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Research Article

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Assessment of the antiproliferative potential of *Cissampelos owariensis* (P. Beauv) methanolic extract in Wistar rats

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ABSTRACT

Cissampelos owariensis is a medicinal plant with a wide range of therapeutic uses. In this study, the objective was to further assess its antiproliferative potential using cell proliferation and tumor suppressor markers. Solvent extraction of the plant leaves was done using methanol. Twenty (20) male albino Wistar rats were randomly divided into four groups 1–4 (n=5) and respectively administered with methanolic extracts of *C. owariensis* at 0, 100, 300 and 500 mg/kg for 30 days. After treatment, the hepatic tissues were processed and examined histologically and immunohistochemically for cell proliferation (Ki-67) and tumor suppressor (p53) proteins. Immunoexpression of the proteins was quantified using image-J software, the data analyzed with SPSS version 20 and values compared using t-test and one-way analysis of variance. The histological results showed no significant variation in hepatic histomorphology of treated Groups 2–4 relative to non-treated Group 1. However, the immunohisto chemical results showed significant ($p< 0.05$) down-regulation in Ki-67 protein expression and a concomitant significant ($p< 0.05$) up-regulation in p53 protein expression in hepatic tissues of treated Groups 2–4 relative to non-treated Group 1.These inverse expression patterns of cell proliferation and tumor suppressor proteins following exposure to methanolic extracts of *C. owariensis* may suggest the antiproliferative potential of the plant extracts.

Keywords: *Cissampelos owariensis*, Antiproliferative potential, Wistar rats

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INTRODUCTION

Medicinal plants are plant with intrinsic therapeutic properties either on the whole or in their parts. These therapeutic properties confer on them high medicinal value especially in developing countries where assess to modern health care remains a challenge for many individuals. Moreover, these easily assessable medicinal plants exhibit significant efficacy and tolerability with insignificant side effects (Okigbo *et al.*, 2008; Adelanwa and Tijani, 2013; Omotoso *et al.*, 2019a; Omotoso *et al.*, 2019b). They have widespread distribution in different parts of the world, constitute vital component of natural plant biodiversity and are actively applied for variety of ethnopharmacological uses (Adelanwa and Tijani, 2013; Omotoso *et al.*, 2020c; Omotoso *et al.*, 2020d). One of such ethnopharmacological applications is their usage as antiproliferative or antitumor or anti-cancer agent. The application of medicinal plants as anti-proliferative or anti-tumor agent in the treatment of cancer is globally on the rise. According to Carraz *et al.*, (2015) different medicinal plant species especially those commonly used for gastric and hepatic tissue related disorders also exhibit antiproliferative effects. Some examples of medicinal plants with documented anti-proliferative effect include *Indigofera aspalathiodes* (Kumar *et al.*, 2012), *Stemona collinsae* (Manosrol *et al.*, 2015), *Annona muricata* (Asare *et al.*, 2015), *Rosmarinus officinalis* (Cattaneo *et al.*, 2015), *Bellis perennis* and *Concolculus galaticus* (Karakas *et al.*, 2015), *Physalis peruviana* (El-kenawy *et al.*, 2015) and *Ageratum conyzoides* (Omotoso and Eze, 2019f).

Cissampelos owariensis (*C. owariensis*) is a tropical, climber or twiner plant found in certain parts of West African countries where it is actively applied in native traditional medicines (Akande *et al.*, 2013). It belongs to the Menispermaceae family of plants which is composed of about 70 genera and over 400 other plant species. Its taxonomic classification includes phylum- Tracheophyta; class- Mangoliopsida;

