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ORIGINAL RESEARCH article

Ascorbic acid has an anxiolytic-like effect in the presence of flumazenil in rats

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Abstract: Ascorbic acid (vitamin C) is a water-soluble vitamin; it is present in the highest concentration in the brain. Ascorbic acid in high doses acts as a potential treatment for various neuropathological and psychiatric conditions. Flumazenil is a benzodiazepine antagonist; it competitively inhibits the activity of benzodiazepine and non-benzodiazepine substances that interact with benzodiazepine receptors site on the GABA/benzodiazepine receptor complex. This study aims to investigate the effect of flumazenil on the anxiolytic action of ascorbic acid using an elevated plus maze model of anxiety in rats. Male Albino Wistar rats weighing between 250 and 320 grams were used. Rats were divided into four equal groups of seven rats each and treated as follows: Group I, the control group received a single dose of 1.0% tween 80; Group II treated with a single dose of 125 mg/kg ascorbic acid; Group III was injected by a single dose of 1.0 mg/kg flumazenil; Group IV received a combination treatment of 125 mg/kg ascorbic acid and 1.0 mg/kg flumazenil. Behavioural measurements using a plus maze were scored 30 min after the administration. The parameters scored are the time spent on the open and closed arms, the lines and number of entries into open and closed arms, and the anxiety measure. Ascorbic acid decreased anxiety measure and increased the total lines and total number of entries; this effect was abolished by the administration of flumazenil with ascorbic acid. Thus, ascorbic acid produces an anxiolytic-like effect in rats; this effect was abolished by flumazenil administration with ascorbic acid. This may indicate that the GABA/benzodiazepine receptor complex has to be stimulated to produce the anxiolytic effect.

Introduction

Ascorbic acid (AA, vitamin C) is a water-soluble vitamin; it reaches its highest concentration in the brain. AA has a neuroprotective function that acts as a cofactor in redox-coupled reactions essential for synthesizing neurotransmitters. AA in high doses has a potential treatment for various neuropathological and psychiatric conditions [1]; it is a necessary nutrient in human diets, and its deficiency results in scurvy disease [2]. Recent research confirms the antioxidant role of AA in preventing oxidative stress injury in the brain [3]. AA has been suggested to play a role in mood regulation [4]. In the previous work, it was found that AA produced dose-dependent anxiolytic effects without sedation in doses up to 500 mg/kg in rats. It was clear that the acute administration of AA was accompanied by increased gamma-aminobutyric acid (GABA) levels in almost all brain areas leading to anxiolytic action. It has been suggested that AA may act as a partial allosteric modulator

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of the $GABA_A$ receptor since it induces a smaller response in their target cells than a full allosteric modulator such as alprazolam. In addition, acute administration of AA (125 mg/kg or 500 mg/kg), selectively increases the ascorbate levels in the striatum, mid-brain, and cerebral cortex [5].

Flumazenil (Ro15-1788) is an imidazobenzodiazepine, developed by Hoffmann la Roche in the 1980s [6]. Flumazenil is a competitive benzodiazepine antagonist; it competitively inhibits the activity of benzodiazepine and non-benzodiazepine substances that interact with benzodiazepine receptors site on the GABA/benzodiazepine receptor complex. It can also reverse the binding of benzodiazepines to benzodiazepine receptors [7]. On the other hand, it does not reverse the effects of other GABAergic sedative-hypnotics such as barbiturates, inhalational aesthetics, propofol, or ethanol, nor does it reverse the effects of opioids [8]. Flumazenil is used in benzodiazepine overdose emergencies. Food and Drug Administration (FDA)-approved clinical uses for flumazenil include reversal agents for benzodiazepine overdose and postoperative sedation from benzodiazepine anaesthetics [9]. Thus, this study aims to investigate the effect of flumazenil on the anxiolytic action of AA using an elevated plus-maze model of anxiety in rats.

Materials and methods

Animals: Adult male Albino Wistar rats weighing between 250 and 320 g, bred in the local animal house of the Faculty of Pharmacy, University of Tripoli, Libya were used. The rats were housed in an animal house under controlled conditions (20-25^{o}C and 12/12-hr light/dark cycle). Rats received free access to a standard pellet diet (Beeky company-Austria) and water *ad libitum.*

Chemical administration: Throughout the studies described in this work, rats were randomly assigned to receive different treatments. Acute drug administration was performed by intraperitoneal route, 1.0% of tween 80 (Riedel-De Haen AG Seelze Hanover, Germany) was used as a suspending agent [10], and all control rats were injected with the vehicle. A volume of injection of 1.0 ml/kg of body weight was adopted in all the experiments [11].

