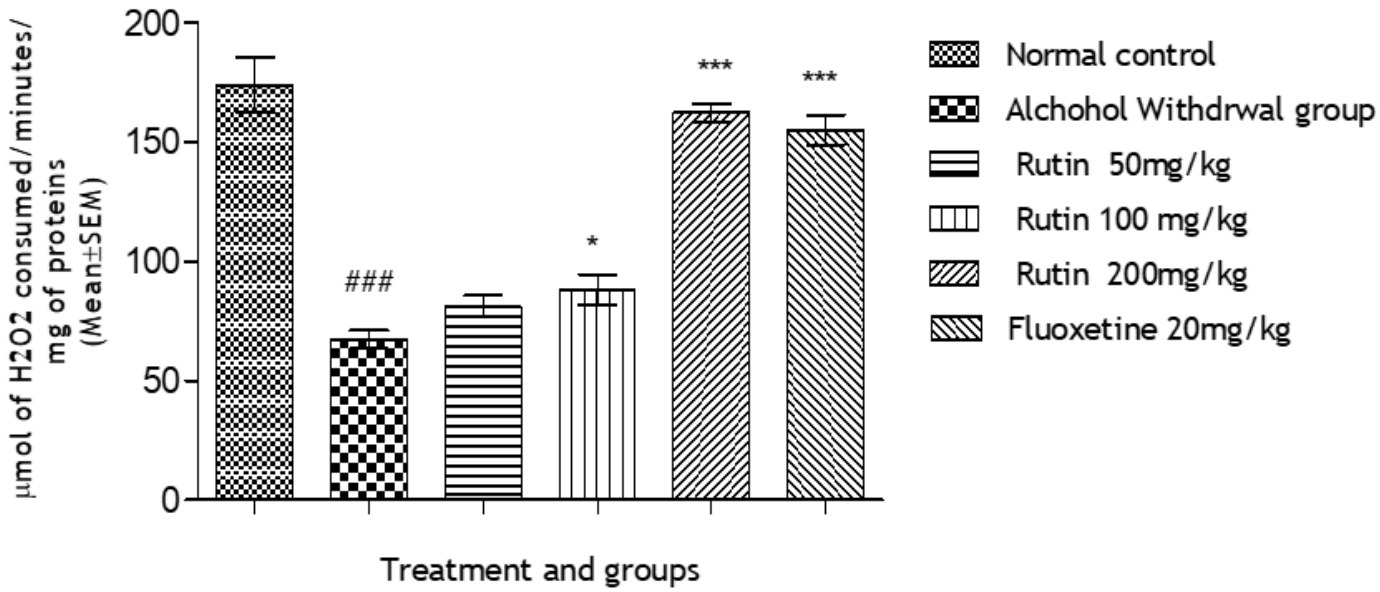

Figure 8

Effect of rutin or fluoxetine on CAT activity in alcohol withdrawal-induced depressive behavior in rats. Data was expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p$<0.0001 compared to normal control group, $^{*}p$< 0.01 $^{***}p$< 0.0001 compared to alcohol withdrawal group

Fig 9.