order- Ranunculales; genus- *Cissampelos* and specie- *owariensis* (Ekeanyanwu *et al.*, 2012). As a medicinal plant, different parts of *C. owariensis* especially the roots, leaves and rhizomes, have been applied for various ethnopharmacological uses. These include treatment of miscarriages and sterility, treatment of wounds, reproductive diseases especially female infertility, gastrointestinal diseases (like dysentery, diarrhoea and enteritis) headache and fever especially typhoid and malaria fevers (Abbiw, 1990; Neuwinger, 2000; Schmelzer, 2008; Erhirhie *et al.*, 2015). However, there is virtually no information on the antiproliferative activity of the plant at the moment. This study was therefore aimed at assessing the anti-proliferative potential of methanolic extract of *C. owariensis* (P. Beauv.) based on the expression of molecular markers for cell proliferation and tumor suppression (Ki-67 and p53 proteins) in hepatic tissue of adult male Wistar rats following exposure to the plant extract.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh *C. owariensis* plant was obtained from suburb of Okada community in Edo State of Nigeria and verified at the Department of Pharmacognosy, Igbinedion University, Okada, Edo State, Nigeria. The bulk quantity of the plant, sufficient for the study, was collected after the verification and prepared for extraction.

Preparation of Methanolic Extract of *C. owariensis*

The detached leaves of the plant were air-dried, pulverised into powdered form, weighed and macerated in methanol for 72 hours with intermittent agitation. The preparation was filtered and evaporated to dryness using a rotary evaporator. The residue was allowed to cool, weighed and used as the methanolic extracts used for this study.

Experimental Protocols

This study involved 20 adult male Wistar rats weighing between 150g - 180g. They were equally divided into four groups (1-4) comprising of five animals per group. Group 1 was administered with distilled water (5 mL/kg body weight) and represented the control group. Groups 2 - 4were administered with 100mg/kg, 300 mg/kg and 500 mg/kg methanolic extract of *C. owariensis* respectively. The selected dosages of extracts for this study were considered safe without toxic effects (Ekeanyanwu *et al.*, 2012).Administration of methanolic extract of *C. owariensis* in this study was done orally using flexible orogastric gavage and lasted for thirty days. After the period of administration, experimental animals were sacrificed via and hepatic tissues harvested and processed.

Tissue Processing

The fixation of hepatic tissue of experimental animals was done using 10% Neutral Buffered Formalin. Further processing involved dehydration using ascending grades of alcohol (which include 70%, 90% and 100% alcohol for 30 minutes each) and clearing was done using xylene for 30minutes. After tissue processing, the hepatic tissues were embedded in molten paraffin and allowed to cool to form tissue blocks in preparatory for sectioning and staining.

Tissue Sectioning and Staining

Blocks of hepatic tissue were cut into sections by using rotary microtome set at 5∝ and 3∝ thickness and sections obtained were mounted on the microscope slides. The 5∝ sections were used for histological staining with Haematoxylin and Eosin (H&E) technique while the 3∝ sections were used for immunostaining of Ki-67 and p53 proteins using monoclonal antibody with Horseradich-peroxidise -3,3-Diaminobenzidine (HRP-DAB)technique.

H & E technique

The 5 µ sections were dewaxed in xylene for 15 minutes and hydrated with descending grades of alcohol (using 100%, 90% and 70% alcohol and distilled water). The tissue sections were stained in Haematoxylin for 10 minutes and rinsed under running tap water for 2 minutes. Tissue sections were differentiated in 1% acid alcohol for 1 minute, blued in Scott's tap water for 10 minutes and rinsed in water. Tissue sections were stained in Eosin for 3 minutes and rinsed in water. Tissue sections were dehydrated with ascending grades of alcohol (using 70%, 90% and 100% alcohol for 2 minutes each), cleared in xylene for 2 minutes and mounted with distrene polystyrene xylene (DPX) (Fischer *et al.*, 2008).

