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EFFECT OF ETHANOL AND BROMAZEPAM ON MONOAMINE OXIDASE ACTIVITY IN RAT BRAIN

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ABSTRACT: The effect of ethanol and bromazepam on monoamine oxidase activity in rat brain was studied. Rats were fed chow with water *ad libitum*, and administered 5% (v/v) ethanol (acute and chronic) and 0.1mg bromazepam per 100g body weight for 14 days. The rats were sacrificed by decapitation, after starving them overnight on the 15th day, and their brains were quickly excised. The mitochondrial fraction of the brain tissues was isolated and the activity of monoamine oxidase determined. Brain serotonin and protein of the rats were also determined. Results indicate that ethanol and bromazepam inhibits monoamine oxidase activity in varying degrees in rat brain. Our data showed that the inhibition varies from mild as in bromazepam injected rats to strong inhibition (in chronic ethanol treated rats) of the monoamine oxidase activity in the brain. Results of this study are significant in the administration of these drugs for treatment of disorders, such as depression associated with neurotransmitter regulation by monoamine oxidase in the brain.

Key Words: Monoamine oxidase; Ethanol; Bromazepam; Neurotransmitters; Rat brain.

INTRODUCTION

Monoamine oxidase (MAO) is an important enzyme in the catabolic breakdown of monoamine neurotransmitters, such as serotonin and catecholamines (1). Monoamine oxidase inhibitors were, therefore, sought for the treatment of disorders associated with neurotransmitter regulation. Mental depression, for example, was treated with such drugs as iproniazine, a potent MAO inhibitor (2). MAO is neuronal in origin and is found in the mitochondria.

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Monoamine oxidases are enzymes found in the mammalian blood plasma, liver, brain tissue, and so on. Although exist in multiple forms, they are monoamine donor cleaving enzyme or deaminating enzyme (Monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4.) (3). They can be categorized into two groups namely, the semicarbazide sensitive and insensitive enzyme groups. For example, mitochondrial and microsomal MAO belong to the semicarbazide sensitive enzyme group. The close association of MAO with mitochondria supports the current belief that MAO functions to maintain the steady-rate of monoamine stores in neurons (4).

MAO is also useful in detoxification of amines formed by the intestinal flora (2). Numerous studies suggest that in chronic schizophrenia the blood platelet level of MAO is low (5). The continual study of monoamine oxidase has profoundly impacted the understanding of the neuropharmacology of the central nervous system (CNS).

The main effects of ethanol which is a non-therapeutic drug, are on the central nervous system (6) where its depressant actions resemble those of volatile anaesthetics. There is biophysical evidence suggesting that ethanol, at pharmacological effective concentration, produces a measurable increase in the structural disorder (that is increased fluidity) of lipid membranes, similar to the effect of volatile anaesthetics. At a cellular level, the effect of ethanol is purely depressant. Its action involves several mechanisms, including inhibition of Ca^{2+}-entry throgh voltage-gated Ca^{2+}- -channels (8) enhancement of Gamma amino butyric acid (GABA) - mediated synaptic inhibition, and antagonism of excitatory amino acids (9).

Ethanol inhibits transmitters release in response to nerve terminal depolarization, without affecting release evoked by calcium ionophores (8). Consistent with this action, it has been shown to inhibit the opening of voltage-sensitive Ca^{2+} -channels in neurons. Ethanol also enhances the action of GABA acting on GABA receptors in a similar way to benzodiazepines (8).

The effects of acute ethanol intoxication in man are well-known, and include slurred speech, motor incoordination, increased self-confidence and euphoria. Intellectual and motor performance and sensory discrimination show uniform impairment by ethanol, but subjects are generally unable to judge this for themselves (6). In addition to the acute effects of ethanol on the nervous system, chronic administration also causes several irreversible neurological syndromes such as dementia, degeneration in specific brain regions, peripheral neuropathy and myopathy. Long-term ethanol consumption causes liver disease, progressing to cirrhosis and liver failure (10).

