Immunoexpression of cell proliferation (Ki-67) and tumor suppressor (p53) proteins in hepatic tissue exposed to aqueous extracts of Ageratum conyzoides Linn using rat model

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ABSTRACT

Objective: Medicinal plant studies have documented diverse pharmacological applications and biological activities of different parts of such plants but still with insufficient knowledge about molecular mechanisms of applications and activities. This study was therefore carried out to assess the expression of molecular markers of cell cycle and proliferation (Ki-67 and p53 proteins) in hepatic tissue of rats exposed to aqueous leaf extracts of A. conyzoides L. and possible effects of exposure on hepatic tissue proliferative ability.

Methods: This study involved 20 male Wistar rats (170-180g) divided into four groups (A-D), groups A was non-treated control while groups B-D were administered aqueous extracts of A. conyzoides L. at dose rate of 100, 300 and 500 mg/kg b.wt. orally for 28 days. After the treatment period, animals were sacrificed, liver tissues were harvested and processed, tissue sections produced were stained with histological staining (H & E) techniques and immunohistochemically stained for cell proliferation (Ki-67) and tumor suppressor (p53) protein with HRP-DAB staining technique (using monoclonal antibody). Stained sections were analyzed using image-J software. Data obtained were statistically analyzed using IBM-SPSS (version 20) and compared using T-test and analysis of variance (ANOVA).

Results: The result of this study showed significant decrease ($p<0.05$) in the Ki-67 protein expression level but no significant difference in the p53 protein expression level in hepatic tissues of all treatment groups relative to the non-treated group.

Conclusion: The aqueous extracts of A. conyzoides L. exhibit significant down-regulatory effect on the expression of cell proliferation (Ki-67) protein in hepatic tissues of study animals and this may indicate prominent anti-proliferative activity of the extracts.

KEY WORDS: Ki-67, p53
Liver
Rats
Ageratum conyzoides

Introduction

Medicinal plants refer to plants that have parts or the entire plant with constituent chemical components that can be applied for therapeutic purposes [1, 2]. One of these plants which represent an important component of natural plant biodiversity in many tropical and temperate regions is Ageratum conyzoides Linn (Asteraceae) (commonly called goat weed). A. conyzoides L. is a tropical medicinal plant with an age-long history of varying ethno-pharmacological applications [3, 4]. Numerous studies have also documented diverse pharmacological applications and biological activities of extracts of A. conyzoides L including anti-proliferative, anti-inflammatory, anti-oxidant and so on [5, 6]. Basically, proliferation of cells is a vital biological process which is regulated by inter-related factors to ensure normal tissue growth and development. The maturation of tissues is usually attained when there is a balance between cell proliferation and death. However, studies have linked tissue pathology and aberrant cell proliferation that results from genomic mutations and mutant gene expression [7, 8, 9]. A major self-regenerating tissue with immense ability to proliferate is the liver.

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The hepatic tissue is largely composed of hepatocytes which possess rapid proliferative ability that in turn accounts for hepatic regenerative ability following partial hepatectomy or tissue necrosis due to hepatotoxin exposure

[10, 11]. The process of hepatic tissue regeneration involves increased proliferation and differentiation of stem cell population which contains oval cells that proliferate at the peri-portal regions and invade the hepatic lobules to differentiate into hepatocytes and biliary epithelium [12, 13, 14]. The process of cell proliferation can be assessed using marker of cell cycle and proliferation – the Ki-67 protein which is a nuclear protein that is expressed in all phases of the cell cycle except the resting (G0) phase in quiescent cells or during DNA damage repair [15, 16]. Over-expression of Ki-67 gene has been implicated in malignancies of most tissues whereby Ki-67 positive pathologic cells become predominant and constitute the proliferative zone [17]. Similarly, the guardian of the genome, the tumor suppressor p53 protein [18] is also a nuclear protein that exhibit vital regulatory role during different events of cell cycle including proliferation, differentiation and death [19, 20]. The mutation of p53 gene had been described as most common cause of tissue malignancy whereby the mutated p53 has not only lost its function as tumor suppressor; it can also function as an oncogene [21, 22]. This study was therefore carried out to assess the expression of molecular markers of cell cycle and proliferation (Ki-67 and p53 proteins) in hepatic tissue of rats following treatment with aqueous leaf extracts of A. conyzoides L. and determine the possible effects of the treatment on hepatic tissue proliferative ability.

