EVALUATION OF THE HEPATOTOXIC POTENTIAL OF METHANOL LEAF EXTRACT OF *MENTA PIPERITHA* USING ANIMAL MODEL

ABSTRACT

The aim of this research work was to evaluate the hepatotoxic potential of methanol leaf extract of *M. piperita*. Freshly harvested leaf of *M. piperita* was dried at room temperature and afterwards ground to fine powder. 500 g of powdered plant sample was soaked in 70% methanol for 72 hr. This was followed by the filtration of the extract which was subsequently concentrated. Twenty five adult male wistar rats were divided into five groups of five rats. Group I was the normal control was fed rat chow and water only. Group II was administered with 100 mg/kg bw of extract orally, Group III was administered with 200 mg/kg bw of extract orally, Group IV was administered with 400 mg/kg bw of extract orally, Group V was administered with 600 mg/kg bw of extract orally. Administration of extract lasted for 14 days after animals were sacrificed and blood sample collected. Biochemical analysis on the sample was determined using standard procedures. The results obtained from this study revealed a non-significant ($P>0.05$) difference in the activity of Alanine transaminase (ALT), Aspartate amino transferase and alkaline phosphatase of rats administered with the extract compared with the normal control (30.00±1.01 IU/L), (32.02±1.01 IU/L) and (20.50±0.04 IU/L) respectively. Similar observation was made on Total and Conjugated Bilurubin as well as the weight of the liver harvested from the rats. In conclusion, it could be deduced from this study that methanol extract of *M. piperita* is not hepatotoxic.

Keywords: *Mentha piperita*; Hepatotoxic; Alanine transaminase; Bilurubin

Introduction

The liver is an embodiment of several cell types originating from embryo and hepatocytes, biliary epithelial cells (cholangiocytes), stellate cells, Kupffer cells, and liver sinusoidal endothelial cells each of which has unique set of functions that cooperatively regulate hepatic function at multiple levels. Human health fails when the function and morphological integrity of the liver is compromised [1]. The liver is known for many physiological functions and these include macronutrient metabolism, blood volume regulation, immune system support,

endocrine control of growth signaling pathways, lipid and cholesterol homeostasis, and the breakdown of xenobiotic compounds etc [1].

Mentha piperita commonly known as peppermint is a hybrid mint, a cross between water mint and spearmint. Although it is indigenous to the Middle East and Europe, the plant is vastly cultivated across the globe. Occasionally, it is found in the wild with its parent species [2]. The plant survives in moist, shaded locations and grows optimally with adequate water supply. Being a hybrid, it is usually sterile, producing very few seeds and reproduces almost by vegetative means, spreading quickly by underground runners [3].

Medicinal plants will continue to play crucial role in the health sector [4]. The use of medicinal plants in developing countries in the treatment of diverse human diseases has been widely observed [5]. Notably, the leaf of *Mentha piperita* is employed in the treatment of common cold, inflammation of the mouth, pharynx as well as gastrointestinal tract disorders such as nausea, vomiting, diarrhea, cramps, flatulence and dyspepsia. It is has antioxidant, antimicrobial, antiviral, anti-inflammatory, and anti-carcinogenic properties [6]. Although research efforts had revealed the effect of some of *Lamiaceae* family, there is paucity of data on the effect of *Mentha piperita* consumption on the liver.

MATERIALS AND METHODS

Collection of Plant Material

Mature leaf of *Mentha piperita* was harvested from a farm in Uturu community in Abia State. The leaf was conveyed in dark polythene bad to the herbarium unit of the Herbarium unit of the Department of Forestry, Umudike Abia State for identification and authentication.

Sample preparation

Leaf of *Mentha piperita* was washed with clean tap water, dried at room temperature after which it was ground with an electric blender. The resulting powder was sieved to obtain fine powder. 500 g of the powdered plant sample was suspended in 2 L of 70% methanol for about 72 hours and was stired intermittently. The extract was filtered and the filtrates concentrated.

Collection of Animals

Adult male wistar rats weighing 100-200 were obtained from the animal house of the animal house of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana Afikpo, Ebonyi State. The rats were kept in plastic cages and were allowed access to food and water *ad libitum*. They were acclimatized for two weeks prior to commencement of experiment.

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Lethal Dose 50

This was performed in accordance with the method described by Lorke [7]. At the start of the experiment, rats were divided into 3 groups of 3 rats per group and were administered with 10 mg, 100 mg and 1000 mg of the extract per kg body weight orally. They were observed for 24 hr for signs of toxicity, including death. In the absence of observable toxicity, the second phase was initiated and involved 4 rats which were divided into 4 groups of one rat each. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Experimental Design

Thirty adult male rats were divided into five groups of five rats each.

Group I: Normal control fed with only normal commercial diet and water *ad libitum.*

Group II: was administered with 100 mg/kg b.w of methanol extract of *M. piperitha* orally.