HRP–DAB technique

The 3 µ thick hepatic tissue sections were hydrated and antigen retrieval was performed by using Citric acid solution (pH 6.0) in a microwave at power 100 Watts and equilibrated by gently displacing hot Citric acid with running tap water. Endogenous peroxidases in sections were blocked using peroxidase block and sections rinsed in phosphate buffer saline (PBS).The tissue sections were treated with Nevocastra protein block, rinsed with PBS, incubated in primary antibody (1/100 dilution ratio) and rinsed with PBS. They were treated with secondary antibody and rinsed with PBS. Polymer was added to sections, allowed to dry for 15 minutes and rinsed twice with PBS. Tissue sections were treated with 3, 3-Diaminobenzidine (DAB) substrate (1/100 dilution ratio) and rinsed with water. Tissue sections were counterstained with Haematoxylin and rinsed in water. Tissue sections were dehydrated, cleared in xylene and mounted with DPX (Omotoso *et al.*, 2019g).

Photomicrography

All stained tissue sections were examined under the microscope and photomicrographs of all sections were produced using a 10MP digital camera for the microscope. The photomicrographs of histological sections were examined for observable histological changes and immunostained photomicrographs were analyzed using image-J software (NIH, USA) to quantify the distribution

of cell proliferation (Ki-67) and tumor suppressor (p53) proteins. All values obtained were recorded for statistical analysis.

Statistical Analysis

Recorded values were statistically analyzed using IBM-SPSS version 20 (IBM Corp, USA) and presented as mean ± standard error of mean (SEM). Relevant statistical values were derived using a *t*-test and one-way analysis of variance. $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Histological Results

The histological profile of hepatic tissues of experimental animals in the treated groups 2–4 showed relatively normal histo-architecture which compares with the normal hepatic histomorphology of the control group 1 animals (Figure 1).

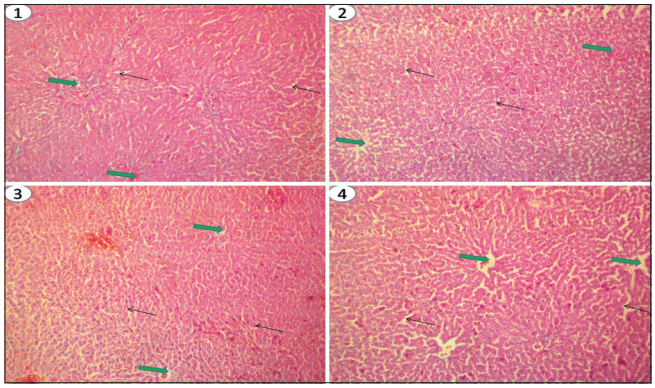

Figure 1: Histological sections of hepatic tissue of controlgroup 1 and treated groups 2-4 (treated with 100, 300 and 500 mg/kg methanol extract of *C. owariensis*, respectively)(H&E X100). Black and green arrows indicate hepatic sinusoids and central veins respectively

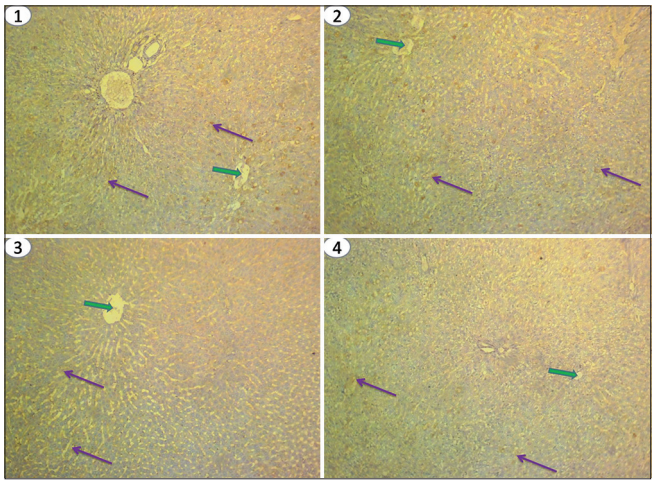

Figure 2: HRP-DAB immunostained section of hepatic tissue of control group 1 and treated groups 2-4 (treated with 100, 300 and 500 mg/kg methanol extract of *C. owariensis*, respectively) (HRP-DAB X100). Purple and green arrows indicate Ki-67 positive sites and central veins respectively

Immunohistochemical Results

The microscopic examination of immunohistochemical sections showed the distribution of Ki-67 and p53proteins in the parenchyma of hepatic tissues of experimental animals in control group 1 and treated groups 2-4 as indicated by typical dark-brown colouration that implied the colour product of HRP-DAB immuno-reaction (Figures 2 and 3). Further quantitative analysis showed the distribution of the Ki-67 and p53 proteins (Figure 4).