Experimental design: Rats were divided into four equal groups of seven rats each and treated as follows: Group I, the control group received a single dose of 1.0% tween 80; Group II, treated with a dose of 125 mg/kg AA (Weishing Pharmaceutical Company, Shijiazhuang, Korea); Group III, injected by a dose of 1.0 mg/kg flumazenil (F. Hoffmann-la Roche Company, Switzerland); Group IV, received a combination of 125 mg/kg AA and 1.0 mg/kg flumazenil. Behavioural measurements using a plus maze were scored 30 min after drug administration. The total time of the test is 240 sec. The methodology and handling of rats were according to the guidelines of animal use of the University of Tripoli, Tripoli, Libya [UOT, 2017].

Behavioural measurements using elevated plus maze: The wooden elevated plus-maze (EPM) apparatus consists of two open arms (45×10 cm each) and two opposite closed arms of the same size, with walls of 40 cm in height. The arms are linked by a central square (10×10 cm). The maze was suspended 50 cm from the room floor, causing aversive stimuli to the rats in the open arms [12]. The rat should be exposed to the plus maze only once after the treatment, otherwise, the anxiolytic-like effects are considerably reduced by pre-exposure to the maze [13, 14]. This phenomenon is known as "one-trial tolerance" [15]. It was found that pre-exposure alters behavioral and pharmacological responses in the EPM [16, 17]. The EPM test was conducted in a closed room with a low illumination [18]. The test was performed between 8:00 am and 4:00 pm, under constant conditions. Rats were placed on the central part of the maze facing the closed arm. The number of entries, lines crossed, and the time spent on the open and closed arms were scored over four min. An entry is defined as having both forepaws on the respective arm. The line crossing is defined as both forepaws crossing the line [16]. Anxiety measure (A.M.) was calculated by dividing the total time spent on closed arms by the total test time; when the rat suffers fear and anxiety, the A.M. ratio is close to 1.0 [19, 20].

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Statistical analysis: Kolmogorov-Smirnov maximum deviation test for goodness of fit to determine whether the observed samples were parametric. If the parameters are normally distributed, the difference among groups is analyzed using one-way ANOVA, followed by a post-hoc test (Duncan and LSD). If the parameters of samples were nonparametric, treatments were compared by applying the Mann-Whitney two samples (non-matched) test. The statistical difference was considered to be significant at $p \leq 0.05$.

Results

In **Table 1**, Ascorbic acid at a dose of 125 mg/kg produced a significant decrease in the anxiety measure ($p \leq 0.05$) compared to the control-treated group ($p \leq 0.05$); while the administration of flumazenil alone treated group (1.0 mg/kg) or flumazenil combined with AA did not change the anxiety measure compared to the control treated group. Administration of AA significantly increases the time spent on open arms and zero area compared to the control-treated group ($p \leq 0.05$). At the same time, it significantly decreases the time spent on closed arms compared to the control-treated group ($p \leq 0.05$). Administration of flumazenil alone or in combination with AA did not show any change in the time spent on closed, open arms, and zero area, compared to the control-treated group (**Table 1**).

Table: 1: Effect of acute administration of flumazenil and ascorbic acid on anxiety measure and time spent on the arms of the plus maze

Treatments	Anxiety measure (AM)	Time spent on open arms (min)	Time spent on closed arms (min)	Time spent on zero area (min)
1.0% tween 80 (5.0 ml/kg)	1.00±0.0	0.00±0.00	240.0±00.0	0.00±0.00
Ascorbic acid (125 mg/kg)	0.551±0.140 *	71.0± 5.92 *	132.29±33.63 *	36.71±17.81 *
Flumazenil (1.0 mg/kg)	0.981±0.015	0.00±0.00	236.14±02.49	3.86±2.49
Ascorbic acid & Flumazenil	0.983±0.010 [a]	0.00±0.00 [a]	235.57±3.66 [a]	4.43±3.66 [a]

