Title: Effects of Jobelyn® on isoniazid-induced seizures, biomarkers of oxidative stress and glutamate decarboxylase activity in mice

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Abstract

Introduction: Isoniazid-induced seizure, often described as *status epilepticus* (SE), is an emergency condition, which is characterized by repeated convulsive episodes and responds poorly to currently available anti-convulsant drugs. This present investigation was designed to ascertain the efficacy of Jobelyn®, an African dietary supplement on seizures, altered oxidative stress and glutamate decarboxylase activity induced by isoniazid in mice.

Methods: Mice (n = 6) received oral doses of JB (10-50 mg/kg), pyridoxine (300 mg/kg), diazepam (5 mg/kg) or distilled water (10 mL/kg) 30 min prior induction of SE with injection of isoniazid (300 mg/kg, i.p.). Thereafter, the mice were then observed for the onset of convulsions for a period of 2 h. Moreover, the effect of JB on glutamate decarboxylase (GAD) activity and biomarkers of oxidative stress [glutathione (GSH) and malondialdehyde (MDA)] were also evaluated in the brain homogenates of another set of isoniazid-treated mice.

Results: JB (50 mg/kg, p.o) prolonged the latency to convulsions but could not prevent the occurrence of seizure episodes produced by isoniazid. Moreover, JB did not show any protection against death nor delay the latency to death caused by isoniazid. The concentrations of MDA and GSH in the brains of mice treated with isoniazid were positively modulated by this dose of JB. The activity of GAD, the enzyme that is responsible for GABA synthesis was increased by JB, which suggest enhanced GABAergic neurotransmission.

Discussion: These findings suggest that JB prolonged the latency to convulsions, enhanced GABAergic neurotransmission and also demonstrated anti-oxidative effect in isoniazid-treated mice.

Keywords: Jobelyn®, Isoniazid, Convulsion, Glutamate decarboxylase, Oxidative stress

1. Introduction

Status epilepticus (SE) is a life-threatening medical condition that is often associated with persistent seizures and recurrent in nature (Bassin, Smith, & Bleck, 2002; Brophy, Bell, & Claassen 2012; Meierkord, Boon, & Engelsen, 2010). It accounts for about 3-5% of all cases of emergency admitted for seizure disorders and also occurred in 2-16 % of individuals with epileptic disorders (Hauser 1990). The incidence of SE was estimated to be 41-61 per 100,000 patients per year (Bronstein et al., 2010; DeLorenzo, Towne, Pellock, & Ko, 1992). Isoniazid, anti-tuberculosis drug, is known to induce SE by depleting brain level of gamma-animobutytic acid (GABA), a major inhibitory transmitter substance in the mammalian brain through inhibition of pyridoxal-5-phosphate-dependent glutamic acid decarboxylase (GAD) (Corda, Costa, & Guidtti, 1982; Uzman et al., 2013). Pyridoxal-5-phosphate is the active form of pyridoxine, a cofactor for GAD, an enzyme required for GABA synthesis (Bassin, Smith, & Bleck, 2002; Brophy and Claassen 2012; Meierkord, Boon, & Engelsen, 2010; Corda, Costa, & Guidtti, 1982; Uzman et al., 2013). The decrease in GABA levels results in recurrent seizures that characterized SE (Corda, Costa, & Guidtti, 1982; Uzman et al., 2013). Although, isoniazid-induced seizure is known to respond poorly to currently available anticonvulsant drugs, intravenous diazepam is still being used to control the seizure episodes in the absence of pyridoxine (Corda, Costa, & Guidtti, 1982; Uzman et al., 2013; Tajender & Saluja 2006; Romero & Kuczler 1998). On this basis, diazepam and pyridoxine serving as reference drugs were utilized for the purpose of comparison with our test substance in this study. However, pyridoxine has been reported as the only effective antidote for isoniazid toxicity and must be given in doses equivalent to the amounts of isoniazid ingested for it to be effective (Uzman et al., 2013; Tajender & Saluja 2006; Romero & Kuczler 1998). But, in a situation where the quantity of isoniazid ingested is unknown, intravenous administration of an initial dose of 5 g of pyridoxine has been recommended in literature (Uzman et al., 2013; Tajender & Saluja 2006). Although intravenous pyridoxine is relatively inexpensive but its immediate availability in sufficient amount in emergency remains uncertain (Tajender & Saluja 2006; Romero & Kuczler 1998). Thus, there is a need to search for new compounds that could serve as alternatives for isoniazid toxicity.