Materials and methods

Plant Material

Ageratum conyzoides L. plant was obtained from the Isihor community, Benin City, Edo State, Nigeria. A sample of the plant was identified at the Herbarium, Department of Plant Biology and Biotechnology, University of Benin, Nigeria and documented with a voucher number 344.

Method of extraction

The leaves of plant were detached, air-dried and pulverised into powdered form using electrical blender. 1000 grams of the powdered leaves was dissolved in 5 litres of distilled water for 72 hours. The preparation was then filtered using Whatman's filter paper and evaporated to dryness using rotary evaporator. The residue was cooled at room temperature, weighed and used as aqueous extracts for this study.

Experimental Design

This study involved 20 male Wistar rats weighing between 170g – 180g and grouped into four groups (A – D). Group A (n=5) were given orally distilled water (5mls/kg body weight). This group represented non-treated control. Group B (n=5) were given orally 100mg/kg aqueous extracts of A. conyzoides L. Group C (n=5) were given orally 300mg/kg aqueous extracts of A. conyzoides L. Group D (n=5) were given orally 500mg/kg aqueous extracts of A. conyzoides L. The treatment period of this study was 28 days and afterwards, experimental animals were sacrificed and liver tissue harvested and prepared for tissue processing. All procedures in this study conformed to the International Standard for experimental animal handling.

Tissue Processing, Sectioning and Staining

The liver tissues were fixed in 10% neutral buffered formalin, dehydrated using alcohol, cleared in xylene and embedded in molten paraffin to form tissue blocks. Blocks of tissue were cut into sections at 3µ and 5µ thickness by using rotary microtome and mounted on microscope slides. The 5µ-thick sections were used for the histological – haematoxylin and eosin (H and E) staining technique while the 3µ-thick sections were used for the immunohistochemical – Horseradich-peroxidise-3, 3-Diaminobenzidine (HRP-DAB) staining technique for ki-67 and p53 proteins using monoclonal antibody.

Photomicrography

Photomicrographs were generated from stained microscope slides using 10MP digital camera for microscope. All photomicrographs examined under microscope and immunostained (HRP-DAB) sections were analyzed using image-J software to quantify the distribution of ki-67 and p53 proteins. All data obtained were recorded and analyzed.

Statistical Analysis

The values recorded were analyzed using IBM-SPSS (version 20) and presented as mean ± SEM. Relevant statistical values were derived using T-test and one-way analysis of

variance (ANOVA). ($P < 0.05$ was considered as statistically significance level).

Results

The histological results of this study showed the histo-architecture of hepatic tissue of experimental animals in non-treated and treated animals (groups A – D) with densely packed hepatic parenchyma and prominent central vein (figure 1). Also, immunohistochemical results showed the distribution of cell proliferation (Ki-67) protein (figure 2) and tumor suppressor (p53) protein (figure 3) of non-treated and treated animals (groups A – D). The protein distribution counts using image-J software were shown in Tables 1 & 2 and Figures 4 & 5.

Table 1. Mean and standard error of mean (SEM) of Ki-67 distribution count in hepatic tissues of non-treated and aqueous extracts-treated groups (groups A-D).

GROUPS	Mean ± SEM
A	9.33 ± 0.88
B	5.67 ± 0.67 *
C	4.00 ± 0.58 *
D	6.67 ± 0.88 *

Table 2. Mean and standard error of mean (SEM) of p53 distribution count in hepatic tissues of non-treated and aqueous extracts-treated groups (groups A-D).

GROUPS	Mean ± SEM
A	6.00 ± 0.58
B	5.67 ± 0.88
C	5.33 ± 0.67
D	5.67 ± 0.88

* $P < 0.05$ was accepted as significant relative to group A

Discussion

The liver is a very active, self-regenerating tissue with rapid proliferation rate especially to constantly recover lost or worn-out cells. In cases of major hepatic tissue injury or resection, studies have shown that the remaining hepatocytes proliferate rapidly to restore hepatic tissue to its original structure, weight, size and function [23, 24]. The nuclear protein (Ki-67) is widely described as marker of proliferation because of its characteristic expression in all phases of cell cycle of proliferating cells. As a marker of cell proliferation, Ki-67 protein distribution can be used to access proliferation of hepatocytes and up-regulation of its expression highlights the rapid proliferative characteristic of hepatic tissue [24].