The values are the means±S.E, * Significantly different from tween 80 treated group at $p \leq 0.05$,
[a] Significantly different from the ascorbic acid 125 mg/kg treated group at $p \leq 0.05$

In **Table 2**, rats treated with AA significantly increased the lines crossed on open arms, and the number of entries into open arms also increased the total number of entries compared to the control-treated group ($p \leq 0.05$). While lines crossed on closed arms, total lines crossed, and number of entries into closed arms were not changed after the administration of AA compared to the control treated group. Treatment with flumazenil alone or combined with AA did not change the lines crossed and the number of entries into a different compartment, even the total lines crossed and the total number of entries were not changed compared to the control treated group (**Table 2**).

Table: 2: Effect of acute administration of flumazenil and ascorbic acid on the lines crossed and number of entries into the arms of the plus maze

Treatments	Lines crossed number			Number of entries number		
	Open arms	Closed arm	Total lines crossed	Open arms	Closed arms	Total entries
1.0% Tween 80 (5.0 ml/kg)	0.00±0.00	5.43±1.15	5.43±1.15	0.00±0.00	1.00±0.00	1.00±0.00
Ascorbic acid (125 mg/kg)	3.14±1.08 *	4.00±0.82	7.14±1.32	1.14±0.26 *	1.00±0.22	2.14±0.34 *
Flumazenil (1.0 mg/kg)	0.00±0.00	4.00±0.69	4.00±0.69	0.00±0.00	1.14±0.14	1.14±0.14
Ascorbic acid & Flumazenil	0.0±0.00 [a]	6.43±1.62	6.43±1.62	0.0±0.00 [a]	1.29±0.18	1.29±0.18 [a]

The values are the means±S.E, * Significantly different from tween 80 treated group at $p \leq 0.05$,
[a] Significantly different from the ascorbic acid 125 mg/kg treated group at $p \leq 0.05$

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Discussion

Anxiety is a chronic severe psychiatric disorder. Thus, several anxiety disorders are highly prevalent and chronic disorders with treatment resistance to current pharmacotherapies occurring in about one in three patients [21, 22]. In the CNS, the major mediators of anxiety disorders are noradrenaline, serotonin, dopamine, and GABA. Peptides, as corticotropin-releasing factors, may also be involved in the hypothalamic-pituitary-adrenal (HPA) axis [23]. Dysfunction of the GABA system activity has been associated with anxiety disorders, and modulation of the GABA system can result in anxiolysis or anxiogenesis [12, 24]. The positive modulators of $GABA_A$ receptors result in anxiolysis and the negative modulators produce an anxiogenic effect [25]. AA is considered an important vitamin that can be used to manage all kinds of stressful conditions that are linked to inflammatory processes and immunity [26]. AA has some other beneficial effects on the management of psychiatric disorders [27], including depression [28, 29], anxiety and schizophrenia, and neurodegenerative diseases [30]. AA has strong antioxidant properties [31, 32] and it increases superoxide dismutase (SOD) and glutathione peroxidase (GPx) effects in the brain. The administration of AA also increases the serum level of brain-derived neurotrophic factor (BDNF) [33].

In the current study, 125 mg/kg of AA produced an anxiolytic-like effect by decreasing the anxiety measure in the elevated plus maze. Thus, the rat spent more time on open arms, an increased number of entries into open arms, and less time on closed arms compared to the control-treated group. It was demonstrated that the pharmacological investigation confirmed that the approach of the open arms was specifically increased by classical anxiolytics such as chlordiazepoxide and diazepam and decreased by anxiogenic substances such as yohimbine, caffeine, and amphetamine [34]. AA is a water-soluble vitamin that has a high concentration in the brain tissue under normal conditions [33]. Ascorbate distribution within different brain areas suggests that it has an important role in the brain. Ascorbate is considered a neuromodulator of glutamatergic, dopaminergic, cholinergic, and GABAergic transmission and related behaviours [35]. Further, AA at 0.5 mM concentration exhibits a neuroprotective effect through interaction with the $GABA_B$ receptor [36]. While another finding indicated that AA exerts an anti-depressant effect in mice at a concentration of 1.0 mg/kg, possibly by interacting with $GABA_A$ and $GABA_B$ receptors [37]. In the human body, AA may act as an endogenous ligand that can potentiate GABA neurotransmission in the CNS [38]. The GABA receptor is the most essential target, to produce anxiolytic and/or antidepressant effects [23]. AA binds to the active sites of $GABA_{A2}$, $GABA_{A5}$, $GABA_{B1}$, and $GABA_{B2}$ proteins [39]. In the rat brain cortical synaptosome, AA stimulates GABA binding capacity [40]. The binding affinities of AA were -5.0, -5.5, and -5.3 kcal/mol with $GABA_{A2}$, $GABA_{B1}$, and $GABA_{B2}$, respectively [39]. AA showed moderate binding affinities with GABA receptor subunits, including $GABA_{A2}$, $_{B1}$, and $_{B2}$, respectively [39].