Group III: was administered with 200 mg/kg b.w of methanol extract of *M. piperitha* orally

Group IV: was administered with 400 mg/kg b.w of methanol extract of *M. piperitha* orally

Group V: was administered with 600 mg/kg b.w of methanol extract of *M. piperitha* orally

Collection of Blood Sample

Rats were administered daily with the extract for a period of two weeks, after which rats were sacrificed and blood sample collected, centrifuged and serum generated stored for use in the evaluation of the activity liver function test.

Evaluation of Serum Hepatomarkers

The colorimetric method was relied upon to determine the activity of Alanine aminotransaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransaminase (AST), bilirubin in the serum using the Randox Diagnostic Kits (USA). Pyruvate solutions of varied concentrations were used to prepare a standard curve from which AST activities were computed as described according to [8] Alanine aminotransaminase (ALT) assay was carried out as was described by AST except that 200 Mm DL-Alanine replaced L-Aspartate in the procedures.

Histopathological Study

Harvested liver tissue was fixed, processed and was subsequently dehydrated in 90% alcohol. The liver tissue was further processed according to the method described by Burki et al [9].

Statistical analysis

Results were expressed as mean ± standard deviation. The data were analysed using analysis of variance (ANOVA). The difference in mean was compared using Multiple Range Test. $P<0.05$ was considered significant.

RESULTS

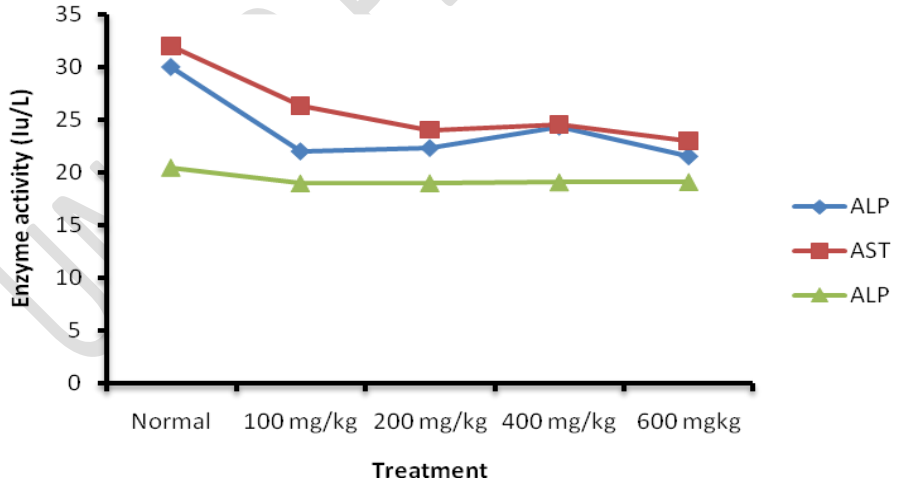

Figure 1: Activity of Liver enzymes in rats administered with methanol leaf extract of *Mentha piperita*

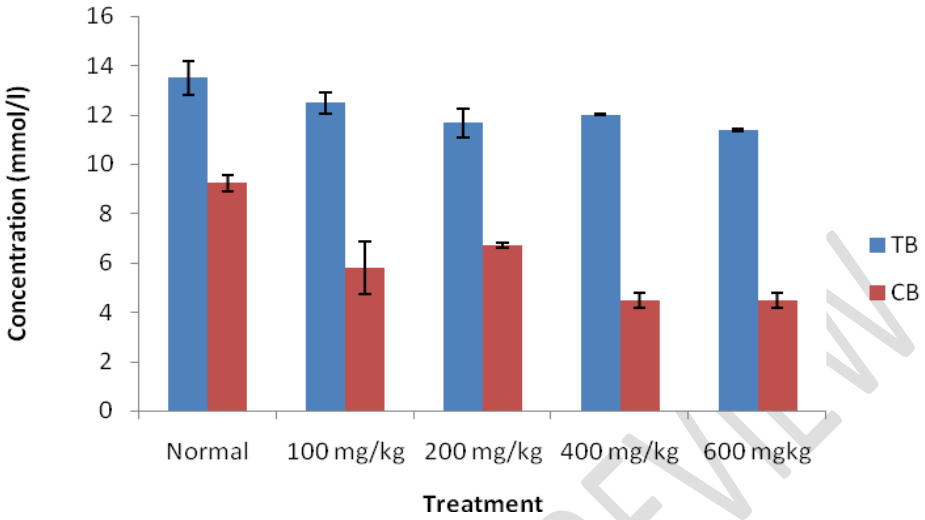

Figure 2: Serum Bilurubin concentration of rats administered with methanol Leaf Extract of *Mentha piperita*