Bromazepam (Lexotan) belongs to a family of drugs called benzodiazepines. Lexotan is hypnotic, sedative, anxiolytic, anticonvulsant and central muscle relaxant action. Lexotan is a powerful psychotropic agent. In low dosage, it selectively reduces tension and anxiety. In high dosage, sedative and muscle-relaxing properties appear.

Lexotan attaches to GABA-receptor/ chloride channel complex, potentiating the effect of GABA, an important inhibitory transmitter in the CNS which acts by opening, chloride ion channels into cells (14). Bromazepam acts chiefly on the brain reticular activating system (reducing sensory input), the limbic system, and the hypothalamus (7).

The ingestion of lexotan causes a relaxation of the central muscles in the body, as such, it shortens the time taken to go to sleep (sleep latency), decreases intermittent awakening and increases total sleep duration (12). Lexotan is used in treating anxiety, panic attacks, insomnia, alcohol withdrawal states and night terrors and somnambulism (in children), but it is however not an antidepressant (12, 13).

From the wealth of literature, there is very scanty or no report on the effect of ethanol and/or bromazepam on monoamine oxidase activity in the brain. Therefore, since alcoholism is increasing in men and women of all ages, and that it has become a public health problem; coupled with the growing usage of sedatives by people, it is imperative to decipher whether or not these drugs affect the activity of monoamine oxidase in the brain. The information obtained from this study will be useful in understanding of the neuropharmacology of the CNS.

MATERIALS AND METHODS

Lexotan (Bromazepam) manufactured by Roche Nig. Limited, Agege, Lagos (with the Batch Number 62061 MFD 05 96, EX 05 2001), was purchased from the Community Pharmacy of the College of Medicine, University of Lagos. The Lexotan contains per tablet 3.0mg 7 -bromo-1, 3-di hydro-5-(2-pyridyl)-2H-1, 4-benzodiazepin-2-one (Bromazepam). The tablets were dissolved in deionized water

to give a concentration of 0.1mg/ml lexotan, by dissolving 3mg tablets in 90ml of deionized water inside a plastic container and then placed in a water-bath at 37^{O}C, stirred continuously for 3min. The drug was administered intra-peritoneally with a dosage of 0.1mg/ml/100g body weight of rat daily.

Seaman's Aromatic Schiedam Schnapps containing 40% ethanol, blended and bottled in Nigeria by Nigeria Distilleries Ltd., Sango Otta, Ogun State was purchased and used as the source of alcohol. Since the alcoholic beverage contained 40% (v/v) ethanol, it was diluted with distilled water in a ratio of 1:7, to obtain 5% (v/v) ethanol, which was used for the study. The 5% (v/v) ethanol was kept in the normal water-drinking container for the rats to drink.

Ninety-six virgin (Sprague-Dawley) male rats weighing 190.2 ± 5.4g, were collected from the Animal Centre of the College of Medicine of the University of Lagos, Lagos. They were put in groups of three rats per cage and kept in a room with a temperature of 28 ± 2^{O}C, and illuminated for 12 hour per day (0700 - 1900h). The rats were fed commercial rat chow containing 21% protein with water *ad libitum* for 14 days acclimatization.

After 14 days of acclimatization, the rats were divided into four groups containing 24 rats each. The first group of rats was fed rat chow with 5% (v/v) ethanol *ad libitum* without water for 14 days. This was also referred to as chronic alcohol consumption group. The second group was fed the chow with water *ad libitum*, and an oral administration of 2ml of (v/v) alcohol per 100g body weight daily using a sterile syring for 14 days. This was also referred to acute alcohol consumption group. The third group of rats was fed chow with water *ad libitum* and an intra-peritoneal administration of 0.1mg/ml lexotan/100g body weight daily for 14 days. The fourth group of rats was fed rat chow with water *ad libitum* for 14 days. This group was used as te control for the study.

The administration of drugs for both lexotan and ethanol to rats, intra-peritoneally and orally respectively was carried out between 0900 and 1100 h daily for 14 days. Feed water and alcohol intake, and their body weights during the 14 days of drug administration were computed.

Preparation of enzyme system:

The metod of Catravas *et al* (15) was adopted to prepare the enzyme systems of monoamine oxidase in the rat brain. At the end of the 14 days of drug administration, all the rats were starved overnight and sacrificed the following day by decapitation. The brains were quickly excised and weighed, and stored at -20^{O}C for further analysis. There was no detectable loss in enzyme activity at this temperature.

