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Research Article

Cissampelos capensis and *Pleiocarpa pycantha* Extracts Protect against N-nitrosodiethylamine-induced Hepatotoxicity in Wistar Rats

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Abstract

Pleiocarpa pycantha (PP) and *Cissampellos capensis* (CC) showed potential phytoprotection in vitro. We then investigated this effects against N-diethylnitrosamine (DEN) induced hepatic tumors and renal function in rat (n=5), treated as follows for 60 days: (1) Control (2) DEN, (3) DEN+PP (4) DEN+CC (5) DEN+PP+CC (6) PP, (7) CC only and group (8) PP+CC. Rats were sacrificed and organs harvested for Biochemical and histological examination. DEN-induced hepatic tumors were counted and measured, liver sections processed for histopathology. Furthermore, markers of oxidative stress, hepatotoxicity and renal function including superoxide dismutase, aspartate transaminase, and creatinine were assessed in serum, in addition to α-fetoprotein and blood glucose levels. DEN treatment resulted in weight loss; elevated serum levels of hepatic transaminases, α-fetoprotein and induction of T2-4 (tumour count) in experimental animals. Tumour incidences were reduced to T1-2, T1-2 and T1 in the presence of PP, CC and PP+CC co-treatment respectively. Histological investigation revealed increased growth in hepatic stellate cells and early fibrosis in DEN only treated group, which were partly reversed by PP and CC. Significant increase in blood glucose level was observed in DEN treated animals compared to PP and CC treated groups. In addition, DEN treated group were low in cellular antioxidant activity relative to other groups, which was elevated in the presence of PP and CC. Our findings indicated that PP and CC co-treatment alleviated DEN-induced hepatic hepatotoxicity in exposed rats, reinforcing earlier in vitro findings. PP and CC phytoprotective effect maybe relevant in protecting the liver against chemical carcinogens.

Key Words: N-Nitrosodiethylamine, *Cissampellos capensis, Pleiocarpa pycantha*, tumorigenesis, hepatic tumors and toxicity.

INTRODUCTION

Nitrosamine and the mechanism of their toxic action have been the focus of attention for several years (Ames 1983; Jen 1994; Pflaum *et al*., 2016) with respect to its carcinogenic role in rodents and humans. The presence of nitrosamine in food is considered a risk factor because of their implication in causing gastrointestinal cancers in addition, to pharyngeal, esophageal, stomach, pancreatic and colorectal cancers (Jen 1994; Chhabra *et al*., 1996; Magee 1996) N-diethylnitrosamine (DEN) is a potent hepatotoxin and if co-administered with promoters like phenobarbital can result in cancer of the liver (Brown 1999; Sullivan *et al*., 1991) DEN can be present in cigarette smoke, residential heating units, power plants and in certain occupational settings (Bostrom *et al*., 2002; Lucier and Hook 1986) . DEN-mediated production of reactive oxygen species (ROS) has direct effect on cell development, growth and survival (Parola and Robino 2001)

Medicinal plants from time immemorial have been in use for treatment of various ailments all over the world (Satyanarayana 1969) . Recent advances emphasize the benefit of functional food derived from plant sources, containing phytochemicals that can be useful as chemopreventive agent against toxicity induced by a wide range of chemical substances (Ferrari *et al*., 2011; Kumar and Pandey 2013; Komakech *et al*., 2017). including DEN. The focus of several studies has been on the functional nutrients that protect or can help prevent damage caused by genotoxic substances (Odin 1997) and their secondary metabolites. Foods such as fruits and vegetable are rich in beneficial phytochemicals including vitamins, selenium, phenolic and flavonoids which can be protective against the onset of human cancers (Peto *et al*., 1981) and other disease development and act as chemopreventive agents in some cell lines (Yu *et al*., 1994). Several phytochemicals derived from edible plant sources have been reported to interfere with a specific stage of the carcinogenic process. However carotenoids can increase the toxic effect of some compounds in certain organs (Peterson 1996) or be completely ineffective in cancer chemoprevention (Astrog *et al*., 1996). Many mechanisms have been shown to account for the anti-carcinogenic actions of dietary constituents, but attention has been focused, on intracellular-signalling cascades as common molecular targets for various chemopreventive phytochemicals (Surh *et al*., 1995). Furthermore, plant products have attracted increased attention for therapeutic intervention in malignant, and neoplastic diseases (Trendowski 2015), plant-derived compounds such as Vincristine, Vinblastine, Irinotecan, Etoposide are used for cancer treatment (Sreepriya and Bali 2005). As such identification of novel medicinal plant sources and their bioactive ingredients to inhibit tumorigenesis in a variety of