Sedative and anxiolytic agents such as benzodiazepine (BZ) exert their action through GABA upon the $GABA_A$ receptor [41]. Classical BZs bind to $GABA_A$ receptors containing α_1, α_2, α_3, and/or α_5 subunits, while binding affinity to α_4-containing and α_6-containing subunits is extremely weak [42]. The $GABA_A$ receptor α_1 subunit is associated with sedation, whereas the $GABA_A$ receptor α_2 and α_3 subunits are involved in anxiolytic effects [43, 44]. Genetic and pharmacological studies suggested that the major role of the α_2 and the α_3 $GABA_A$ receptor subunit is in mediating anxiolysis [42, 45, 46]. It is found that AA at higher doses (10^{-3} M) markedly inhibits, and at lower doses (10^{-6} M) highly stimulates 3H-GABA binding capacity in the rat brain cortical synaptosomes [40, 47]. It seems that the AA dose used in this study is considered low dose leading to stimulation of GABA binding capacity and producing an anxiolytic effect. In this work, flumazenil is used to investigate the possible mechanism of action of AA as an anxiolytic agent in rats. Flumazenil is best known as a competitive antagonist at the B-binding site on the $GABA_A$ receptor and has long-standing clinical use as an emergency treatment for BZ overdose [48, 49]. It is essentially used as an antidote in the treatment of BZ overdoses [50]. Two sub-types of BZ receptors were identified; namely, BZR_1 was found through the brain,

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predominantly in the cerebellum, while BZR_2 is located principally in the cortex, hippocampus, and spinal cord. Compounds with selectivity towards the BZR_1 subtype would be non-sedative anxiolytics, and in contrast, BZR_2 selective compounds might be non-anxiolytic sedatives [51].

In this study, the administration of flumazenil alone in a dose of 1.0 mg/kg did not change the anxiety measure or the spontaneous motor activity compared to the control-treated group. Therefore, flumazenil represents a competitive antagonist blocking the enhancing effect of agonists and the depressant effect of "inverse agonists" on GABAergic synaptic transmission [52]. Flumazenil was originally reported to lack effects on behaviour when given on its own, although it was later found to possess some 'anxiogenic' activity [9, 53]. Flumazenil administration alone in a dose of 1.0 mg/kg did not change the time spent on any areas. Flumazenil has no or only weak intrinsic efficacy for changing GABAergic transmission [52]. It has weak intrinsic agonist activity on the $GABA_{AR}$ [49, 54]; but depending on basal conditions, tests, dosage, and measurements performed. Flumazenil had weak agonist-like and weak inverse agonist-like properties [55]. Therefore, it has no or only weak effects when given to animals or humans, but can inhibit the effects of BZ receptor agonists and inverse BZ receptor agonists [52]. Weak intrinsic agonist-like or inverse agonist-like pharmacological activity of flumazenil is unlikely to be of clinical importance [55]. According to the previous explanation, flumazenil did not change the anxiety measure or the spontaneous motor activity with the dose used. This study revealed that the anxiolytic action of AA in the EPM was abolished by the administration of flumazenil. According to a model in mice, flumazenil antagonizes the anxiolytic effect of BZs [56]. It may act by binding to the CNS BZ receptors and competitively block BZ activation of inhibitory GABAergic synapses. Also, flumazenil can inhibit the enhancement of GABA currents [49]. Administration of flumazenil with AA decreased significantly the time spent on open arms and increased the anxiety measure significantly when compared with the AA alone treated group. The present findings suggested that AA as an endogenous ligand can potentiate GABA neurotransmission in the CNS [37]; it may stimulate GABA binding capacity [39]. At the same time, BZR1 has to be stimulated by endogenous benzodiazepine for AA to produce its anxiolytic effect. Endogenous BZ of natural origin has been found in human blood and brains as well as in medicinal plants and foods; it acts as an agonist at the BZ receptors of central type. It is considered a natural anxiolytic and sedative [57]. These compounds could act as physiological regulators of the $GABA_A$ receptors [58]. AA could produce an anxiolytic effect through stimulation of GABA neurotransmission. Flumazenil abolishes the effect of AA, through blocking the BZR. This effect of flumazenil will block BZ activation of inhibitory GABAergic synapses, leading to antagonizing the anxiolytic effect of AA.

