



International Journal of Pharmacy and Natural Medicines

CODEN (USA): IJPNRC | ISSN: 2321-6743

Journal Home Page: www.pharmaresearchlibrary.com/ijpnm



RESEARCH ARTICLE

Adenopus Breviflorus Restores Glucose-6-Phosphate Dehydrogenase-Dependent Antioxidant Suppression caused by Clozapine in Female Wistar Rats

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ABSTRACT

Oxidative damage is an important feature of cardiometabolic syndrome. Clozapine (CLO) is a typical antipsychotic medication that alters protein and lipid metabolism with consequent oxidative stress. The G6PD-dependent antioxidant barrier is an important cellular defense against oxidative damage. Nevertheless, Adenopus Breviflorus (AEAB) has been shown to contain beneficial antioxidant phytochemicals and inhibits mitochondrial lipid peroxidation. This study therefore aimed at testing AEAB fruit aqueous extract antioxidant property in CLO-induced redox imbalance. Twenty male Wistar rats weighing between 135-140 g were used for the experiment. They were grouped into Control (CTR; Standard feed and Water), Clozapine only (CLO; 25mg/kg of Clozapine), Extract only (AEAB; 200mg/kg of AEAB) and Clozapine +Extract (CLO+AEAB; 25 mg/kg of Clozapine for first twenty-eight days and AEAB for the last fourteen days), where n=5. All administrations were done orally, and the experiment lasted for forty-two days after a week of acclimatization. The animals were euthanized with 0.2ml of ketamine hydrochloride, blood samples were collected measured for plasma G6PDH, Catalase, Sodium Dismutase, NADPH, GPX, and GSSG using standard spectrophotometric method. The results showed that CLO reduced plasma G6PD, NADPH, GPx, and cardiac NADPH. There was a slight reduction in cardiac G6PD, SOD, GSSG, and GPx with CLO treatment. However, AEAB reversed the alterations caused by CLO treatment in the heart and plasma of animals. AEAB elicits considerable antioxidant activity by limiting the oxidant effects of CLO in the plasma, heart, and plausibly other tissues. AEAB may be considered for therapy in the treatment of oxidative stress-related diseases.

Keywords: Adenopusbreviflorus, Clozapine, Antioxidant, Oxidative damage, Anti-inflammatory

ARTICLE INFO

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ARTICLE HISTORY: Received 05 February 2020, Accepted 25 April 2020, Available Online 15 June 2020

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Citation: Okesina Akeem Ayodeji, et al. Adenopus Breviflorus Restores Glucose-6-Phosphate Dehydrogenase-Dependent Antioxidant Suppression caused by Clozapine in Female Wistar Rats. *Int. J. Pharm. Natural Med.*, 2020, 8(1): 21-24.

CONTENTS

1. Introduction. 22

2. Materials and Methods	22
3. Results and Discussion	23
4. Conclusion.	24
5. References.	24

1. Introduction

Clozapine has been used to treat severe schizophrenia symptoms in people that have not responded to other medications.[1] Clozapine can cause Drug-Induced Metabolic Syndromes (DIMS), which may be useful in cellular and molecular changes related to both Metabolic Syndrome (MetS) and Drug-Induced Metabolic Syndromes,[2] in Glucose-6- phosphate dehydrogenase pathway.[3] Furthermore, clozapine is an atypical antipsychotics drug, which can induce oxidative stress and oxidative cell injury by promoting oxidation of protein. [4] Alterations in protein oxidation could be caused by the generation of free radicals and reactive oxygen species by drug-induced oxidative stress. [4] Studies suggested typical and atypical psychotics drugs, to induce both lipid peroxidation and total protein peroxidation.[5–7] Therefore, protein oxidation is a mechanism by which atypical antipsychotics increase the risk for metabolic alteration. [5,7] Also, mitochondria produce reactive oxygen species (ROS) in living cells and diabetes-associated metabolic disorders can cause mitochondrial dysfunction and excess ROS production, which promotes cardiovascular disorders as a result of oxidative stress.[8] Antioxidants are known to reduce the oxidation of proteins, lipids, and molecules, consequently in this study, *Adenopusbreviflorus* (*Lagenariabreviflora*) was employed owing to its antioxidant property. *Adenopusbreviflorus* belongs to the family of Cucurbitaceae. It is commonly called Wild colocynth in English.[9] *AdenopusBreviflorus* has been shown through phytochemical studies to contain saponins with tannins, which suggests it as triterpenoids.[10] Saponins have been used as traditional and industrial applications, and as anti-inflammatory, antioxidant, molluscicidal, antimicrobial, antispasmodic, antidiabetic, anti-tumor as well as adjuvants in medicine.[11] Furthermore, ethyl acetate and methanol fractions of *Adenopusbreviflorus* modulate mitochondrial permeability transition pore opening, inhibit mitochondrial ATPase activities, and reduce iron-induced mitochondrial lipid peroxidation.[12] Therefore, *Adenopusbreviflorus* aqueous extract was used to suppress the expression of free radicals produced because of the influence of clozapine in male adult Wistar rats.