The brain tissues were thawed and homogenized in 10 volume of 0.25M Sucrose containing 1mM $MgCl_2$ using Teflon glass homogenizer placed in ice-bath. The brain homogenate was centrifuged at 1500g for 10 min. and the resultant supernatant was centrifuged at 10,000g for 30 min. The supernatant fluid was carefully decanted and the mitochondrial pellet was suspended in an equal volume of homogenization medium, and was used for the enzyme assay. No MAO activity was observed in the 10,000g supernatant.

Determination of monoamine oxidase activity:

Monoamine oxidase activity was assayed by a modification of the method of Weissbach *et al* (16). The assay mixture contained 0.5ml of 0.05M Tris-HCl buffer, pH 7.4. 1mg of serotonin (5-hydroxytryptamine creatinine sulphate) and 0.2ml of the brain homogenate. The final volume of the incubation mixture was made to 3.5ml with deionized water.

The incubation was carried out at 37^{O}C for 30 min. in a water-bath. This assay of monoamine oxidase activity is by observing the rate of serotonin disappearance. It utilizes a direct spectrophotometric method read against a blank cuvette prepared by replacing serotonin with water. Increase in absorbance is measured at 330nm using DU - 7400 Beckman spectrophotometer expressed as the change in the absorbance at 330nm per unit of time.

Determination of protein:

The protein determination was performed according to the method of Lowry *et al* (17).

Determination of serotonin in concentration in rat brain:

Brain serotonin concentrations in rats treated with or without ethanol or bromazepan on the first and the 14th day of drug administration were determined by methods of Udenfriend *et al* (18) and Ebuehi and Akinwande (19).

Statistical analysis:

Data were subjected to analysis of variance (20). Significant differences were further tested by the Duncan's Test (21).

RESULTS

The body weights, feed intake, water or alcohol intake of rats fed rat chow with 5% (v/v) ethanol *ad libitum*, rat chow with water *ad libitum* and an oral administration of 2ml of 5% (v/v) ethanol per 100g body weight, rat chow with water *ad libitum* and an intra-peritoneal administration of 0.1mg/ml bromazepam per 100g body weight, rat chow with water *ad libitum*; for 14 days are presented in Figures 1, 2 and 3.

The results obtained showed that the body weights of rats treated with or without drug increases linearly with days of drug administration. However, the increase in body weight with days of drug administration was significantly ($P < 0.01$) higher in rats fed chow with water and an oral administration of 5% ethanol or rats fed chow with water *ad libitum* than in rats fed chow with 5% ethanolor chow with water *ad libitum* and an intra-peritoneal administration of bromazepam (Figure 1).

Results of the feed intake of rats showed that there was an increase in the feed intake of all the rats treated with or without drugs during the duration of drug administration. However, the increase in feed intake with days of drug administration was significantly ($P<0.01$) higher in rats fed chow with water and an intra-peritoneal administration of bromazepam of chow with water *ad libitum* than in rats fed chow with water, and an oral administration of 5% ethanol or chow with 5% ethanol for 14 days (Figure 2).

The water or alcohol intake of rats showed that there was an increase in the water or alcohol intake of all rats treated with or without drug and with no remarkable change in water intake of rats fed chow with water and bromazepam, during the duration of drug administration. However, there was a significant ($P<0.01$) increase in water intake of the rats fed chow with water than in other rats treated with drugs (Figure 3).

The absorbance at 330nm of the brain assay mixture containing monoamine oxidase in rats fed chow with water, 5% ethanol and 0.1mg bromazepam per 100g body weight of rats is presented in Table 1. There was a linear increase in absorbance at 330nm in rats fed chow with water with the time of incubation. However, significant increase in the absorbance at 330nm in rats treated with ethanol or bromazepam within 10min. of incubation, were reported, which later slightly decrease or plateaus between 10 - 30min. of incubation of rat brain assay mixture containing monoamine oxidase.