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animal models is gaining considerable attention (Aggarwal *et al*., 2011) that can be extended for therapeutic uses in human. Here we focus on two plants: (1) Cissampelos capensis (Menispermaceae) (CC), well known for their medicinal uses, associated with their rich diversity of isoquinoline alkaloids (Barbosa-Filho *et al*., 2000; De Wet and Van Wyk 2000).

Traditionally CC is used to treat a variety of ailments including glandular, ulcers and skin cancers (Van Wyk 2000).. Phytochemical screening shows that CC is rich in alkaloids (De-Freitas *et al*., 1995) . Some of these alkaloids have been implicated in antitumour activities (De Wet and Van Wyk 2000) (2) Pleiocarpa pycnantha (Apocynaceae) (PP), its widespread from the Sahara to southern Africa and popular in folk medicine for abating various ailment (Burkhill 1985). Some indole alkaloids such as pycnanthine, pleiocarpamine, quebrachamine and macusine have been isolated from PP roots and bark in which pleiocarpamine has demonstrated anticancer potential (Keawpradub *et al*., 1997)..

With the foregoing in mind we explored the potential effect of these medicinal plants on DEN-induced hepatotoxicity and hepatic tumorigenesis. We also examined the antioxidant status, lipid profiles and genotoxicity of CC and PP. Finally, we present the results of our in vivo findings on the effect of CC and PP derived stem extracts which has shown potential anti-carcinogenic properties in vitro, on DEN-induced hepatic tumors and their role in kidney function using albino Wistar rats as a model organism.

MATERIALS AND METHODS

Chemical and Assay kits : *N*-nitrosodiethylamine (DEN) was purchased from Sigma Chemical (St Louis, MO, USA). Randox™ diagnostic kit (Randox™ Laboratories, United Kingdom) used for the determination of hepatic transaminases - alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin assessment. All other reagents were of the highest quality available commercially.

Plant extracts: Extracts of *CC* and *PP* for this study was a generous contribution by a collaborator after extraction from the plant sources.

Plant Materials: Stem bark of *CC* and *PP* were sourced from the Noun region of Cameroon and South Africa respectively, and the specimens were appropriately authenticated in the Department of Botany, University of Ibadan, Ibadan. *CC* and *PP* were washed thoroughly and air-dried in the dark at room temperature before being pulverized separately using Hammer mill. *CC* (2000g) and *PP* (1000g each) were macerated in the cold using methanol in a ratio of 1:10 respectively for 72hrs. The resulting decoction was filtered with a Whatman No.1 filter paper to remove any residual cellulose fiber under pressure Buchner flask. Extract of *CC* and *PP* were concentrated using a rotary evaporator (Buchi Model 240) at 35^0C to dryness. The extracts of *CC* and *PP* were scrapped out of the round bottom flask and weighed with approximate yield for *CC* (12%w/w) and *PP* (18.7% w/w). The extracts were preserved in a refrigerator at 4^0 C until needed for further experiment.

Animals: Healthy male Wistar rats (120-150 g) purchased from the primate colony, University of Ibadan, Nigeria were used for this study. Experimental animals were acclimatized for seven days before commencement of the study**,** and were humanely treated in adherence to the conditions required for the Care and Use of laboratory experimental animals as stipulated by the National Institute of Health (NIH). All procedures were done following authorization by the University of Ibadan Ethical Committee. The animals were, declared disease-free with no obvious physical deformity, and were randomly grouped into eight group of 5 rats each. Each group was housed in polycarbonated plastic cages placed in a well-ventilated room. The animals were provided with standard rat pellets purchased from Ladokun™ feeds, Mokola, Ibadan, Nigeria and were provided water *ad libitum.* All experimental were subjected to natural photoperiod of 12-hour light: dark cycle, during acclimatization (7 days) and experimental (60days) period until they were sacrificed.