2. Materials and Methods

and make up with 100ml by using 0.1 N HCL (100µg/ml). From this 10ml was taken and make up with 100 ml of 0.1 N HCL)

Animal Treatments: The study was carried out on twenty male Wistar rats weighing between 135-140 g, after they have been acclimatized for one week. The rats were fed ad libitum with grower's + finisher's mash purchased from Ogo-Oluwa Feed Mill, Ilorin, Nigeria. The study was conducted following the guidelines and care and use of laboratory animals,[13], and the experimental approval for

this study was gotten from the college of health sciences, University of Ilorin (UERC/ASN/2016/357) ethical review committee. The rats were randomly divided into four groups (CTR, CLO, AEAB and CLO+AEAB, n= 5 per group). The animals in the CTR group was administered 0.5mls of water orally, the animals in the CLO group received 25 mg/kg of clozapine dissolved in 3 ml of distilled water, the AEAB group took 200 mg/kg of Aqueous extract of *Adenopusbreviflorus* dissolved 3 ml of distilled water orally and the last group; CLO+AEAB was given 25 mg/kg of clozapine for four weeks + 200 mg/kg of aqueous extract of *Adenopusbreviflorus*. All administration was through the oral route and lasted for two weeks.

Preparation of Drugs: Clozapine tablets (100 mg/ tablet) were obtained from the local pharmacy. 25 mg of clozapine was measured and portioned using the sensitive weighing scale and freshly dissolved in 3ml of distilled water every day of the drug treatment.

Collection and Preparation of Aqueous Extract of *Adenopusbreviflorus*:

Fresh samples of *Adenopusbreviflorus* fruit were bought in Bodija Market, Ibadan, Nigeria, and authenticated by the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. Authentication was done with a voucher specimen (form number: 0254) and was deposited in the herbarium of the same department. Large quantity (7.0 kg) of fresh specimens of whole fruit of *Adenopusbreviflorus* were washed free of debris and sliced into pieces, the pieces were soaked in distilled water in an air-tight container for seventy-two hours, after which the filtrate collected was concentrated to dryness in a water bath. The aqueous extract was kept at cool temperature after use for preservation. The extract was administered to the rats with the use of an oropharyngeal cannula at a dosage of 200 mg/kg.

Determination of Biochemical Parameters:

After twenty-four hours of the last treatment, the animals were anesthetized with 20 mg/kg of ketamine. Thereafter, they were dissected, and blood samples were collected into well-labeled heparin bottles by cardiac puncture while 0.1g of the tissue samples of the heart were homogenized in 0.4 ml of 5% sucrose solution. The blood and tissue samples were spun for 10 minutes at 4000 rpm and the supernatants from each bottle were transferred into a plain bottle and stored at 20°C before assaying for plasma Glucose-6-phosphate dehydrogenase (G6PDH), Catalase, Superoxide Dismutase (SOD), Nicotinamide Adenine dinucleotide phosphate (NADPH), Glutathione Peroxidase (GPX), glutathione disulfide (GSSG) using standard spectrophotometric method.