Conclusion: Ascorbic acid produces an anxiolytic-like effect in rats, using an elevated plus maze model of anxiety; this effect was abolished by flumazenil administration with ascorbic acid. This indicates that the GABA/benzodiazepine receptor complex has to be stimulated to produce the anxiolytic effect.

References

1. Ballaz SJ, Rebec GV (2019) Neurobiology of vitamin C: Expanding the focus from antioxidant to endogenous neuromodulator. Pharmacological Research. 146. 104321. doi: 10.1016/j.phrs.2019.104321
2. Barnes M, Kodicek E (1972) Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. Vitamins and Hormones. 30: 1-43. doi: 10.1016/S0083-6729(08)60793-1
3. Ferrada L, Barahona MJ, Salazar K, Vandenabeele P, Nualart F (2019) Vitamin C controls neuronal necroptosis under oxidative stress. Redox Biology. 29: 101408. doi: 10.1016/j.redox.2019.101408
4. Han Q, Wu F, Li H, Cao Y, Chen G, Wang F (2022) SVCT2-mediated ascorbic acid uptake buffers stress responses via DNA hydroxymethylation reprogramming of the S100 calcium-binding protein A_4 gene. Redox Biology. 58: 102543. doi: 10.1016/j.redox.2022.102543
5. Ben Saad JM, Eldrogi AF, Al-Tubuly RA, Aburawi SM (2016) Neurobehavioral effect of alprazolam in presence of ascorbic acid using albino rats. Lebda Medical Journal. 2 (1): 68-82. doi: Nil.

