Histopathological effect of aqueous bitter leaf extract (Vernonia amygdalina) on acetaminophen-induced liver damage in albino rat (Rattus norvegicus)

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Abstract

Liver diseases are a worldwide problem. Medicinal plants are being increasingly utilized to treat a wide variety of diseases, liver disorder inclusive. The effect of aqueous leaf extracts of Vernonia amygdalina on acetaminophen-induced liver damage via the assessment of the histopathological examination was examined. Five groups of albino rats were used (n = 5). Animals in group 1 were fed normal laboratory pellet and water ad libitum (Control); those in group 2 received 3000mg/kg of acetaminophen alone. Groups 3, 4 and 5 received 3000mg/kg of acetaminophen and in addition 50mg/kg Vernonia amygdalina leaf extract, 100mg/kg Vernonia amygdalina leaf extract and 200mg/kg Silymarin, respectively for 14 days. Twenty-four hours after the last administration, the animals were sacrificed. The organs (liver) was excised and used for the histopathological examination. Histological examinations of the liver showed acetaminophen-induced hepatotoxicity. Histology of the liver revealed altered cellular architecture (poor architecture, moderate to severe congestion of the portal vein as well as severe peri portal infiltration of inflammatory cells, the liver parenchyma also showed area with destroyed liver plates with severe hemorrhage and necrosis. The morphology of the hepatocytes showed some degenerated liver cells, the liver parenchyma also showed focal area of moderate aggregate of inflammatory cells) following administration of acetaminophen (Group 2). Normal histological features were restored after treatment with aqueous leaf extract of Vernonia amygdalina. The study has demonstrated that aqueous leaf extract of Vernonia amygdalina ameliorate liver of rats against acetaminophen-induced toxicity and modulates the adverse effects of acetaminophen on the liver.

Keywords: Albino rats, Histopathology, Liver, Acetaminophen, Vernonia amygdalina

Introduction

Toxicity is the extent at which a compound causes harm to animal and plant tissue or organs. It can be acute, sub chronic, or chronic. There is a wide distribution of biologically-active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition. The knowledge that these compounds elicit both toxic and advantageous biological responses has given rise to several investigations in recent times as to their possible physiological implications in various biological systems. Toxicity is expressed generally as a dose–response relationship, involving the quantity of substance to which the organism is exposed to. Furthermore, it is well known that many plant products contain some toxic substances which when consumed could accumulate gradually in the body and later cause some damages to cells, tissue or
The toxic effects of environmental toxins and drugs on the human and animal system have become a major health concern (Nawal, 2015). Paracetamol (acetaminophen) as a pain killer is widely used for the treatment of mild pain and pyrexia. Paracetamol, being an analgesic has been proven to cause hepatic centrilobular necrosis when given at over dose. Da Silva Melo et al. (2016) reported that paracetamol (acetaminophen), an over-the-counter drug, is widely used for the treatment of mild pain and pyrexia. Because of its over-the-counter status, cases of both accidental and intentional paracetamol (APAP) overdose are numerous. As a result of this high rate of abuse, paracetamol has been described by Larson et al. (2010) as the most common cause of drug induced liver failure in the United States. At overdose level, paracetamol causes hepatic centrilobular necrosis (James et al., 2014) which has been linked to excessive generation of N-acetyl-p-benzoquinoneimine (NAPQI). It had been observed that mitochondria may be the primary targets in acetaminophen (APAP) toxicity with particular attention on the mitochondrial permeability transition. Involvement of other generated reactive oxygen species such as nitric oxide and superoxide anion cannot also be discounted in paracetamol-induced hepatocyte death (Chang and Trivedi, 2014). A number of antidotes had been suggested for the treatment of APAP-induced liver damage, one of which is Vernonia amygdalina. Vernonia amygdalina is well known as a medicinal plant with several uses attributed to it, including for diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache, and joint pain associated with AIDS (Ohigashi et al., 2010). These leaves are exported from several African countries. The roots of V. amygdalina have been used for gingivitis and toothache due to its proven antimicrobial activity (Jisaka et al., 2012). Vernonia amygdalina (compositae) is a small shrub that grows predominantly in the tropical Africa. In Nigeria, the plant is locally called bitter leaf due to its bitter taste. The macerated leaves of the plant are used in making soup while the water extract serves as a tonic drink. In the local community, the leaves are used as anthelmintic, a laxative and an antimalarial because they contain quinine substitute. It is noted that the leaves contain stigmastane-type saponin such as vernoniosides A1, B1, A2, A3, B2, D2, A4 and C which have been identified in the leaves (Jisaka et al., 2012). Earlier investigation on Vernonia amygdalina showed that purified chloroform fractions identified as vernodaline, vernolide and vernomygdine elicited cytotoxic effects in human carcinoma involving narsopharynx cells with IC50 values of 1.8, 2.0 and 1.5 =g/ml respectively. It was concluded that the activities were dependent on their possession of the (alphamethyl-gamma-lactone group) as their structures (Jisaka et al., 2012). For years now since the discovery of Vernonia amygdalina there has been a store of information on the clinical value of this plant. However less than 10% of the world's flora had been studied chemically in detail to determine their active constituents; phytochemical components and histopathological effects of this plant when consumed (Yang and Health, 2014). Silymarin (extracted from the seeds of Silybum marianum or milk thistle) can also be used in the treatment of liver diseases. Silymarin may be of use as an adjuvant in the therapy of alcoholic liver disease. This study revealed the histopathological effect of Vernonia amygdalina and Silymarin on acetaminophen-induced liver damage in albino rat (Rattus norvegicus) concurrently administered with toxic doses of
paracetamol.