The recurrent seizures that typified SE have been linked to increased oxidative stress that create a vicious cycle for neurodegeneration and manifestation of other neurological complications including memory deficits (Cicek et al., 2005; Adebayo et al., 2012). The oxidative damage due to isoniazid has been shown to be associated with the generation of reactive oxygen species, which initiate lipid peroxidative tissue damage (Cicek et al., 2005; Adebayo et al., 2012; Ahadpour et al., 2016; Cevik et al., 2012). It also depletes endogenous antioxidant status of the cells thereby making the body organs more prone to the deleterious effect of oxidative stress (Adebayo et al., 2012; Ahadpour et al., 2016; Cevik et al., 2012). However, the brain is more susceptible to oxidative stress damage because of high content of oxidizable fatty acids, high demand of oxygen and low levels of antioxidant status (Moreira et al., 2008). Thus, the combination of INH with a potent antioxidant agent like jobelyn® (Benson et al., 2013) may help to reduce brain damage and thus, prevent the manifestations of various neurological complications associated with isoniazid neurotoxicity.

Jobelyn® (JB) is a dietary supplement obtained from *Sorghum bicolor* L. (Gramineae) that has won international recognition for anemia, arthritis and relief of stress (Benson et al., 2013; Okochi, Okpuzor, Okubena, & Awoyemi, 2003). The most active compounds in JB include apigenin, luteolin and naringenin, which have been shown to possess various biological activities (Benson et al., 2013; Umukoro et al., 2015). Previous investigations have revealed that JB has potent antioxidant and anti-inflammatory properties (Benson et al., 2013; Umukoro et al., 2015). Moreover, JB was listed as one of the herbal remedies used by the populace for the management of febrile seizures (Oshikoya, Senbanjo, Njokanma, & Soipe, 2008). We have previously established that JB prolonged the latency to convulsions induced by pentylenetetrazole in mice (Umukoro, Omogbiya, & Eduviere, 2013), which suggests its possible effectiveness in controlling convulsive episodes (Umukoro, Omogbiya, & Eduviere, 2013). However, this study was carried out to investigate the possible protective effect of JB against INH-induced convulsion, altered oxidative stress and glutamate decarboxylase activity in mice.

2. Materials and Methods

2.1. Laboratory Animals

Male Swiss mice (20-22 g) used in the study were purchased from the Central Animal House, University of Ibadan, Nigeria. They were housed in plastic perplex cages at room temperature, had free access to rodent pellet diet and water *ad libitum*. The experimental procedures were carried out in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs and Chemicals

Jobelyn® (JB) (Forever Product Lagos, Nigeria), isoniazid (Hamex Medica Ltd., UK), diazepam (Swipha Pharma, UK), trichloroacetic acid-TCA (Burgoyne Burbidges & Co., Mumbai, India), thiobarbituric acid-TBA (Sigma, Germany), 5,5□dithio-bis(2-nitrobenzoicacid)□DTNB (Sigma Aldrich, Germany), glutamate (Sigma Aldrich, Germany) and pyridoxine (Pauco Pharamceutical Industry, Nigeria) were used in the study. Jobelyn® and other drugs were dissolved in distilled water immediately before use. The doses of JB were chosen based on previous studies (Umukoro, Omogbiya, & Eduviere, 2013).

2.3. Effect of Jobelyn® on the onset of seizure

The effect of JB on INH-induced convulsion was assessed as earlier described (Corda, Costa, & Guidtti, 1982). The animals were divided into different treatment groups (n = 6) and were given distilled water (10 mL/kg) and JB (10, 25, 50 mg/kg) orally. Thirty minutes later, mice were given isoniazid (300 mg/kg) intraperitoneally and were then observed for 2 h for latency to convulsion and death.

2.4. Biochemical studies

New set of animals were pretreated with JB (10, 25, 50 mg/kg), diazepam (5 mg/kg) or pyridoxine (300 mg/kg) 30 min prior to intraperitoneal injection of isoniazid (300 mg/kg). Thereafter, the mice were sacrificed 30 min post-isoniazid injection through cervical dislocation under ether anaesthesia. The brains were removed, weighed and kept in 10% w/v phosphate buffer (0.1M pH 7.4). The whole brain were then homogenised in 10% w/v phosphate buffer and the supernatants were used for the biochemical studies.