ISSN: 2789-1895 online
ISSN: 2958-3101 print

6. Sivilotti LA (2016) Flumazenil, naloxone and the 'coma cocktail'. British Journal of Clinical Pharmacology. 81 (3): 428-436. doi: 10.1111/bcp.12731
7. An H, Godwin J (2016) Flumazenil in benzodiazepine overdose. Canadian Medical Association Journal. 188 (17-18): E537. doi: 10.1503/cmaj. 160357
8. Kucken AM, Teissére JA, Seffinga-Clark J, Wagner DA, Czajkowski C (2003) Structural requirements for imidazobenzodiazepine binding to $GABA_A$ receptors. Molecular Pharmacology. 63 (2): 289-296. doi: 10.1124/mol.63.2.289
9. Shoar NS, Bistas KG, Patel P, Saadabadi A (2024) Flumazenil. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Bookshelf ID: NBK470180. PMID: 29262246.
10. Collinge J, Pycock CJ, Taberner PV (1983) Studies on the interaction between cerebral 5-hydroxytryptamine and gamma-aminobutyric acid in the mode of action of diazepam in the rat. British Journal of Pharmacology. 79 (3): 637-643. doi: 10.1111/j.1476-5381.1983.tb10000.x
11. Aburawi S, Ahmed S, Elhwuegi A, Saad SF, Attia AS (2001) Brain glycine levels in triazolam-treated albino rats. Journal of Neural Transmission. 108: 527-539. doi: 10.1007/s007020170054
12. Sherif FM, Harro J, El-Hwuegi A, Oreland L. Anxiolytic-like effect of the GABA-transaminase inhibitor vigabatrin (gamma-vinyl GABA) on rat exploratory activity. Pharmacology, Biochemistry, and Behaviour. 1994 Dec;49(4):801-5. doi: 10.1016/0091-3057(94)90226-7
13. Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology. 92 (2): 180-185. doi: 10.1007/BF00177912
14. File SE (1990) One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psychopharmacology. 100 (2): 281-282. doi: 10.1007/BF02244419
15. Schneider P, Ho Y-J, Spanagel R, Pawlak CR (2011) A novel elevated plus-maze procedure to avoid the one-trial tolerance problem. Frontiers in Behavioral Neuroscience. 5 (43): 1-8. doi: 10.3389/fnbeh.2011.00043
16. File SE, Zangrossi H Jr (1993) "One-trial tolerance" to the anxiolytic actions of benzodiazepines in the elevated plus-maze, or the development of a phobic state? Psychopharmacology. 110 (1-2): 240-244. doi: 10.1007/BF02246980
17. Bertoglio LJ, Carobrez AP (2002) Anxiolytic effects of ethanol and phenobarbital are abolished in test-experienced rats submitted to the elevated plus maze. Pharmacology, Biochemistry, and Behaviour. 73 (4): 963-969. doi: 10.1016/s0091-3057(02)00958-9
18. Garcia AMB, Cardenas FP, Morato S (2005) Effect of different illumination levels on rat behavior in the elevated plus-maze. Physiology and Behaviour. 85 (3): 265-270. doi: 10.1016/j.physbeh.2005.04.007
19. Pawlak CR, Karrenbauer BD, Schneider P, Ho Y-J (2012) The elevated plus-maze test: Differential psychopharmacology of anxiety-related behavior. Emotion Review. 4 (1): 1-19. doi: 10.1177/1754073911421374
20. Aburawi SM, Elhwuegi AS, Ahmed SS, Saad SF, Attia AS (2003) Behavioral effects of acute and chronic triazolam treatments in albino rats. Life Sciences. 73 (24): 3095-107. doi: 10.1016/s0024-3205(03)00612-x
21. Aburawi SM, Sadaa KA, Elshalakani MH, Altubuly RA (2013) Effect of sildenafil citrate on behaviour and excitatory and inhibitory amino acids levels in Albino rat's brain. Jordan Journal of Pharmaceutical Sciences. 6 (2): 242-257. doi: 10.12816/0000370
22. Pitsikas N, Tarantilis PA (2020) The $GABA_A$-benzodiazepine receptor antagonist flumazenil abolishes the anxiolytic effects of the active constituents of *Crocus sativus* L. Crocins in rats. Molecules. 25 (23): 5647. doi: 10.3390/molecules25235647
23. Gallo AT, Addis S, Martyn V, Ramanathan H, Wilkerson GK, Bennett KS, Hood SD, Stampfer H, Hulse GK (2023) The role of flumazenil in generalised anxiety disorder: a pilot naturalistic open-label study with a focus on treatment resistance. Therapeutic Advances in Psychopharmacology. 13. doi: 10.1177/20451253231156400
24. Sherif FM, Ahmed SS (1995) Basic aspects of GABA-transaminase in neuropsychiatric disorders. Clinical Biochemistry. 28 (2):145-54. doi: 10.1016/0009-9120(94)00074-6
25. Szuhany KL, Simon NM (2022) Anxiety disorders: A review. Journal of the American Medical Association. 328 (24): 2431-2445. doi: 10.1001/jama.2022.22744
26. Sorice A, Guerriero E, Capone F, Colonna G, Castello G, Costantini S (2014) Ascorbic acid: its role in immune system and chronic inflammation diseases. Mini Reviews in Medicinal Chemistry. 14 (5): 444-452. doi: 10.2174/1389557514666140428112602
27. Moretti M, Fraga DB, Rodrigues ALS (2017) Ascorbic acid to manage psychiatric disorders. CNS Drugs. 31 (7): 571-583. doi: 10.1007/s40263-017-0446-8
28. Liu T, Zhong S, Liao X, Chen J, He T, Lai S, Jia Y (2015) A meta-analysis of oxidative stress markers in depression. PLoS ONE. 10 (10): e0138904. doi: 10.1371/journal.pone.0138904
29. Shivavedi N, Kumar M, Tej GNVC, Nayak PK (2017) Metformin and ascorbic acid combination therapy ameliorates type 2 diabetes mellitus and comorbid depression in rats. Brain Research. 1674: 1-9. doi: 10.1016/j.brainres.2017.08.019