Materials and methods

Study area

The study was carried out at the Animal Production unit, Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria.

Plant materials

Samples of Bitter leaf (Vernonia amygdalina) were obtained from a private farm in Ado Ekiti, air dried in the laboratory, pulverized and then stored in an airtight container.

Reagents and chemicals

All reagents and chemicals were all of analytical grade

Preparation of bitter leaf extract

Vernonia amygdalina were air-dried for 30 days at room temperature. The air-dried samples were ground to fine powder using a blender. The powdered leaf (500g) was soaked in 2000 ml of distilled water for 72 hours. It was then filtered using a cheese cloth, and freeze-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 4°C for further studies.

Experimental design

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Group 1: Normal control (NC)</td>
<td>Distilled water only for 14 days</td>
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<tr>
<td>Group 2: Induced untreated</td>
<td>3000mg/kg acetaminophen alone for a single administration</td>
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<tr>
<td>group (Positive Control)</td>
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<tr>
<td>Group 3</td>
<td>3000mg/kg acetaminophen + 50 mg/kg Vernonia amygdalina extract for 14 days</td>
</tr>
<tr>
<td>Group 4</td>
<td>3000mg/kg acetaminophen + 100 mg/kg Vernonia amygdalina extract for 14 days</td>
</tr>
<tr>
<td>Group 5</td>
<td>3000mg/kg acetaminophen + 200 mg/kg Silymarin for 14 days</td>
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</tbody>
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Dissection of rats

The rats were dissected and liver was excised using scissors and forceps, washed in distilled water, blotted with filter paper and weighed.

Histopathological analysis

Histopathological Analysis was carried out according to the method of Avwioro (2010).

Results

The photomicrograph of the liver of experimental animals showed a high power magnification (x400 mag) of the hepatocytes. Photomicrographs showed the
architecture and histo-morphological manifestation of the hepatocytes.

**Positive Control (Induced Untreated Group)**

Photomicrograph of a liver section stained by Haematoxylin and Eosin showed poor architecture, there was moderate to severe congestion of the portal vein (white arrow) as well as severe peri portal infiltration of inflammatory cells (black arrow), the liver parenchyma also showed area with destroyed liver plates with severe hemorrhage and necrosis (green arrow), the morphology of the hepatocytes showed degenerated liver cells (blue arrow), the liver parenchyma also showed focal area of moderate aggregate of inflammatory cells, the sinusoids appear normal and not infiltrated (slender arrow).

**Negative Control (Normal Control)**

Photomicrograph of a liver section stained by Haematoxylin and Eosin showed normal central venules without congestion (white arrow), the morphology of the hepatocytes appeared normal (blue arrow), the sinusoids appeared normal and not infiltrated (slender arrow), no pathological lesion seen.
3000mg/kg acetaminophen +50mg/kg *Vernonia amygdalina*

**LIVER (X400)**

Photomicrograph of a liver section stained by Haematoxylin and Eosin showing poor architecture, there was moderate to severe congestion of the portal vein (white arrow) as well as severe peri portal infiltration of inflammatory cells (black arrow) and the morphology of the hepatocytes show some degenerated liver cells (green arrow).