Changes in monoamine oxidase activity in rats fed chow with 5% ethanol, water and 5% ethanol and bromazepam for 14 days is presented in Table 2. Results obtained showed a decrease in the specific activity of monoamine oxidase in rat brain of all the rats treated with or without drugs. However, a gradual decrease in monoamine oxidase activity in rats fed chow with water was reported unlike the sharper decrease in monoamine oxidase activity of the other rats treated with drugs.

Table 1: Absorbance measurement at 330nm of the brain assay mixture.

Time of incubation (min)	Rat chow with 5% (v/v) ethanol	Rat chow with water and 5% (v/v) ethanol	Rat chow with water and 0.1 mg/ml bromazepam	Rat chow with water
0	0.0178 + 0.0034	0.0525 + 0.0031	0.0690 + 0.0035	0.0634 + 0.0040
10	0.1419 + 0.0045	0.1816 + 0.0044	0.1907 + 0.0036	0.1803 + 0.0061
20	0.1485 + 0.0082	0.1305 + 0.0036	0.1855 + 0.0061	0.2975 + 0.0054
30	0.1386 + 0.0067	0.1236 + 0.0052	0.1857 + 0.0079	0.3493 + 0.0082

*Values are expressed as means ± S.E. Absorbance measurement at 330nm of the brain assay mixture containing monoamine oxidase in rats fed rat chow with 5% (v/v) ethanol, rat chow with water and an oral administration of 2ml of 5% (v/v) ethanol per 100g body weight, rat chow with water and an intra-peritoneal administration of 0.1mg/ml bromazepam per 100g body weight and rat chow with water *ad libitum* for 14 days.

Table 2: Specific activity of monoamine oxidase in rat brain.

Time of incubation (min)	Rat chow with 5% (v/v) ethanol	Rat chow with water and 5% (v/v) ethanol	Rat chow with water and 0.1 mg/ml bromazepam	Rat chow with water
10	0.120 ± 0.004^{a}	0.148 ± 0.005^{b}	0.225 ± 0.007^{c}	0.404 ± 0.005^{d}
20	0.074 ± 0.005^{e}	0.134 ± 0.003^{b}	0.218 ± 0.005^{c}	0.405 ± 0.006^{d}
30	0.033 ± 0.006^{f}	0.083 ± 0.004^{g}	0.154 ± 0.006^{h}	0.332 ± 0.004^{i}

Values are presented as means ± S.E. and expressed as micromoles/60min./mg protein. Values carrying different superscripts horizontally or vertically are significantly different ($P<0.01$).
Changes in monoamine oxidase activity in rats fed rat chow with 5% (v/v) ethanol, rat chow with water and an oral administration of 2ml of 5% (v/v) ethanol per 100g body weight, rat chow with water and an intra-peritoneal administration of 0.1mg/ml bromazepram per 100g body weight and rat chow with water *ad libitum* for 14 days.

