ISSN 2955-1226 (Print)
ISSN 2955-1234 (Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Effect of Ethanol and N-Hexane Combined Extracts of Selected Plants on Liver Enzymes in Wistar Rats

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ABSRTACT

Medicinal plant are commonly used for the treatment of various diseases including liver disease. The ameliorative roles of these plants depends on their phytochemical contents, which is obtained based on the extracting solvent of choice. This study aims at determining the effect of ethanol and n-hexane combined extracts of selected plants on liver enzymes in wistar rats. A total of 18 wistar rats weighing 150 - 250 grams were separated into 3 groups of 6 rats each and 2 groups were administered with 200 mg/kg each of ethanol and n-hexane combined extracts for 7 days. Result showed that serum levels of alanine aminotransferase (118.50±5.99 and 102.17±3.73) and aspartate aminotransferase activities (162.83 ± 4.12 and 154.67 ± 2.16 IU/L) respectively were reduced more in ethanol extracts, while serum level of alkaline phosphatase (89.50±4.89 and 98.50±3.73) were reduced more in n-hexane treated group compared to control with (141.17±2.64) ALT, (173.17±2.13) AST and 108.17±2.32) ALP. This study revealed that administration of combined extracts of ethanol or n-hexane improved liver condition of wistar rats.

Keywords: Liver enzymes, Medicinal plants.

Received 20 September 2023
Revised 30 October 2023
Accepted 03 November 2023
Published online 02 December 2023

Introduction

Liver, the largest gland is a vital organ, is the metabolic "engine-room of the body". Almost all the drugs, foods and water constituents are metabolized and detoxified in the liver.[1] And as such it is often exposed to maladies resulting in a number of liver diseases that may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in liver fibrosis).[3] Unfortunately, treatments of choice for liver diseases are controversial because conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects.[2] But since ancient times, mankind has made use of plants in the treatment of various ailments because their toxicity factors appear to have lower side effects.[5] Many of the currently available drugs were derived either directly or indirectly from medicinal plants.[5] Recent interest in natural therapies and alternative medicines has made researchers pay attention to traditional herbal medicine. In the past decade, attention has been centered on scientific evaluation of traditional drugs with plant origin for the treatment of various diseases, including liver diseases.[4] due to their effectiveness, with presumably minimal side effects in terms of treatment as well as relatively low costs, herbal drugs are widely prescribed, even when their biologically active constituents are not fully known.

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Citation: Ogar VO, Itam YB, Akataobi US, Ukam EE, Ogar EA. Effect of Ethanol and N-Hexane Combined Extracts of Selected Plants on Liver Enzymes in Normal Albino Rats. Trop J Phytochem Pharm. Sci. 2023; 2(4):114-116. http://www.doi.org/10.26538/tjpps/v2i4.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The utility of natural therapies for liver diseases has a long history. Despite the fact that most recommendations are not based on documented evidence, some of these combinations do have active constituents that are dependent on the method/solvent of extraction, with confirmed antioxidant, anti-inflammatory, anti-obesity or anti-diabetic properties, disease conditions that have been associated with liver dysfunction.[8] Although large number of these plants have been investigated, the studies were mostly unsatisfactory. For instance, the therapeutic values, in most of these studies, were assessed against a few chemicals-induced subclinical levels of liver damages in rodents. In this study, we investigated the effect of medicinal plants locally used in the treatment of disease conditions reported to have adverse effect on the liver.

Materials and methods

Collection of plant material

Fresh leaves of *Ipomea alba* (ufuk ikot), *Emilia sonchifolia* (awak mmong), *Hygrophylia polysperma* (mmeme), *Eremomastax polysperma* (edem ididout), *Dissotis rotundifolia* Triana (eyen ndang), *Eremomastax speciose* (ikpo ikong ukebe), *Piper umbellatum* (mweweb), as well as seeds of *Piper guinenses* (mfri etinkeni), were obtained from Okuku market in Yala Local Government Area of Cross River State, Nigeria. The plant was authenticated by a Botanist in the Department of Botany, University of Calabar and assigned a voucher identification number: Herb/Bot/UCC/0067 in January 2021. The leaves were air dried at room temperature, in Biochemistry Laboratory, Department of biochemistry, University of Calabar. After which the leaves were pulverized with an electric blender into powder forms, weighed and extracted according to the method described in Extraction Technologies for Medicinal and Aromatic Plant page 23-24 using 200ml of ethanol and n-hexane respectively.

The pulverized plant samples, 100g each were placed in No1 Whiteman filter paper, folded and placed in chamber of the Soxhlet apparatus and extracted with 200ml of ethanol or n-hexane extraction

solvent heated in a flask. The extracts were subsequently concentrated to about 10 percent of their original volumes, using a rotary evaporator at $<40^0$C and dried to a semi solid form in a water bath.

Preparation of plant extract
Equal weights (0.2 g) of all the ethanol extracts were measured into a mortar and dissolved with 4 ml mixture of Dimethyl sulfoxide (DMSO) water (0.2:3.8 v/v) using a pestle until a homogenized phase were obtained. Similarly, n-hexane extracts were prepared with 4 ml mixture of DMSO-water (0.2:3.8 v/v).