Statistical Analysis: The statistics were calculated with Statistical Package for Social Sciences (SPSS) software (version 20.0; SPSS Inc, IL., USA) for windows. One-Way Analysis of Variance (ANOVA) was used to evaluate the

significance between the groups studied, comparison tests were performed by fixing the significance level at $p > 0.05$.

3. Results and Discussion

Adenopusbreviflorus augments plasma G6PD-dependent antioxidant levels in CLO-treated animals:

Clozapine treatment reduced plasma G6PD, SOD, NADPH, and GPx but showed no significant change in plasma catalase and GSSG when compared with control. Nevertheless, Adenopusbreviflorus treatment with or without CLO increased plasma G6PD, SOD, NADPH, and GPx compared with CLO-treated animals (fig.1). However, treatment with only Adenopusbreviflorus increased NADPH and GPx compared with the control group (fig.1).

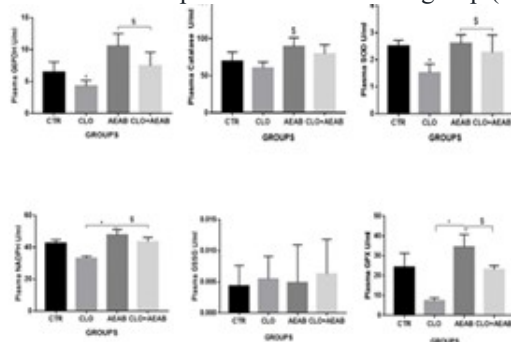


Fig 1: Adenopusbreviflorus augments plasma G6PD-dependent antioxidant levels in CLO-treated animals.

Data were represented as mean $\pm$ SEM (n=6) * $P < 0.05$ vs CTR, $P < 0.05$ vs CLO. CTR=Control, CLO=Clozapine, AEAB=Aqueous Extract of *Adenopusbreviflorus*, CLO+AEAB=Clozapine, Aqueous Extract of *Adenopusbreviflorus*

Adenopusbreviflorus improved cardiac G6PD-dependent antioxidant in CLO-treated animals:

CLO slightly but insignificantly reduced cardiac G6PD, SOD, GSSG, GPx, and NADPH but did not show alteration in cardiac catalase, increased cardiac G6PD and NADPH significantly but did not significantly alter cardiac SOD, GSSG, catalase and GPx despite a slight increase compared with CLO-treated animals without Adenopusbreviflorus treatment (fig.2). Treatment with Adenopusbreviflorus without CLO exposure significantly increased cardiac SOD and NADPH compared with CLO-exposed animals without Adenopusbreviflorus treatment but were not altered compared with control (fig.2).

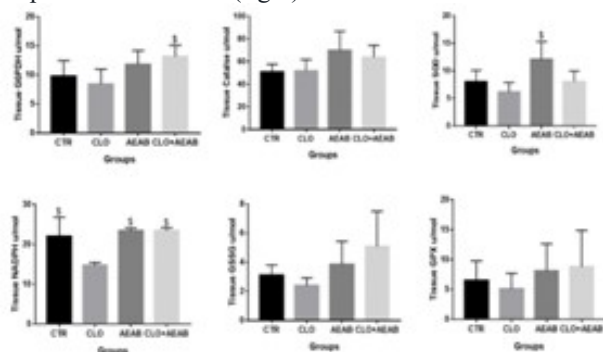


Fig 2: Adenopusbreviflorus improved cardiac G6PD-dependent antioxidant in CLO-treated animals.