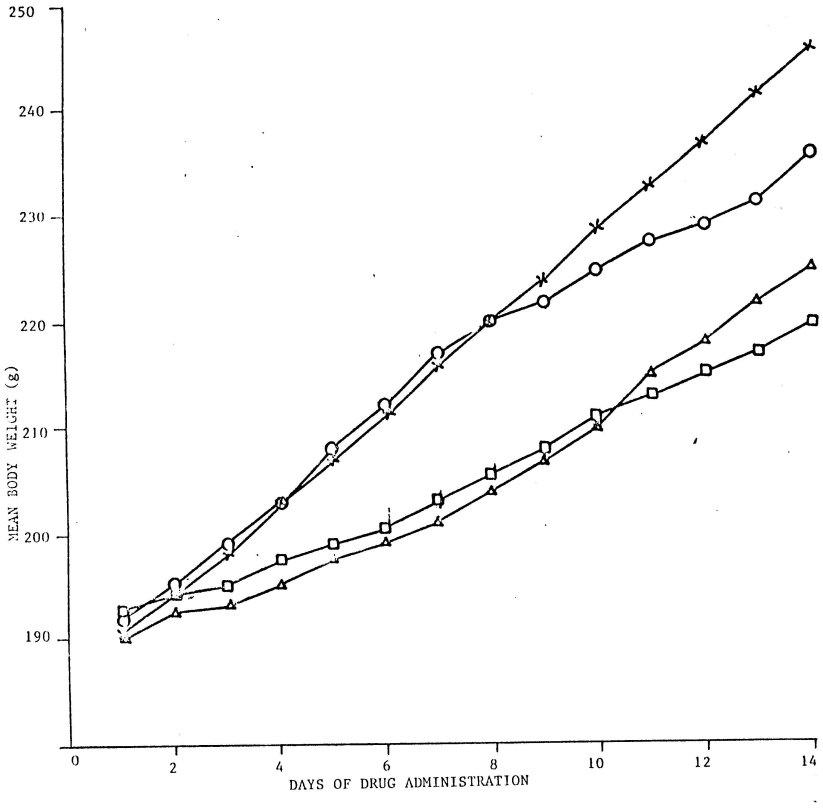

Fig. 1: Mean body weight of rats fed chow with 5% ethanol (□—□), chow with water and an oral administration of 5% ethanol (x—x); chow with water and an intra-peritoneal administration of bromazepam (Δ—Δ); and chow with water (O—O); for 14 days.

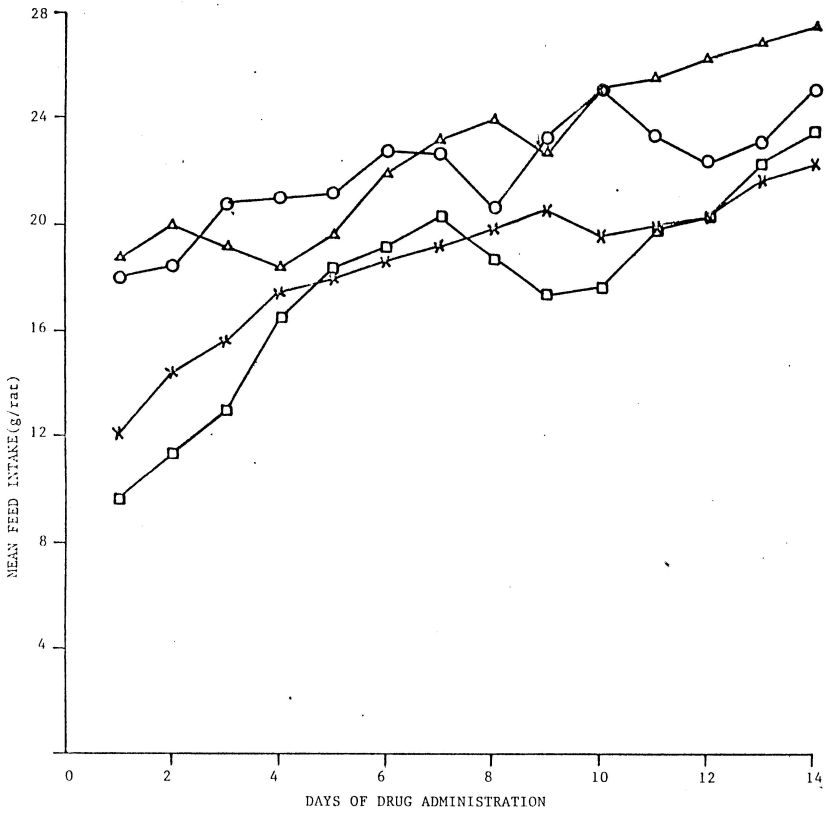

Fig. 2: Mean feed intake of rats fed chow with 5% ethanol (□—□), chow with water and an oral administration of 5% ethanol (x—x); chow with water and an intra-peritoneal administration of bromazepam (Δ—Δ); and chow with water (O—O); for 14 days.