Figure 1. Photomicrograph of hepatic tissue section of non-treated and aqueous extracts-treated experimental animals (groups A-D) showing normal histomorphology with densely packed parenchyma and prominent central vein (H&E X100).

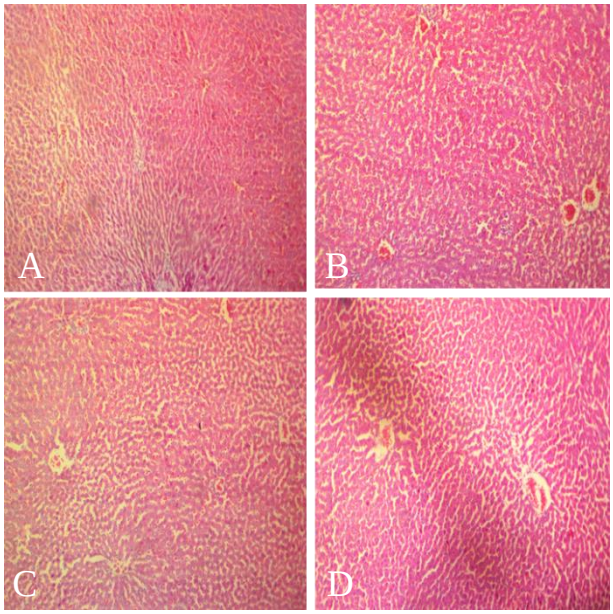
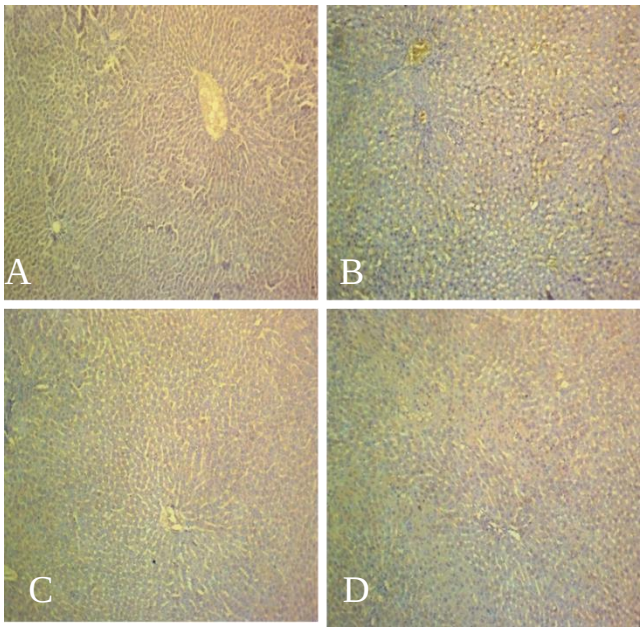


Figure 2. Photomicrograph of immuno-stained hepatic tissue sections of non-treated and aqueous extracts-treated experimental animals (groups A-D) showing Ki-67 protein distribution in the hepatic parenchyma (HRP-DAB X100).



According to the result of this studyF (Table 1, Figure 4), exposure to aqueous extracts of A. conyzoides L. results into significant ($P < 0.05$) reduction of cell proliferation (Ki-67) protein expression in hepatic tissues of all treatment groups relative to the expression level of the non-treated group. This outcome highlights the possible anti-

proliferative activity of the extracts in hepatic tissues of the study animals.

Figure 3. Photomicrograph of immuno-stained hepatic tissue sections of non-treated and aqueous extracts-treated experimental animals (groups A-D) showing p53 protein distribution in the hepatic parenchyma (HRP-DAB X100).