Data were represented as mean $\pm$ SEM (n=6) * $P < 0.05$ vs CTR, $P < 0.05$ vs CLO. CTR=Control, CLO=Clozapine, AEAB=Aqueous Extract of *Adenopusbreviflorus*, CLO+AEAB=Clozapine and Aqueous Extract of *Adenopusbreviflorus*

Discussion

The outcome of the present study showed that CLO can depress both circulating and cardiac G6PD-dependent antioxidant defense. The effect of the drug was more pronounced in the plasma than in the heart. However, AEAB fruit aqueous extract restores circulating and cardiac G6PD-dependent antioxidants which were reduced by clozapine exposure. This shows that fractions of AEAB fruit aqueous extract contain phytochemicals that have detectable G6PD-dependent antioxidant enhancing property. G6PD-dependent antioxidant system includes molecules like glutathione and enzymes like glutathione peroxidase. The involvement of G6PD in furnishing the glutathione antioxidant system is indirect through the production of NADPH. [14] Although SOD and catalase are not dependent on G6PD, their roles in cellular defense significantly complements the G6PD-dependent antioxidant system. In the present study, exposure to a 4-week CLO treatment caused a significant reduction in circulating G6PD-dependent antioxidants along with a reduction in SOD and catalase. This status agreeably predicts that spillover of systemic tissue production of G6PD-dependent antioxidants, SOD, and catalase were reduced with CLO treatment. This reveals that individuals exposed to chronic CLO treatment can have depressed cellular antioxidants in multiple organs leading to oxidative damage if not curtailed. CLO activity might directly or indirectly involve the reduction of G6PD production or activity without directly affecting the glutathione system since reduced G6PD activity can affect NADPH production and consequently the glutathione system.

Also, CLO treatment led to a slight alteration in cardiac G6PD-dependent antioxidant. Although the changes observed here were not significant, the consistent downward trend of the antioxidant status shows that CLO might cause cardiac oxidative stress as it is noted for by attenuating G6PD-dependent antioxidants. Besides, CLO showed a tendency for reducing cardiac SOD. This might indicate that CLO can affect cardiac nuclear erythroid 2-related factor 2 (Nrf2) since it is required as a nuclear factor in the synthesis of glutathione, SOD, and other cellular antioxidants.[15] Further studies are required to elucidate the exact mechanism by which CLO induces oxidative stress. However, this study proposes that G6PD and Nrf2-related mechanisms are involved in CLO oxidant activity. Nevertheless, AEAB fruit aqueous extract preparation has a reputation for preventing mitochondrial lipid peroxidation,[12] which describes the fact that it has the potential to alleviate redox imbalance. Here, AEAB juice improved circulating G6PD and led to improved circulating NADPH, and GPx in CLO-treated animals. This indicates increased activity in the glutathione system. AEAB also significantly improved circulating SOD but slightly and insignificantly increased catalase in CLO-treated animals. Adenopusbreviflorus therefore might enhance Nrf2

signaling by preventing its degradation through the KEAP1/Nrf2 pathway [16] leading to improved production of glutathione, SOD, and catalase in multiple organs during CLO exposure. However, the G6PD-NADPH-glutathione pathway was positively affected by AEAB fruit aqueous extract showing that AEAB assuredly demonstrates notable antioxidant property by enhancing G6PD dependent antioxidants. Furthermore, in the heart, G6PD and NADPH were enhanced by AEAB, but SOD and catalase only showed an insignificant upward trend in CLO-treated animals. It is evident that like what is probable in other tissues, AEAB protects the heart against CLO-induced redox imbalance by enhancing, more importantly, the G6PD-dependent antioxidant pathway than the SOD/catalase pathway. It is therefore necessary to identify the exact molecules responsible for the antioxidant property of AEAB fruit aqueous extract since it showed notable tendencies to protect the heart and plausibly other tissues. This study also sets the stage for further investigation of the cellular pathways involved in the activation of especially G6PD and plausibly Nrf2 by AEAB.

4. Conclusion

In conclusion, CLO induces antioxidant dysmetabolism by suppressing the G6PD-dependent antioxidant pathway in the heart and circulation. This CLO action was abated by AEAB fruit aqueous extract leading to improved G6PD-dependent antioxidants in the heart and plausibly other tissues. Therefore, AEAB plant can be subjected to thorough scientific studies to establish its potential as a therapeutic candidate in combating excessive oxidant production in conditions like metabolic syndrome with very high global prevalence.

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