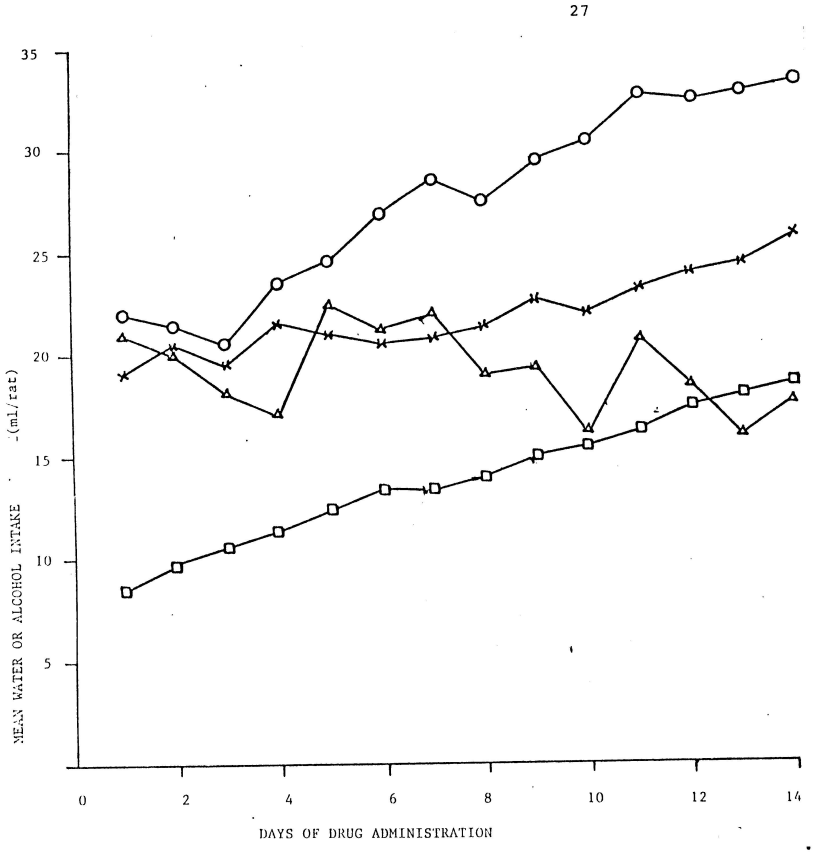

Fig. 3: Mean water and alcohol intake of rats fed chow with 5% ethanol (□—□), chow with water and an oral administration of 5% ethanol (×—×); chow with water and an intraperitoneal administration of bromazepam (Δ—Δ); and chow with water (○—○); for 14 days.