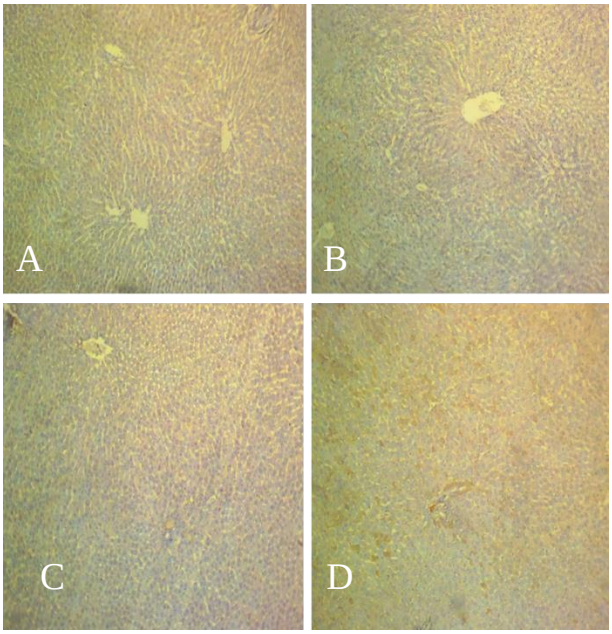
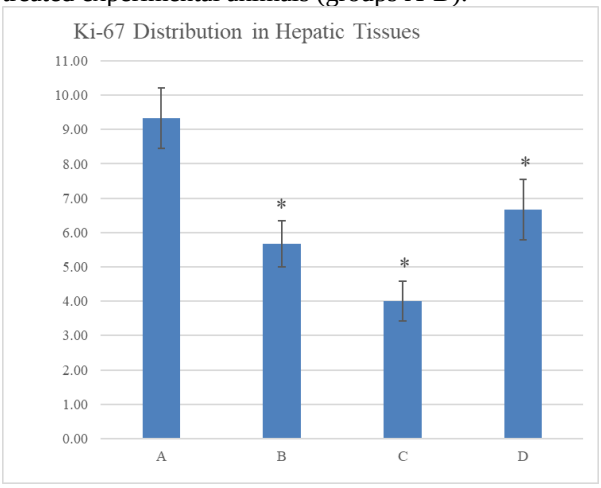
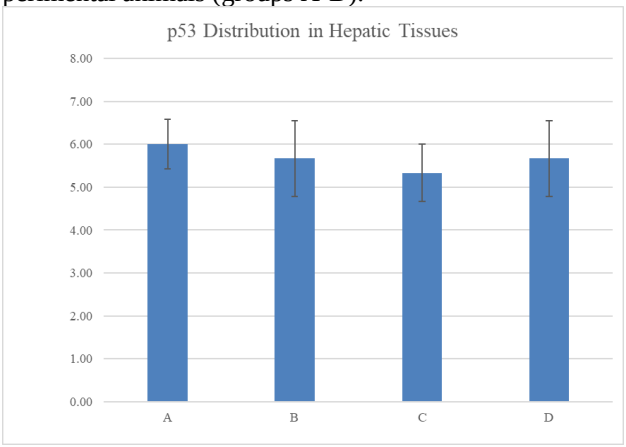


Figure 4. chart showing the mean Ki-67 distribution count in hepatic tissues of non-treated and aqueous extracts-treated experimental animals (groups A-D).



Furthermore, the tumor-suppressor (p53) protein is a product of tumor-suppressor gene whose expression is up-regulated by DNA damage and function to arrest the progression of cell cycle to allow repair of genomic alterations. In case of widespread genomic damage or impairment of DNA repair machinery, p53 protein stimulates apoptosis for affected cells through up-regulation of pro-apoptotic proteins (such as Bax, Bid, Noxa and others) or death ligands and receptors (such as Fas and TRAIL) to prevent accumulation of genomic distortions and division of genetically impaired cells [24, 25].

Figure 5. chart showing the mean p53 distribution in hepatic tissues of non-treated and aqueous extracts-treated experimental animals (groups A-D).



According to the result of this study (Table 2, Figure 5), the exposure to aqueous extracts of A. conyzoides L. results into no significant changes of tumor suppressor (p53) protein expression in hepatic tissues of all treatment groups relative to the non-treated group. This outcome highlights no genomic deleterious effects in hepatic tissues of the study animals following exposure to the aqueous extracts of A. conyzoides L.

The aqueous extracts of A. conyzoides L. exhibit significant down-regulatory effect on the expression of cell proliferation (Ki-67) protein in hepatic tissues of study animals and this may indicate prominent anti-proliferative activity of the extracts.

Conflict of Interest

We declare that we have no conflict of interest.

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