ISSN: 2789-1895 online
ISSN: 2958-3101 print

30. Aburawi SM (2021) Vitamin C and human diseases: An overview. Mediterranean Journal of Pharmacy and Pharmaceutical Sciences. 1 (4): 25-36. doi: 10.5281/zenodo.5805947
31. Cort WM (1982) Antioxidant properties of ascorbic acid in foods; American Chemical Society: Washington, DC, USA. 533-550. ISBN: 9780841206328.
32. Yen GC, Duh PD, Tsai HL (2002) Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food and Chemistry. 79 (3): 307-313. doi: 10.1016/S0308-8146(02)00145-0
33. Delrobaei F, Fatemi I, Shamsizadeh A, Allahtavakoli M (2019) Ascorbic acid attenuates cognitive impairment and brain oxidative stress in ovariectomized mice. Pharmacological Reports. 71 (1): 133-138. doi. 10.1016/j.pharep.2018.10.001
34. Cryan JF, Sweeney FF (2011) The age of anxiety: role of animal models of anxiolytic action in drug discovery. British Journal of Pharmacology. 164 (4): 1129-1161. doi: 10.1111/j.1476-5381.2011.01362.x
35. Harrison FE, May JM (2009) Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. Free Radical Biology and Medicine. 46 (6): 719-730. doi: 10.1016/j.freeradbiomed.2008.12.018
36. Naseer MI, Lee HY, Kim MO (2010) Neuroprotective effect of vitamin C against the ethanol and nicotine modulation of $GABA_B$ receptor and PKA-alpha expression in prenatal rat brain. Synapse. 64 (6): 467-477. doi: 10.1002/syn.20752
37. Rosa PB, Neis VB, Ribeiro CM, Moretti M, Rodrigues ALS (2016) Antidepressant-like effects of ascorbic acid and ketamine involve modulation of $GABA_A$ and $GABA_B$ receptors. Pharmacological Reports. 68 (5): 996-1001. doi: 10.1016/j.pharep.2016.05.010
38. Calero CI, Vickers E, Moraga Cid G, Aguayo LG, von Gersdorff H, Calvo DJ (2011) Allosteric modulation of retinal GABA receptors by ascorbic acid. Journal of Neuroscience. 31 (26): 9672-9682. doi: 10.1523/JNEUROSCI.5157-10.2011
39. Hossain R, Khan RA, Sarkar C, Islam MS, Dey D, Jain D, Faria F, Akbor R, Atolani O, Oliveira SM, Siyadatpanah A, Pereira MD, Islam MT (2021) Quercetin and/or ascorbic acid modulatory effect on phenobarbital-induced sleeping mice possibly through $GABA_A$ and $GABA_B$ receptor interaction pathway. Pharmaceuticals. 14 (8): 721. doi: 10.3390/ph14080721
40. Grigor'ev IP, Neokesariĭskiĭ AA (1986) Effect of ascorbic acid on the binding of 3H-GABA and 3H-glutamic acid to synaptosomes of the rat cerebral cortex. Biulleten Ekspweimental noi Biologii i Meditsiny. 102 (9): 288-289. PMID: 2875748.
41. Tan KR, Rudolph U, Lüscher C (2011) Hooked on benzodiazepines: GABAA receptor subtypes and addiction. Trends in Neurosciences. 34 (4): 188-197. doi: 10.1016/j.tins.2011.01.004
42. Rudolph U, Möhler H (2004) Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annual Review of Pharmacology and Toxicology. 44: 475-98. doi: 10.1146/annurev.pharmtox.44.101802.121429
43. Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR (2004) Different $GABA_A$ receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proceedings of the National Academy of Sciences of the United States of America. 102 (3): 915-920. doi: 10.1073/pnas.0405621102
44. Vinkers CH, Klanker M, Groenink L, Korte SM, Cook JM, Van Linn ML, Hopkins SC, Olivier B (2009) Dissociating anxiolytic and sedative effects of $GABA_A$-ergic drugs using temperature and locomotor responses to acute stress. Psychopharmacology. 204 (2): 299. doi: 10.1007/s00213-009-1460-4
45. Atack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, Cook SM, Collins I, Wafford K, McKernan RM, Dawson GR (2005) Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing $GABA_A$ receptors. British Journal of Pharmacology. 144 (3): 357-366. doi: 10.1038/sj.bjp.0706056
46. Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, Hallett D, Humphries AC, Thompson SA, Atack JR, McKernan RM, Dawson GR, Reynolds DS (2005) Evidence for a significant role of alpha 3-containing $GABA_A$ receptors in mediating the anxiolytic effects of benzodiazepines. Journal of Neurosciences. 25 (46): 10682-10688. doi: 10.1523/JNEUROSCI.1166-05.2005
47. Islam MT, Molla S, Zihad NK, Umer M, Rahman S, Zaman F, Das AK, Afzal MI, Salehi B, Akter MS, Mubarak MS, Martins N, Imran M, Chaudhary N, Iqbal Z, Sharifi-Rad J (2020) Ascorbic acid antagonizes the sedative effect of diazepam possibly through inhibition of $GABA(A\rho_1)$ and $GABA(B_1)$ receptors. Cellular and Molecular Biology. 66 (4): 15-19. doi: 10.14715/cmb/2020.66.4.3
48. Ishola IO, Chatterjee M, Tota S, Tadigopulla N, Adeyemi OO, Palit G, Shukla R (2012) Antidepressant and anxiolytic effects of amentoflavone isolated from Cnestis ferruginea in mice. Pharmacology, Biochemistry, and Behavior. 103 (2): 322-331. doi: 10.1016/j.pbb.2012.08.017
49. Safavynia SA, Keating G, Speigel I, Fidler JA, Kreuzer M, Rye DB, Jenkins A, García PS (2016) Effects of γ-aminobutyric acid type a receptor modulation by flumazenil on emergence from general anaesthesia. Anaesthesiology. 125 (1): 147-58. doi: 10.1097/ALN.0000000000001134