Dose response studies and Treatment protocol:: DEN (25µg/g body weight) was administered weekly by intraperitoneal (i.p.) injection following established protocol (Heindryckx *et al*., 2009). Doses of *CC* and *PP* -suspended in distilled water- used for all experiment were selected from a one-week dose response study using doses of *PP* and *CC* 50, 100 and 180 mg/kg body weight. There were no toxic responses in treated animals, limit food intake or abnormal body weight change in the dose response study (Data not shown). A dose of 180mg/kg body weight for *PP* and *CC* was therefore selected for further experimental purposes.

Group 1: *Control*
Group 2: *DEN: (25µg/g body weight) weekly by intraperitoneal (i.p.) injection.*
Group 3: *DEN + PP (180mg/Kg body weight) orally thrice weekly*
Group 4: *DEN + CC (180mg/Kg body weight) orally thrice weekly*
Group 5: *DEN+PP + CC (180mg/Kg body weight each) orally thrice weekly.*
Group 6: *PP (180mg/kg body weight) orally, thrice weekly*
Group 7: *CC (180mg/kg body weight) orally, thrice weekly*
Group 8: *PP+CC (180mg/kg body weight each) orally, thrice weekly*

Terminal point sacrifice of experimental animals: Twenty-four hours after the last treatment, the animals were sacrificed by cervical dislocation. Blood was collected in non-heparinized tubes by ocular puncture and allowed to clot for serum separation by centrifugation (4000g, for 15 minutes) with a table centrifuge. Liver were harvested quickly rinsed in ice-cold 1.15% potassium chloride solution, blotted dry, weighed, and processed for histopathological and biochemical assays. For biochemical assays, pieces of liver section were homogenized in ice-cold phosphate buffer, pH 7.4 weight/volume as required for specific biochemical assays. Liver-somatic index was estimated according to the formula: (rat liver weight / rat final body weight) x 100% and expressed as percentage.

Estimation of tumor burden, count and sizes: The incidences of DEN-induced tumors were counted macroscopically on each lobe of the liver and the sizes were calculated using a Delcast™ Digital Caliper Model (DCAL-02).

Assessment of α-fetoprotein, blood glucose : The serum α-fetoprotein content was quantified using the rat α-fetoprotein ELISA kit whereas blood glucose was assayed using glucometer according to manufacturers' instructions.

Biochemical analysis

Determination of serum ALT and AST : The quantitation of ALT and AST was carried out using Randox™ Assay kits. The serum ALT activity was determined as previously described (Reitman and Frankel 1957).

Determination of Bilirubin: Total bilirubin was determined by colorimetric method as described (Heinemann G, Vogt 1988).

Determination of reduced Glutathione (GSH), Glutathione peroxidase (GPX) activities, Catalase (Cat) and Superoxide dismutase (SOD): Reduced glutathione (GSH) was estimated by the method of Beutler *et al*., (1977) and glutathione peroxidase (GPX) activity as described by Rotruck *et al*., (1973) with modifications. Superoxide dismutase (SOD) activity was estimated by the method of Misra and Fridovich (1972).

Histopathology: Limited histopathological analysis was conducted on sections of liver tissue. Tissues were fixed in 10% phosphate buffered formalin for 24 hours, and subsequently embedded in paraffin following dehydration serially in an ethanol gradient followed by xylene. Sections of 5-µm thickness were cut, fixed on glass slides, de-paraffinized and rehydrated. Liver sections were stained with hematoxylin and eosin (H&E) and examined by Carl Zeiss light microscope.

Statistical analysis: All values were expressed as the mean ± S.D of five animals. Data were analyzed using one-way analysis of variance (ANOVA) of biochemical data using SPSS (10.0) statistical software and graph pad prism for the plotting of graphs. P values < *0.05* were considered statistically significant.