Medicinal plants are plants with intrinsic therapeutic properties which can be efficiently applied in the treatment and management of disease conditions. The diverse therapeutic activities of medicinal plants extracts or herbal preparation are essentially a function of their constituent phytochemicals which

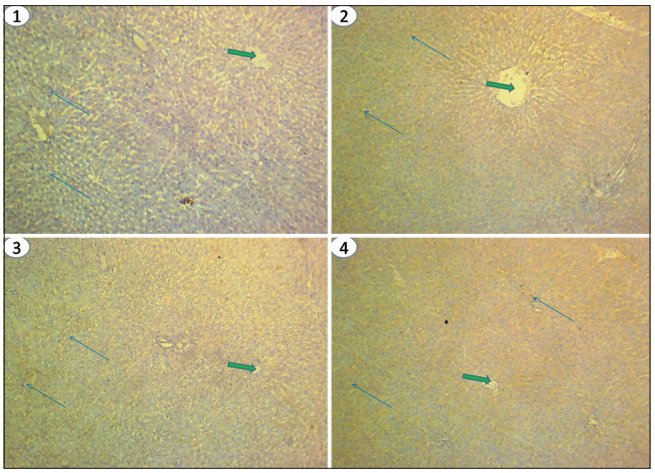

Figure 3: HRP-DAB immunostained section of hepatic tissue of non-treated control group 1 and groups 2-4 (treated with 100, 300 and 500 mg/kg methanol extract of *C. owariensis*, respectively) (HRP-DAB X100). Blue and green arrows indicate p53 positive sites and central veins respectively

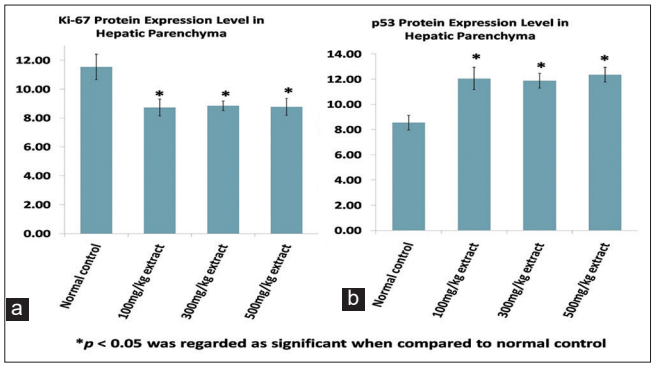

Figure 4: Distribution of Ki-67 (a) and p53 (b) proteins in the hepatic parenchyma of experimental animals treated with 100, 300 and 500 mg/kg methanol extract of *C. owariensis*, respectively

are basically their secondary metabolites often referred to as bioactive components or active principles (Duraipandiyan *et al.*, 2006; Omotoso *et al.*, 2020e; Banso and Adeyemo, 2007). These phytochemicals primarily confer medicinal value or therapeutic (including antiproliferative) activity on medicinal plant extracts or herbal preparations. In a study by Halabi and Shelkh (2014), the concentration dependent trend of antiproliferative effect of *Cymbopogon citrates* extracts was correlated with the phytochemical constituents of the plant. Similarly, the study by Amar *et al.*, (2017) using phenolic extracts of *Rosmarinus officinalis* highlighted the antiproliferative effects of the extract as a function of its phytochemical properties especially the antioxidant activity of phenolic compounds.