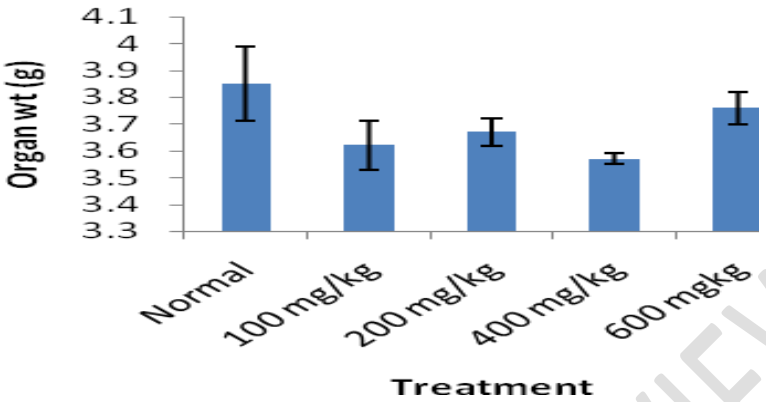

Figure 3: weight of liver obtained from wistar rats administered with methanol leaf extract of *Mentha piperita*

DISCUSSION

The liver is known for many physiological functions and these include macronutrient metabolism, blood volume regulation, immune system support, endocrine control of growth signaling pathways, lipid and cholesterol homeostasis, and the breakdown of xenobiotic compounds etc [1]. Thus, when damaged could have some grave consequences. Although known for its potential to detoxify diverse arrays of substances, it can be its integrity could be compromised following exposure to herbal substances. Figure 1 shows the activity of liver enzymes in rats administered with methanol leaf extract of *Mentha piperita* indicating a non-significant ($P>0.05$) difference in the activity of Alanine transaminase (ALT), Aspartate amino transferase and alkaline phosphatase of rats administered with the extract compared with the normal control (30.00±1.01 IU/L), (32.02±1.01 IU/L) and (20.50±0.04 IU/L) respectively. Similar observation was made on Total and Conjugated Bilurubin as well as the weight of the liver harvested from the rats. This outcome of this study is consistent with the finding of made by Rajesh et al. [10] which reported a reduced serum hepatomarkers in rats with hepatic lesions administered with 400 mg/kg bw of ethanolic leaf extract of *Mentha arvensis* a member of the *Lamiaceae family* to which *Mentha piperita* belongs. Similarly essential oil derived from *Mentha spicata* another member of the family restored hepatic health in rats induced with hepatic damage using manganese and lead Mostapha et al. [11].

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

[1] Trefts E, Williams AS and Wasserman DH (2015). Exercise and the regulation of hepatic metabolism. In Progress in Molecular Biology and Translational Science, (1st edition), Amsterdam: Elsevier.

[2] Geneva: World Health Organization (2002). pp. 188, 199. ISBN 92-4-154537-2.Retrieved October 29, 2010.

[3] Paul Rita and Datta K Animesh (2011) An updated overview on peppermint *(Mentha piperita L*). Int. Res. J. Pharm. 8(1)10.

[4] Hoareau H, DaSilva EJ(1999). Medicinal plants: a re-emerging health aid' Electronic Journal of Biotechnology. Issue of August 15, Available online at http://www.ejb.org/content/vol2/issue2/full/2/

[5] UNESCO, author. Culture and Health, Orientation Texts-World Decade for Cultural Development 1988-1997. Paris, France: 1996. Document CLT/DEC/PRO - 1996. pgs. 129.

[6] Valente JSS, Fonseca AOS, Denardi LB, Dal Ben VS, Filho FSM et al (2016) In Vitro susceptibility of *Pythium insidiosum* to *Melaleuca alternifolia*, *Mentha piperita* and *Origanum vulgare* essential oils combinations. Mycopathologia. 181(7-8):617-22.

[7] Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol. 54(4):275-287.doi: 10.1007/BF01234480

[8] Varley H, Gowenlock A H, Bell M.(1980). Practical Biochemistry. (5th ed) London : W.Heinemann

[9] Burki S, Burki ZG, Ahmed I, Jahan N, Owais F, Tahir N (2020) GC/MS assisted phytochemical analysis of *Ajuga parviflora* leaves extract along with antihepatotoxic effect against anti-tubercular drug induced liver toxicity in rat. Pak. J Pharm. Sci.; 33:325-31.

[10] Rajesh K, Vishwanath AH, Shivakmar SI, Joshi V, Kurnool AN (2013) Hepatoprotective and antioxidant activity of ethanol extract of Mentha arvensis leaves against carbon tetrachloride induced hepatic damage in rats. *Int. J*. Pharm. Technol. 5, 426-430.

[11] Mostapha B, Djallal E, Houari A, Miloud S, Wafaa A, Narimane T, Khaled K (2019) Evaluation of the therapeutic effects of *Mentha spicata* essential oil at the liver level in developing wistar rats co-exposed to lead and manganese. Carpathian J. Food Sci. Technol. 11(2), 152-164.