RESULTS

Effect of DEN, CC and PP treatment on organ and body weight and food intake: Table 1 shows changes in body weight in experimental animals post treatment expressed as weight gained and percentage increase in weight. These significant ($p<0.001$) increase in weight are more visible in the control group compared to DEN treated group only, where reduction in feed intake was noticeable, this trend in weight gained was observed in animals treated with extract (CC and PP) alone compared to the DEN and extracts co-treated group

Table 1:
Effect of Diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*) on body, organs and relative organ weight of male

	IBW (g)	FBW (g)	WC (g)	LW (g)	RLW (g)	KW (g)	RKW (g)
Control	199.00 ± 1.00	270.00 ± 2.82	70.00 ± 2.82	**7.28 ± 0.67**	**2.69 ± 0.12**	**0.54 ± 0.01**	**0.2 ± 0.01**
DEN	183.33 ± 2.88	229 ± 5.65	46.33 ± 1.52	**8.36 ± 1.55**	**3.65 ± 0.22**	**0.63 ± 0.01**	**0.28 ± 0.01**
DEN+*PP*	177.00 ± 2.82	193.33 ± 6.02	13.66 ± 1.52	**6.60 ± 1.37**	**3.41 ± 0.15**	**0.51 ± 0.01**	**0.26 ± 0.01**
DEN+*CC*	190.00 ± 11.54	194.50 ± 2.50	22.50 ± 7.77	**7.34 ± 0.60**	**3.77 ± 0.21**	**0.51 ± 0.01**	**0.26 ± 0.01**
DEN+*PP*+*CC*	171.66 ± 7.63	193.33 ± 6.02	13.66 ± 1.52	**7.30 ± 1.09**	**3.75 ± 0.17**	**0.54 ± 0.01**	**0.28 ± 0.01**
PP	190.00 ± 14.4	228.00 ± 9.89	52.50 ± 4.94	**5.80 ± 0.40**	**2.54 ± 0.13**	**0.50 ± 0.01**	**0.22 ± 0.01**
CC	111.33 ± 2.30	206.00 ± 9.60	91.10 ± 4.24	**7.12 ± 0.31**	**3.45 ± 0.29**	**0.45 ± 0.01**	**0.22 ± 0.01**
PP+*CC*	116.66 ± 7.63	224.00 ± 4.24	105 ± 2.64	**7.17 ± 0.56**	**3.20 ± 0.19**	**0.48 ± 0.01**	**0.21 ± 0.01**

*Values are expressed as mean (n=5) ± S.D. *Figures are significant ($p < 0.05$) compared with normal control and #compared to DEN only. IBW: Initial body weight; FBW: final body weight; WC: weight change; LW: liver weight; RLW: relative liver weight, KW: kidney weight, RKW: relative kidney weight*

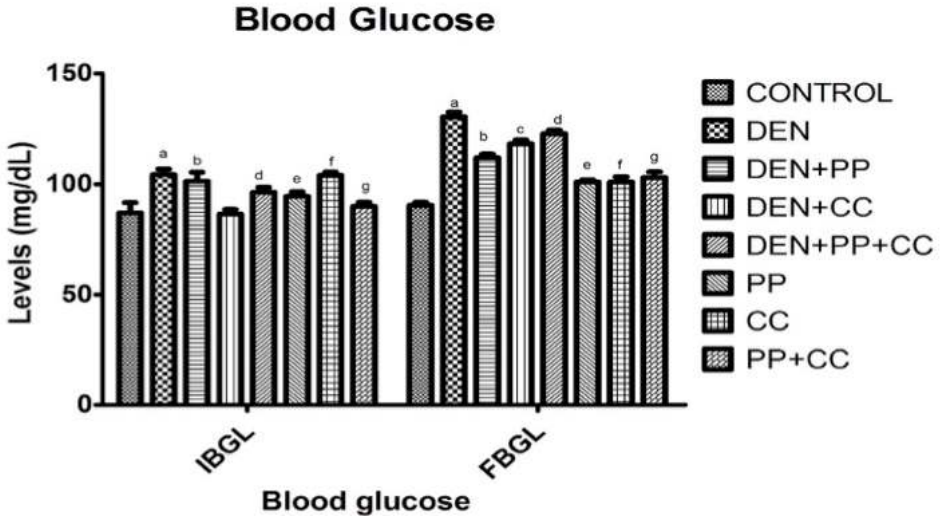

Figure 1
Effect of diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*) on blood glucose level in rats.
Values are expressed as mean, ± S.D. Significantly different ($p < 0.001$) compared to control; DEN only [a] , DEN+*PP* [b] , DEN+*CC* [c] , DEN+*PP*+*CC* [d] , *PP* [e], *CC* [f] ,*PP*+*CC* [g].