2.4.1. *Determination of glutathione (GSH) concentration*

The brain level of reduced GSH was determined as earlier described (Moron, Depierre, & Mannervik, 1979). Briefly, the supernatant (0.4 mL) of the brain tissues was added to 20% trichloroacetic acid (0.4 mL) and then centrifuged at 10,000 rpm for 20 min at 4°C. This mixture (0.25 mL) was added to 2 mL of 0.6 mM DTNB and the final volume was made up to 3 mL with phosphate buffer (0.2M, pH 8.0). The absorbance was then read at 412 nm against blank reagent using a spectrophotometer. The brain levels of reduced GSH were expressed as micromoles per gram tissue (□mol/g tissue).

2.4.2. *Estimation of brain concentration of malondialdehyde*

MDA concentrations in the brain supernatants were determined as previously described (Ohkawa, Ohishi, & Yagi, 1979). Briefly, distilled water (0.5 mL) and 10% TCA (1.0 mL) were added to 0.5 mL of each homogenate of the brain tissues. This mixture was then centrifuged at 3000 rpm for 10 min and 0.1 mL of thiobarbituric acid (0.375%) was added to 0. 4 mL of the supernatant. This mixture was then incubated in a water bath at 80°C for 40 min. After cooling, the absorbance of the supernatant was read at 532nm using a spectrophotometer. The brain levels of MDA were expressed as □mol/g tissue.

2.4.3. *Determination of glutamate decarboxylase activity*

Brain glutamate decarboxylase activity was determined as earlier described (Cozzani 1970). The brain supernatant (1 mL) was adjusted to pH 7 and then incubated at 37°C for a period of 5 min. Thereafter, the reaction was started by adding 100 □L glutamate (10 mM) and decarboxylation of glutamate was measured at 340 nm against a blank having all the components except glutamate using spectrophotometer (Cozzani 1970).

2.5. Statistical Analysis

Data were analysed using Graph Pad Prism software, version 4.0 and expressed as mean □ S.E.M. Statistical analysis was carried out using one-way ANOVA followed by Newman-keuls post- hoc test. P-values less than 0.05 were considered statistically significant.

3. Results

3.1 Jobelyn® prolongs latency to seizure

As shown in Table, i.p. injection of isoniazid (300 mg/kg) produced 100% convulsion and death, as all the animals convulsed and died within the experimental period. However, JB (50 mg/kg, p.o) prolonged the latency to seizure episodes ($p < 0.05$) but failed to prevent occurrence of seizure episodes or death (Table 1). In contrast, diazepam but not pyridoxine offered 30% protection against convulsions and death in isoniazid-treated mice (Table 1).

3.2. Jobelyn® reduces isoniazid-induced increased oxidative stress in mouse brain

Figure 1A and B showed the effect of JB on the brain MDA and GSH levels in isoniazid-treated mice. Isoniazid (300 mg/kg, i.p) altered the concentrations of these biomarkers of oxidative stress relative to control ($p < 0.05$). As presented in Fig. 1A and B), JB (10, 25, 50 mg/kg, p.o) or diazepam (5 mg/kg, p.o) attenuated the altered MDA and GSH levels in the brains of mice treated with isoniazid ($p \square 0.05$).

3.3. Effect of Jobelyn® on glutamic acid decarboxylase activity in mice brains

The effect of JB on isoniazid-induced decrease in glutamic acid decarboxylase (GAD) activity is presented in Figure 2. Jobelyn (50 mg/kg, p.o) but not diazepam nor pyridoxine significantly attenuated isoniazid-induced decrease in brain GAD activity in mice.

4. Discussion

The results of this study revealed that JB did not prevent the occurrence of seizures induced by isoniazid in mice. However, the highest dose of JB delayed the onset of seizures in a significant manner. In contrast, diazepam but not pyridoxine offered 30% protection against convulsions and death in isoniazid-treated mice. Moreover, JB and diazepam decreased the brain concentrations of MDA and increased the contents of GSH suggesting antioxidant property. However, JB but not diazepam nor pyridoxine produced a significant increase in GAD activity, which suggest its ability to elevate GABA levels in the brains of mice treated with isoniazid.