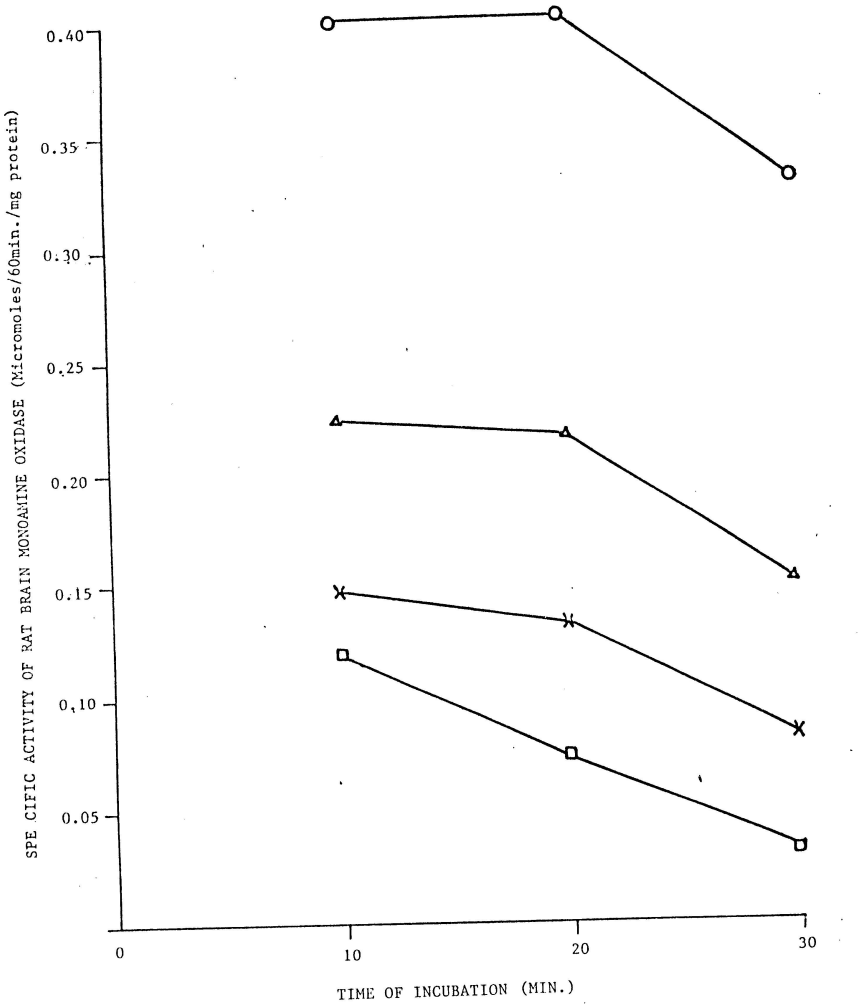

Fig. 4: Changes in monoamine oxidase activity in rats fed chow with 5% ethanol (□—□), chow with water and an oral administration of 5% ethanol (x—x); chow with water and an intra-peritoneal administration of bromazepam (Δ—Δ); and chow with water (O—O); for 14 days.

Table 3: Brain serotonin concentration (mg/g tissue).

Days of drug administration	Rat chow with 5% (v/v) ethanol	Rat chow with water and 5% (v/v) ethanol	Rat chow with water and 0.1 mg/ml bromazepam	Rat chow with water
1*	0.64 ± 0.04^{a}	0.64 ± 0.04^{b}	0.64 ± 0.04^{a}	0.64 ± 0.04^{a}
14	0.74 ± 0.08^{b}	0.70 ± 0.06^{c}	0.68 ± 0.03^{a}	0.63 ± 0.06^{a}

Brain Serotonin concentration in rats fed chow with 5% (v/v) ethanol, rat chow with water and an oral administration of 2ml of 5% (v/v) ethanol per 100g body weight, rat chow with water and an intraperitoneal administration of 0.1mg/ml bromazepram per 100g body weight and rat chow with water *ad libitum* for 14 days.

*Represents the day of commencement of drug administration. Values carrying different superscripts horizontally or vertically are significantly different ($P<0.01$).

The concentration of brain serotonin in rats fed rat chow with water, 5% ethanol and 0.1mg bromazepam per 100g body weight of rat is represented in Table 3. There was a significant ($P<0.01$) increase in the brain serotonin concentration in rats fed chow with 5% ethanol or water and ethanol intra-peritoneally. Rats fed chow with water and 0.1mg bromazepam had a slight increase in their brain serotonin concentration; but there was no change in brain serotonin concentration in rats fed chow with water for 14 days.

DISCUSSION

The effect of acute and chronic administration of ethanol, and of bromazepam on the monoamine oxidase activity in rat brain was investigated. There was a linear increase in the body weight of all the rats treated with or without ethanol or bromazepam for 14 days. Results of body weight of rats indicate that the administration and dosage of 5% ethanol and 0.1mg bromazepam/100g body weight do not affect body growth. However, rats fed chow with water and an oral administration of 5% ethanol or chow with water *ad libitum* had a significantly higher body weight. This may suggest that chronic administration of 5% ethanol and 0.1mg bromazepam/100g body weight impair body weight within 14 days of administration.

This finding corroborates the results of the feed intake of rats, which increased in all rats treated with or without drug. One could infer that the administration of 5% ethanol or 0.1mg bromazepam/100g body weight of rat, did not inhibit feed intake. In addition, the water intake of rats increased in all the rats treated with or without drug, with the days of administration, which also suggest that these drugs do not affect water consumption.

Data obtained for the changes in brain monoamine oxiadase activity of rats treated with or without drug, indicate that there was inhibition to varying degrees by 5% ethanol (in both acute and chronic administration) and 0.1mg bromazepam per 100g body weight. This inhibition varies from mild as in bromazepam injected rats to strong inhibition (in chronic alcohol treated rats) of the monoamine oxidase activity in the brain. Our findings agree with previous reports by Pletscher (22) and Eadie (230) on monoamine oxidase inhibitors.