Ethical approval
Ethical approval for the treatment and handling of experimental animal and human subjects was obtained from the Faculty Animal Research Ethics Committee on Use and Care of Experimental Animals, Faculty of Basic Medical Sciences, University of Calabar with the approval number; 184BCM2024.

Experimental animals
Eighteen (18) Wistar rats of both sexes, weighing between 150 - 250g were obtained from the animal house of the department of Biochemistry, University of Calabar, Nigeria. The animals were allowed to acclimatize for 7 days in ventilated cages under 12 hours' light/dark cycle. The animals were fed rat chow and allow4444444ed free access to water *ad libitum* throughout the period of the experiment. The rats were randomly grouped into 3 experimental groups of six (6) rats each. Group 2 rats were administered with a calculated doses of 200 mg/kg body weight combined plant extracts of ethanol. Group 3 rats were administered with a calculated doses of 200 mg/kg body weight combined plant extracts of n-hexane. While Group 1 the control group were similarly given water. All treatment lasted for seven days.

Collection of blood samples for biochemical analysis
At the end of 7days, the rats were anaesthetized using ketamine, dissected and blood samples were collected through cardiac puncture using a 5ml syringe and needle into properly labeled sample bottles. The blood samples were allowed to stand for 10 minutes and centrifuged at 3500 rpm for 15 minutes and serum obtained transferred into new labeled sample bottles, for the determination of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase according to the method described by Randox®.[13]

Statistical analysis
Data obtained from this study were analyzed by using SPSS (2.1), and mean compared presented as mean ± standard error of mean (Mean ± SEM).

Results and Discussion

Table 1 shows the effects of treatment on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALP) and alkaline aminotransferase (ALT). The result demonstrated that the combined ethanol or n-Hexane plants extracts caused a reduction in the level of the AST, ALP and ALT compared to the control group (Group 1). Previous studies have shown that each liver function test by itself is neither highly sensitive nor specific but when combined and interpreted together, the results may provide useful information about the patient's liver condition, as well as ameliorative roles of the plants bioactive components on the liver, in experimental condition that evaluated plant products as a treatment of choice in liver disease.[6] ALT and AST are sensitive indicators of hepatocellular injury but have been reported to lack specificity as liver damage indicators because they are also present in muscle (cardiac and skeletal), kidney, and RBCs and may increase or decrease in disease conditions affecting these organs/cells.[7] Results of this study showed that both were reduced significantly in n-hexane treated group, ALT 102.17±3.73 and AST 154.67± 2.16 (Group III) compared to control 141.17±2.67 and 173.17 (Group I) and ethanol group 118.50± 5.99 and 162.83 respectively (Group II). Apart from the liver, ALP is also present in other organs for instance in the bone, kidney, intestine and placenta, studies have reported that liver ALP present on canalicular surfaces, is raised in any condition of biliary obstruction (intrahepatic and extra-hepatic). it has also been shown that in most disease conditions, injury in hepatocyte often result in normal ALP or marginally increased ALP level, reason for the enzyme been used alongside other liver indices to determine liver condition and low level of these liver enzymes are an indication of healthy liver.[7,10] This report is in line with the result of this current study that demonstrated that administration of the combined extract of the plants both from ethanol and n-hexane extracts improved liver condition by reducing the level of ALP in a similar manner as AST and ALT, but unlike these enzymes ALP was reduced more in ethanol (Group II) with 89.50±4.89 compared to n-hexane (Group III) with 98.50±3.73 and control (Group I) with 108.17±2.32. This result suggests ability of the plants to promote healthy liver. Furthermore, previous reports on the use of medicinal plants in the treatment of diseases have shown that, numerous phytomedicines or polyherbal formulations are now being used in the management or treatment of liver disease or hepatotoxicity.[8] This is because the plant contain phytochemicals such as flavonoid, terpenoids, polyphenols, alkaloids, saponins, vitamins, enzymes, polysaccharides, lignins, xanthones and pigments etc., which possess strong antioxidant activities.[9] The antioxidants contents carryout the ameliorative roles of these plants in protecting the cells from damage caused by highly reactive oxygen compounds, the free radicals.[11] This may account for the response recorded in this current study on the ability of the combined plants extracts to induce hepatoprotective role in the wistar rats.[2]

Conclusion

This study revealed that the combined extracts of ethanol or n-hexane significantly reduced the level of liver enzymes measured. AST and ALT were reduced more in n-Hexane combined extracts while ALP was reduced more in ethanol combined extracts compared to the control. Thus, this study revealed that the combined extracts ethanol or n-hexane was able to improve liver condition in wistar rats

Conflict of Interest

The authors declare no conflict of interest.

Table 1: Shows serum levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase

Parameters	Normal Control	200 mg/kg body weight of ethanol extract.	200 mg/kg body weight of n-hexane extract.
Aspartate aminotransferases, (AST) activity (IU/L).	173.17 ± 2.13	162.83 ± 4.12	154.67 ± 2.16
Alanine aminotransferase (ALT) activity (U/L).	141.17 ± 2.64	118.50 ± 5.99	102.17 ± 3.73
Alkaline phosphatase (ALP) activity (U/L).	108.17 ± 2.32	89.50 ± 4.89	98.50 ± 3.73

Mean ± Std. deviation (n=6).

 ISSN 2955-1226 (Print)
ISSN 2955-1234 (Electronic)

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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