Status epilepticus (SE) is one of the key features of isoniazid poisoning or overdose and it is known to be refractory to conventional anticonvulsant drugs (Bassin, Smith, & Bleck, 2002;

Brophy, Bell, & Claassen, 2012; Meierkord, Boon, & Engelsen, 2010). SE induced by isoniazid has been shown to be related to inhibition of GAD, an enzyme required for GABA synthesis (Bassin, Smith, & Bleck, 2002; Bronstein et al., 2010; Corda, Costa, & Guidtti, 1982; Cevik et al., 2012). Thus, the depletion of brain GABA levels is known to be associated with continuous seizures seen in animals exposed to high dose of isoniazid. Treatment approach for INH toxicity involves intravenous injection of pyridoxine, an essential co-factor required for GABA synthesis ((Bassin, Smith, & Bleck, 2002; Brophy, Bell, & Claassen, 2012; Meierkord, Boon, & Engelsen, 2010. However, the uncertainty of the amount of INH ingested and its immediate availability are often the major limitations to the usefulness of pyridoxine in managing INH toxicity in clinical settings (Minns, Ghafouri, & Clark, 2010). In this study, the reasons as par why pyridoxine unlike diazepam could not prevent nor delay INH-induced convulsions in mice were not apparent in this investigation except that previous studies have shown that pyridoxine was ineffective against INH-induced seizures in mice (Bonner, 1999). Moreover, the reason (s) why pyridoxine protected rats but not mice against convulsions induced by isoniazid was not also obvious from the study of Bonner, (1999).

Previous studies have shown that anticonvulsant effect of a novel agent does not absolutely depend on prevention of convulsions but also based on its ability to prolong the latency to seizures (Kendall, Fox, & Enna, 1981). Moreover, compounds that only delay the latency to convulsions have been found to halt the spread of seizures in an epileptic brain (Corda, Costa, & Guidtti, 1982). Thus, the finding that JB prolonged the latency to isoniazid-induced convulsions suggests anticonvulsant potentials in mice. The finding that JB also increased brain activity of GAD, an enzyme responsible for the synthesis of GABA suggests its ability to elevate GABA levels in the brain. It is instructive to note that reduced brain GABA concentrations have been implicated in the pathophysiology of convulsion (McNamara, 1994). Thus, increasing the brain levels of GABA is one of the major therapeutic approaches for the treatment of patients with seizures (McNamara, 1994). Nevertheless, more studies are necessary to establish the role of GAD in the anticonvulsant potential of JB in INH-induced convulsions in mice.

Increased oxidative stress and subsequent manifestation of other neurological complications have been shown to be associated with isoniazid toxicity (Cicek et al., 2005; Adebayo et al., 2012; Ahadpour et al., 2016; Cevik et al., 2012). Isoniazid-induced oxidative tissue damage has been reported to be linked to the formation of reactive oxygen species, which in turn initiate lipid peroxidation in most organs of the body especially the liver and the brain (Cicek et al., 2005; Adebayo et al., 2012; Ahadpour et al., 2016; Cevik et al., 2012). It also depletes endogenous antioxidant status of the cells thereby exposing body organs to the deleterious effect of oxidative stress (Adebayo et al., 2012; Ahadpour et al., 2016; Cevik et al., 2012). The brain cells however, are known to more prone to deleterious effect of oxidative stress because of high content of \ oxidizable fatty acids, high oxygen demand and low levels of antioxidant molecules (Moreira et al., 2008). In recent years, a great number of natural products are being sought from plants for the mitigation of oxidative stress-mediated pathologies (Mahmoud, Germoush & Soliman, 2014). For example, berberine, an alkaloid found in variety of plants has been shown to protect the liver against isoniazid-induced hepatotoxicity via inhibition of oxidative stress (Mahmoud, Germoush & Soliman, 2014). Also, several phytochemicals have been reported to demonstrate neuroprotection and are still being evaluated as potential therapeutic agents for neurological diseases (Uttara et al., 2009; Natalie et al., 2010). Moreover, previous preclinical investigations have revealed that JB has antioxidant and neuroprotective properties in various experimental models (Benson et al., 2013; Oyinbo, Dare, Avwioro, & Igbigbi, 2015). Meanwhile, Jobelyn® also demonstrated antioxidant property in this present studies as it attenuated isoniazid-induced increase in brain concentrations of MDA, a well known marker of oxidative stress implicated in lipid perioxidative tissue damage. This finding is in agreement with the reports of previous studies (Oyinbo, Dare, Avwioro, & Igbigbi, 2015) and further suggests that JB supplementation may protect the neurons against isoniazid-induced neurotoxicity. On the other hand, isoniazid-treated mice had a significant decrease in brain GSH content. Glutathione is a key endogenous antioxidant agent that protects cellular constituents against the harmful effect of oxidative stress by scavenging ROS (Franco et al., 2007). However, pretreatment with JB inhibited depletion of GSH suggesting its ability to scavenge ROS generated by isoniazid in mice brains. Thus, it may be assumed that the antioxidant effect exhibited by JB suggest that it has potential benefits in preventing neurological complications associated with isoniazid therapy.