ISSN: 2789-1895 online
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50. Nelson LS, Howland MA, Lewin NA, Smith SW, Goldfrank LR, Hoffman RS (2019) Goldfrank's toxicologic emergencies, 11e. McGraw-Hill. Kepler Std by Cenveo® Publisher Services. ISBN: 978-1-259-85961-8.
51. Doble A, Martin IL (1992) Multiple benzodiazepine receptors: no reason for anxiety. Trends in Pharmacological Sciences. 13 (2): 76-81. doi: 10.1016/0165-6147(92)90027-4
52. Polc P, Bonetti EP, Schaffner R, Haefely W (1982) A three-state model of the benzodiazepine receptor explains the interactions between the benzodiazepine antagonist Ro15-1788, benzodiazepine tranquilizers, beta-carbolines, and phenobarbitone. Naunyn-Schmiedeberg's Archives of Pharmacology. 321 (4): 260-264. doi: 10.1007/BF00498510
53. Nutt DJ, Glue P, Lawson C, Wilson S (1990) Flumazenil provocation of panic attacks: evidence for altered benzodiazepine receptor sensitivity in panic disorder. Archives of General Psychiatry. 47 (10): 917-925. doi: 10.1001/archpsyc.1990.01810220033004
54. Votey SR, Bosse GM, Bayer MJ, Hoffman JR (1991) Flumazenil: a new benzodiazepine antagonist. Annals of Emergency Medicine. 20 (2): 181-188. doi: 10.1016/s0196-0644(05)81219-3
55. Brogden RN, Goa KL (1991) Flumazenil. A reappraisal of its pharmacological properties and therapeutic efficacy as a benzodiazepine antagonist. Drugs. 42 (6): 1061-1089. doi: 10.2165/00003495-199142060-00010
56. Dhaliwal JS, Rosani A, Saadabadi A (2024) Diazepam. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Bookshelf ID: NBK537022. PMID: 30725707.
57. Baraldi M, Avallone R, Corsi L, Venturini I, Baraldi C, Zeneroli ML (2009) Natural endogenous ligands for benzodiazepine receptors in hepatic encephalopathy. Metabolic Brain Disease. 24 (1): 81-93. doi: 10.1007/s11011-008-9111-8
58. Izquierdo I, Medina JH (1991) GABAA receptor modulation of memory: the role of endogenous benzodiazepines. Trends in Pharmacological Sciences. 12 (7): 260-265. doi: 10.1016/0165-6147(91)90567-c

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