Kashimoto and Armstrong (24) reported that rabbits given monoamine oxidase inhibitors showed an increase in precursor compounds involved in the synthesis of various neurotransmitters in brain tissues. The increase in these compounds (for example tryptophan for serotonin) suggests the possibility of change in the

pathway of tryptophan metabolism. Chronic ethanol consumption in rats results is striking structural and functional alterations in liver and other organs (25) . Functional consequences of this disruption are probably mediated by proteins such as monoamine oxidase in the brain.

In the current study, we sought to ascertain whether or not ethanol or bromazepam can affect the activity of monoamine oxidase in the brain, and our results indicate that ethanol and bromazepam inhibit monoamine oxidase activity. This finding was further corroborated with the results of serotonin concentration in rat brain treated with or without the drugs. For instance, rats exposed to chronic ethanol consumption had a significantly ($P<0.01$) lower brain serotonin concentration than in rats treated with bromazepam for 14 days. Data suggest that the inhibition of monoamine oxidase resulted in increased brain serotonin concentration, since serotonin is a substrate of monoamine oxidase. It could be implied that enzyme-substrate complex formation was impaired by the inhibition of monoamine oxidase, since its activity was reduced due to its dependence in forming the active complex. It is also indicative of a decrease in the formation of biogenic aldehydes as a result of the decrease in monoamine activity.

Evidence on the effect of ethanol on serotonin has been circumstantial and contradictory (26, 27). Some investigators reorted decreases (28, 29) and others have found increases (30, 31) while a large number have reported no change in serotonin level (32). But very scanty or no information exists on the
effect of bromazepam on monoamine oxidase and serotonin.

Additionally, a conflicting data also prevail on the effect of ethanol on monoamine oxidase activity, while some workers report a decrease in activity, others indicate an increase since monoamine oxidase oxidatively deaminates serotonin. It could be suggested that a degradation of serotonin will result in less of it being available for release in response to axon depolarization (23). Therefore, if monoamine oxidase is inhibited the serotonin concentration will increase. Monoamine oxidase is inhibited the serotonin concentration will increase. Monoamine oxidase inhibitors currently in widespread clinical use are non-specific, for example, phenelzine, tranylcypromine and pargyline. They raise central synaptic catecholamine and serotonin concentrations, and thus help in the therapy of depression and some phobic states. Monoamine oxidase inhibitors produce adverse effects mostly related to the nervous system, such as agitation, insomnia, heightened anxiety and tremor. However, some of the behavioural characteristics were observed in the rats used for this study, for instance the chronic, ethanol treated rats were sleeping most of the time, while the acute alcohol treated rats were more hyperactive.

Ethanol is a non-therapeutic drug which acts as a general CNS depressant, by the inhibition of calcium channel opening and enhancement of GABA - action (7). On the other hand, bromazepam is a powerful psychotropic agent, which acts by binding to a specific regulatory site on the GABA - receptor, thus enhancing the neuronal inhibitory effect of GABA (13).

The study of the activity of monoamine oxidase in the brain of mammals require the isolation of the brain tissue mitochondria fraction. This is necessary in order to allow for a proper assessment and observation of the monoamine oxidase activity. Serotonin, a neurotransmitter synthesized from tryptophan (33) is a major substrate of monoamine oxidase in the mammalian brain. Therefore, the rate of monoamine oxidase activity can be determined by measuring the rate of disappearance of this protein in the tissue in question, or the rate of formation of the product of the reaction; 4 - hydroxyquinoline (15). The pharmacological action of monoamine oxidase inhibitors is central stimulation, and effect which coincides with increased brain neurotransmitter levels such as norepinephrine (7, 34). This is shown to be linked to the relief of mental depression and may benefit manic depressives by relaxing the nervous and muscular systems of the body. This finding corresponds to the known effects of ethanol and bromazepam ingestion in the body, and supports their role as monoamine oxidase inhibitors as found in

this study; albeit in varying degrees of inhibition by ethanol and bromazepam.

It is concluded that the results of this study indicate that ethanol and bromazepam inhibits monoamine oxidase in rat brain. However, there is still room for growth in the application of these findings for the treatment of disorders associated with neurotransmitter regulation by monoamine oxidase in the brain.

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