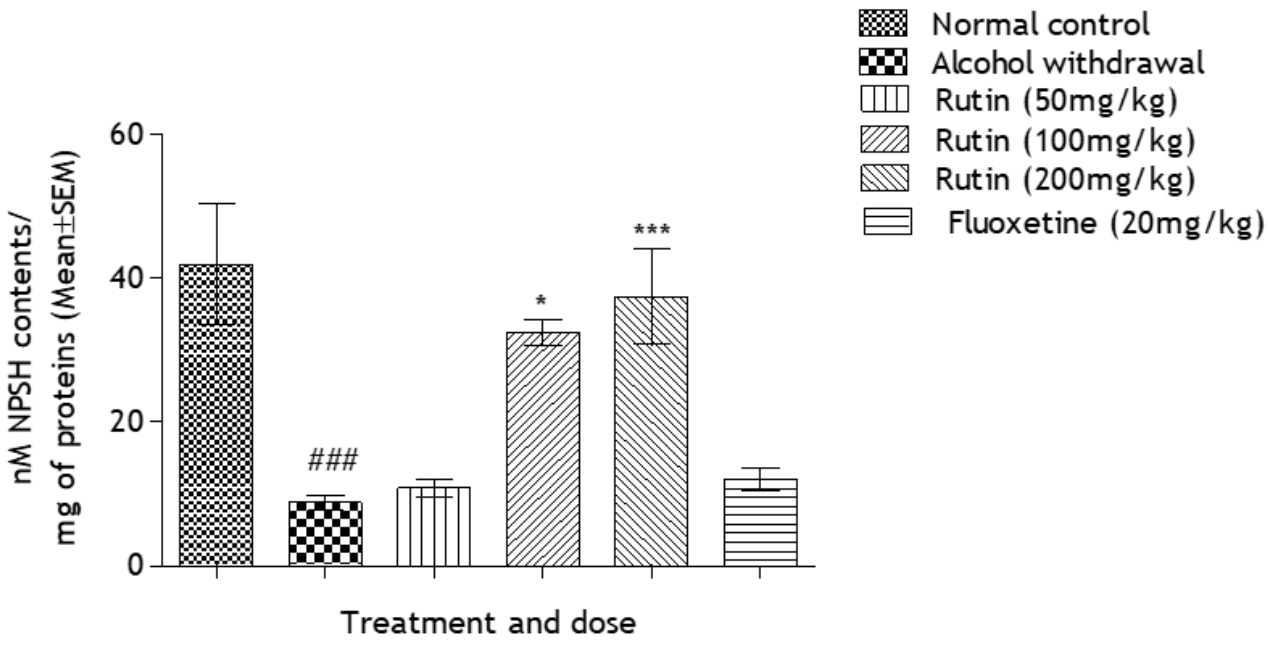

Figure 9

Effect of rutin or fluoxetine on NPSH contents (GSH levels) in alcohol withdrawal-induced depressive behavior in rats. Data was expressed as mean ± SEM (n=6) followed by one-way ANOVA and Bonferroni post-test, $^{###}p<0.0001$ compared to normal control group, $^{*}p< 0.01$ $^{***}p< 0.0001$ compared to alcohol withdrawal group

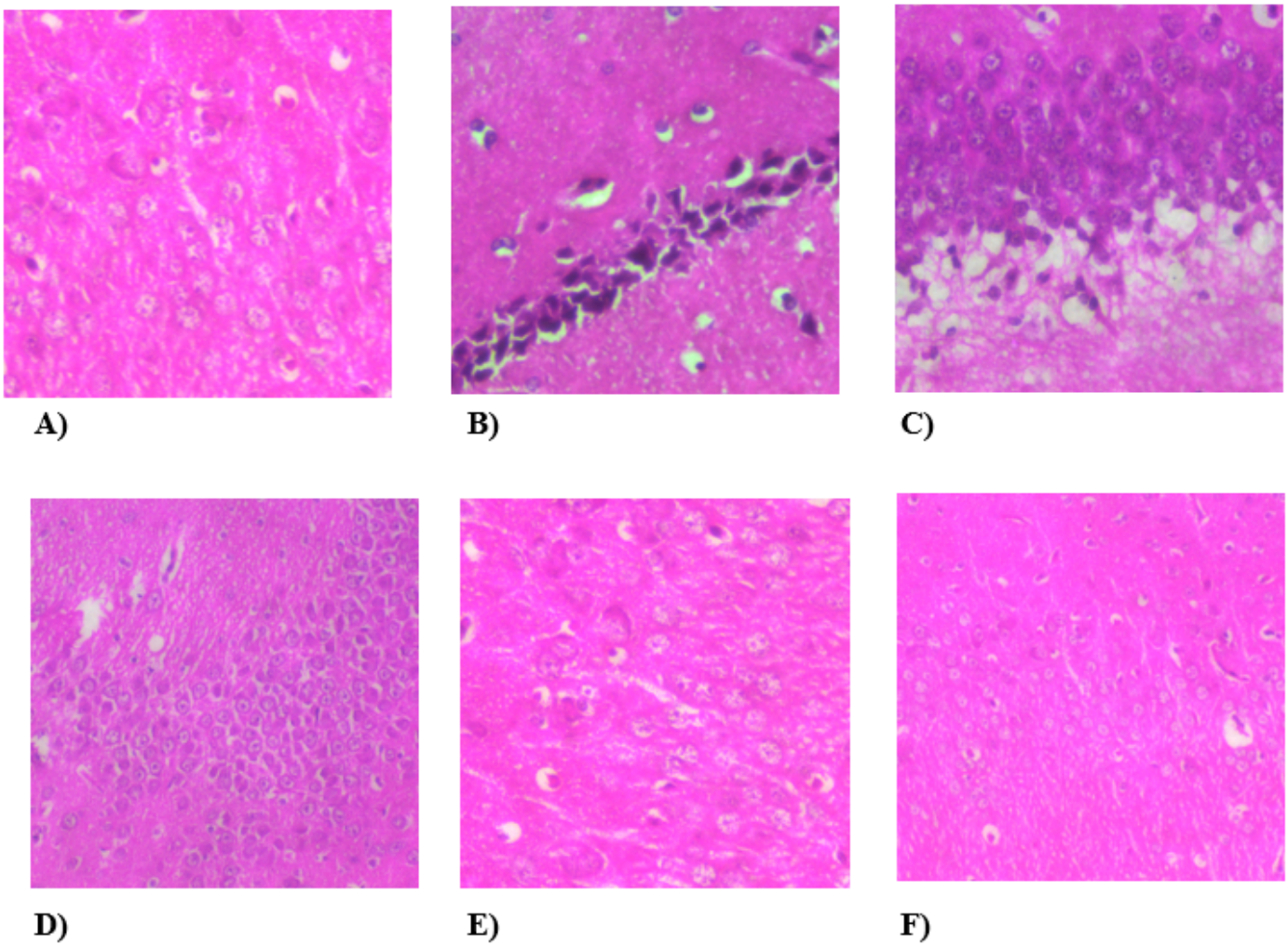

Figure 10

Histopathological sections of rat brain Hematoxylin and eosin stained histopathological sections of rat brain (A=Normal control, B- alcohol withdrawal group; C- Rutin 50mg/kg, , D- Rutin 100mg/kg, E- Rutin 200 mg/kg, F- Rutin 200 mg/kg).