According to the findings of Ekeanyanwu *et al.* (2012), the alcoholic extract of *C. owariensis* contains a very high concentration of flavonoids, saponins, tannins and fairly high concentration of alkaloids. These phytochemical compounds especially flavonoids and tannins have been described to exhibit diverse biological activity including antioxidant and antiproliferative effects (Altemimi *et al.*, 2017; Singh *et al.*, 2017). As earlier noted and in other studies by Bonofiglio *et al.*, (2016) and Kabir *et al.*, (2017), the antioxidant activity of medicinal plant extracts represents one of the molecular bases of their antiproliferative or anti-tumor effects. Furthermore, from the results of this study, the histological profile of hepatic tissues of treated experimental animals in Groups 2-4 showed no significant difference from the non-treated Group 1 animals (Figure 1). This implied no prominent changes in the hepatic histomorphology of the experimental animals following exposure to the plant extract. However, the immunohistochemical evaluation of proliferation markers showed a significant ($p < 0.05$) reduction in Ki-67expression and concomitant significant ($p < 0.05$) increase in p53 expression in the hepatic tissues of treated groups 2-4 animals compared to the control group 1 animals (Figure 4).

These results revealed an inverse expression pattern of Ki-67 and p53 proteins in the hepatic parenchyma of experimental animals following exposure to the plant extract. Essentially, these protein molecules, which are among the key markers of cellular proliferation and inducer of apoptosis respectively, have been indicated in molecular mechanisms of antiproliferative activity of medicinal plants (Kooti *et al.*, 2017). On the whole, the mechanisms of antiproliferative effect of medicinal plants involve the ability of their constituent bioactive components to inhibit proliferation and/or activate apoptotic pathways which are of interest during prevention and treatment of tumorigenesis or carcinoma (Leelawat and Leelawat, 2017; Wang *et al.*, 2019). The cell proliferation Ki-67 is one of the typical molecular markers of cellular proliferation which is expressed in all phases of cellular division within the nucleus of normal or neoplastic cells (Kaya *et al.*, 2015; Jurikova *et al.*, 2016). The inhibition of cellular proliferation signals leading to arrest of cell cycle and activation of cellular death signals leading to cellular death has been described as the major sub-cellular mechanisms of antiproliferative activity in tissues.

According to the studies by Kim *et al.*, (2015) and Sdiri *et al.*, (2018), antiproliferative mechanisms of medicinal plants include inhibition of AKT/mTOR and Wnt signalling pathways as well as down-regulation of c-myc expression and activation of cellular death pathways. Essentially, the down-regulation or inhibition of AKT/mTOR, arrest of cell cycle progression and induction of apoptosis have been associated with up-regulation of p53 expression (Li *et al.*, 2019). Studies by Turan *et al.*, (2017) and Kumnerdkhonkaen *et al.*, (2018) also described the antiproliferative mechanisms to include reduction of cell viability, inhibition of cell cycle progression and induction of autophagy or apoptosis and findings by Koutsoulas *et al.*, (2019) and Daddiouaissa *et al.*, (2019) further highlighted antiproliferative mechanisms to involve arrest of the cell cycle at G_0/G_1 or G_2/M phase. Zhang *et al.*, (2019) also noted that cellular proliferation inhibition, arrest of cell cycle at G_1 phase and induction of apoptosis due to an up-regulation of wild type p53 expression culminated in antiproliferative activity of anti-tumor agents. In essence, the inverse expression patterns which include the down-regulation of cell proliferation Ki-67 and up-regulation of tumor suppressor p53 protein observed in this study may indicate the antiproliferative potential of the methanolic extracts of *Cissampelos owariensis* (P. Beauv.). However, further studies with tumorigenic cells *in vivo* and tumorigenic cell lines *in vitro* are required to ascertain the anti-proliferative activity of the plant extract and its underlying mechanisms.

CONCLUSION

The concomitant down-regulation of cell proliferation Ki-67 and up-regulation of tumor suppressor p53 protein expression in the hepatic parenchyma of treated experimental animals observed in this study may indicate antiproliferative potential or possible antiproliferative effect of methanolic extracts of *Cissampelos owariensis* (P. Beauv).

COMPETING INTERESTS

The authors declare no competing interests in this study.

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