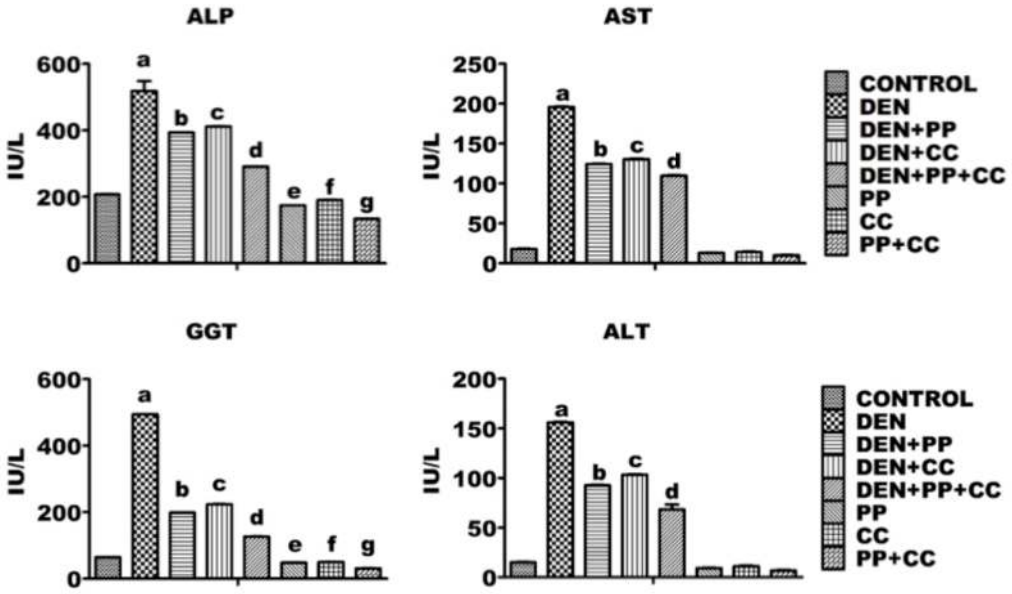

Figure 2
Effect of diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*) administration on hepatic transaminases in rat serum.
Values are expressed as mean, ± S.D. Significantly different ($p < 0.001$) compared to control, DEN only [a], DEN+*PP* [b], DEN+*CC* [c], DEN+*PP*+*CC* [d], *PP* [e], *CC* [f], *PP*+*CC* [g].

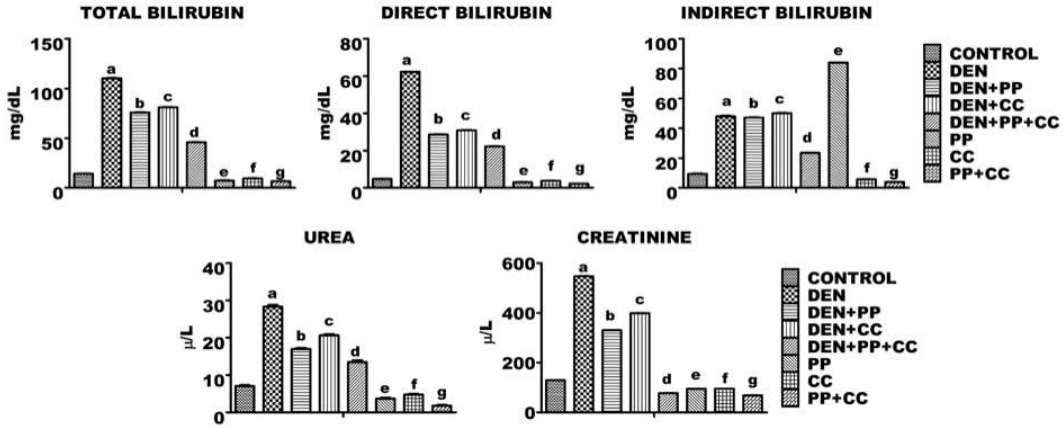

Figure 3
Figure 3: Effect of diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*) on total bilirubin (direct and indirect) urea and creatinine in rats. Values are expressed as mean; ± S.D; values are significant ($p < 0.001$) compared to control, DEN only [a], DEN+*PP* [b], DEN+*CC* [c], DEN+*PP*+*CC* [d], *PP* [e], *CC* [f], *PP*+*CC* [g].