Conclusion

The results of this study suggest that increased GAD activity might contribute to the ability of Jobelyn® to prolong the latency to convulsions induced by isoniazid. Our data also suggest that JB supplementation might be useful as a potential antioxidant agent for preventing oxidative stress-induced neurological complications associated with anti-tuberculosis therapy with isoniazid.

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Table 1. Effect of Jobelyn (JB), diazepam (DZ) and pyridoxine on isoniazid-induced seizure episodes in mice

Treatment	Onset of Seizure (min)	Seizure (%)	Latency to death (min)	Death (%)
Control	48.67□1.687	100	53.83□1.302	100
JB(10mg/kg)	48.17□1.493	100	51.50□3.085	100
JB(25mg/kg)	52.67□3.518	100	58.50□3.008	100
JB(50mg/kg)	62.33□3.870*	100	62.50□4.552	100
DZ(5mg/kg)	85.17□2.072*	70	88.83□10.95*	70
Pyridoxine (300mg/kg)	35.83□2.496*	100	35.67□2.565	100

Values represent mean □S.E.M for 5 animals per group. *P□0.05 compared to control (ANOVA followed by Newman Keul test).

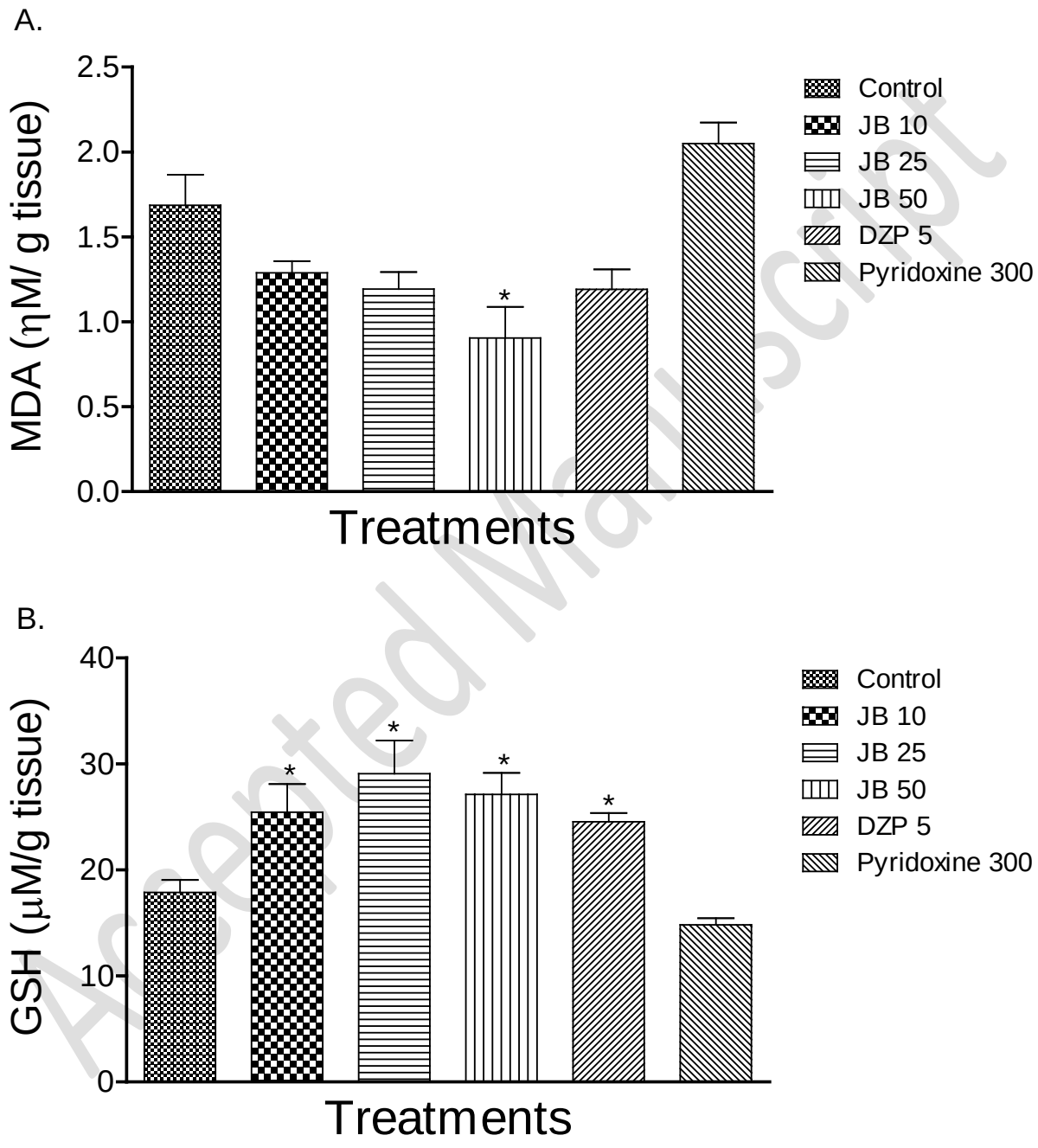

Figure 1: Effect of Jobelyn (JB), diazepam (DZ) and pyridoxine on the brain levels of malondialdehyde (MDA, panel A) and glutathione (GSH, panel B) in isoniazid-treated mice.

Each column represents mean ☐S.E.M for 6 animals per group. *P* ☐0.05 compared to control (ANOVA followed by Newman Keuls test).

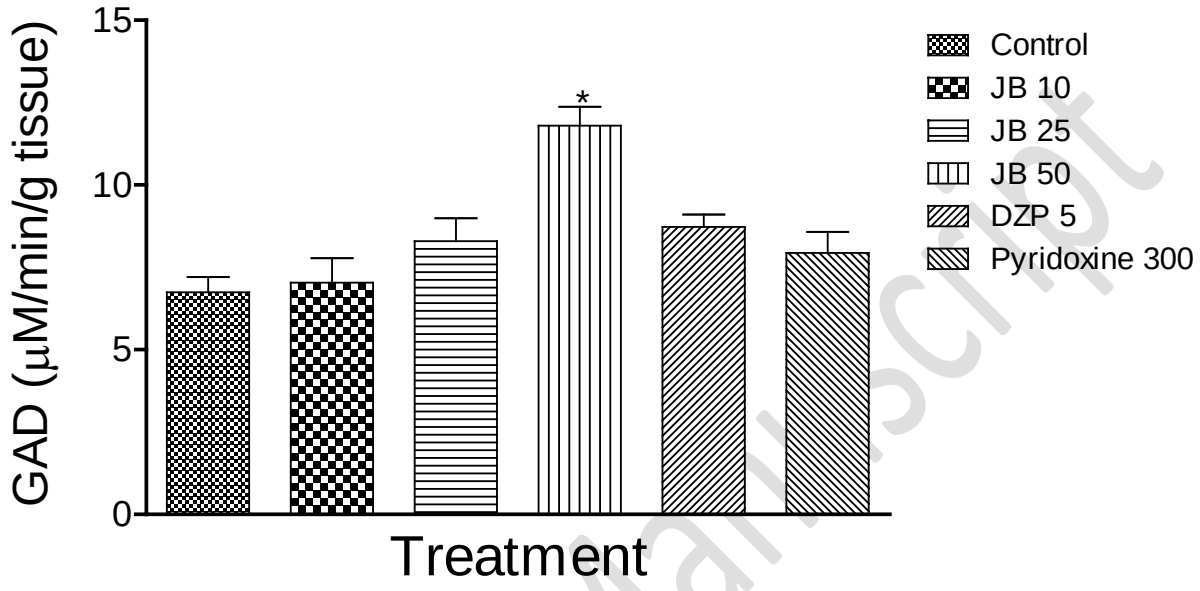

Figure 2: Effect of Jobelyn (JB), diazepam (DZ) and pyridoxine on glutamate decarboxylase activity. Each column represents mean ☐S.E.M for 6 animals per group. P☐0.05 compared to control (ANOVA followed by Newman Keuls test).