3000mg/kg acetaminophen +100mg/kg *Vernonia amygdalina*

**LIVER (X400)**

Photomicrograph of a liver section stained by Haematoxylin and Eosin showed normal central venules (white arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow), no pathological lesion seen.

3000mg/kg acetaminophen +200mg/kg Silymarin

**LIVER: normal liver tissue (X400)**
Histopathological effect of aqueous bitter leaf extract (Vernonia amygdalina)

Photomicrograph of a liver section stained by Haematoxylin and Eosin showing normal central venules and portal tract without congestion (white arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow), no pathological lesion seen.

**Discussion**

Histopathological changes of the liver tissue manifesting as, hepatocellular necrosis and lymphocytes infiltration are indicative of hepatotoxicity (Nabeshima et al., 2012). Severe histoarchitectural distortion of the liver parenchyma observed as diffused necrosis of hepatocytes, infiltration of the inflammatory cells and congestion of the portal vein in the liver section of Wistar rats treated with acetaminophen only when compared with the liver sections of the control is indicative of acetaminophen related hepatotoxicity. Necrosis is a type of cell death that occurs after abnormal stresses such as chemical injury or toxin. Necrotic cells are unable to maintain membrane integrity as they leak out their content and this may elicit inflammation in the surrounding tissue (Kumar et al., 2010). This result is in accordance with reports on CCl₄ administration namely, loss of the normal liver histo-architecture and cytoto-architecture (Soufy et al., 2012). Inflammation is a protective response, the ultimate goal, which is to get rid of the initial cause of the cell injury (e.g., acetaminophen, toxin; CCl₄) and the consequence of such injury (necrotic cells and tissues) (Kumar et al., 2010). Histo-architectural distortion, such as, infiltration of inflammatory cells and congestion, a local increase in the volume of blood as a consequence of impaired outflow from the hepatic tissue, were observed in the liver section of rats treated with acetaminophen. Histopathological changes were, however, mild in group treated with 50mg/kg Vernonia amygdalina when compared with the liver sections of the group administered acetaminophen only as the cyto-architecture of the liver parenchyma was preserved. Treatment with Vernonia amygdalina at 50 mg/kg followed by the administration of acetaminophen revealed histo-architectural preservation of the liver parenchyma against acetaminophen intoxication manifesting as mild histopathological changes when compared with the severely damaged liver sections of acetaminophen treated group. Administration of the extract Vernonia amygdalina at 100 mg/kg dose and Silymarin at 200mg/kg dose improved the histo-architecture of the liver and by extension restored its functionality. The groups administered with Vernonia amygdalina at (100 mg/kg) dose and Silymarin at (200mg/kg) dose demonstrated a distinct regenerative capacity over the other 50mg/kg Vernonia amygdalina extract. Histoarchitectural distortion, such as, hepatocellular necrosis, central vein congestion and inflammatory cells infiltration observed in the liver sections is resultant of acetaminophen intoxication, while observed histoarchitectural preservation is consequent to treatment with plant extract Vernonia amygdalina at 100 mg/kg and silymarin at 200mg/kg. This finding is supportive of medicinal plant related studies that have reported hepatoprotective activity of plant extracts; (Al-Qarawi et al., 2012) treatment with plant extract decrease the severity of histopathological changes induced by CCl₄. Silymarin is a well-established hepatoprotective drug and used as a reference drug for comparison of hepatoprotective activity of plants principle (Pradhan et al., 2011). The ability of Silymarin in preventing drug induced hepatotoxicity is associated with its ability to act as a radical scavenger, thereby
protecting membrane permeability (Song et al., 2016). Extract Vernonia amygdalina at (100 mg/kg) dose presented histoarchitectural preservation of the liver parenchyma when compared with the standard drug, Silymarin-treated group. Treatment 100mg/kg dose demonstrated the plant extract potentials as a free radical scavenger and lipid peroxidation inhibitor, thus helping to maintain the integrity and permeability of cell membranes and protects cells and tissues against oxidative stress induced by free radicals (Naik et al., 2017).

Conclusion and Recommendation
The result of this study suggested that Vernonia amygdalina was effective histologically in preventing acetaminophen-induced acute liver injury in Wistar rats, especially at dose of 100 mg/kg body weight. The hepatoprotective activity of the plant extract which could be of therapeutic potentials might be consequent to the antioxidative activities of constituent phytochemicals. Therefore, it is recommended that extract of Vernonia amygdalina can act as an adjunct in chronic treatment of liver injury.

References
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