Effect of DEN, *CC* and *PP* treatment on blood glucose levels: There was an increase in the glucose levels of the experimental animals' priors to the treatment with DEN (Figure 1). The highest mean blood glucose level was observed in DEN only (130.4mg/dL) treated compared to all other groups. Whereas blood glucose level was lowest in control only treated animals (90.50mg/dL). Blood glucose levels were increased significantly ($p<0.001$) in the presence *PP* alone and in combination with DEN treated animals. Blood glucose in *CC* only groups decreased compared to the final levels of *CC* only treated animals.

Effect of DEN, *CC* and *PP* treatment on hepatic transaminases in serum: Figure 2 shows the changes in hepatic transaminases in treated animals; there is a significant ($p<0.001$) increase in the activities of aspartate amino transferase (AST) alanine amino transferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in the DEN only treated group compared to control animals. Administration of *CC* and *PP* respectively caused a significant ($p<0.001$) decrease in the elevated activities of AST, ALT, ALP and GGT in rat serum compared to DEN only treated group. Combination of *CC* and *PP* was more effective in ameliorating the damaging effect of DEN in hepatocyte resulting in the elevated levels of transaminases observed in serum.

Effect of DEN, *CC* and *PP* treatment on total and direct bilirubin (TB and DB) and markers of kidney function: There is a significant ($p<0.001$) increase in the total bilirubin (TB) and direct bilirubin (DB) levels in the DEN-only treated group compared to the control (Figure 3). Administration of

CC and *PP* respectively caused a significant decrease in the elevated activities of TB and DB observed in experimental rat serum compared with the DEN only treated group. Co-administration of *CC* and *PP* acted synergistically in abrogating effectively the effect of DEN toxicity relative to either *CC* or *PP* alone, marked by reduced activities of TB and DB in serum. In addition there was a significant increase in serum creatinine and urea in the DEN only treated animal compared to control group, however treatment with *CC* and *PP* abrogated these observed increase.

Effect of DEN, *CC* and *PP* treatment on hepatic tumor number, sizes and α-feto protein: DEN treatment induced tumor formation in all lobules of the liver with varying numbers and sizes. The presence of the tumors on the liver may have responsible for the increase in liver weight observed. In addition, treatment with DEN significantly (p<0.001) elevated the levels of alpha-feto protein (figure 4) in DEN only treated groups compared to control animals.

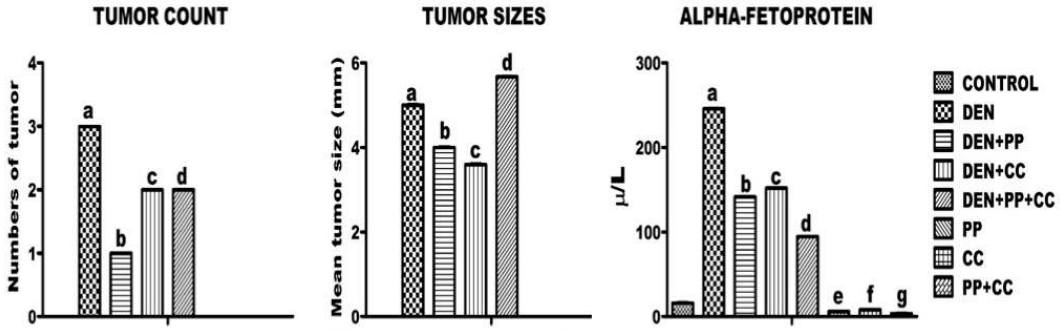

Figure 4:
Effect of diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*) on total tumor in liver, mean tumor sizes and α-fetoprotein in rats. Values are expressed as mean, ± S.D; values are significant (p < 0.001) compared to control, DEN [a] only, DEN+*PP* [b], DEN+*CC* [c], DEN+*PP*+*CC* [d]; *PP* [e], *CC* [f], *PP*+*CC* [g].

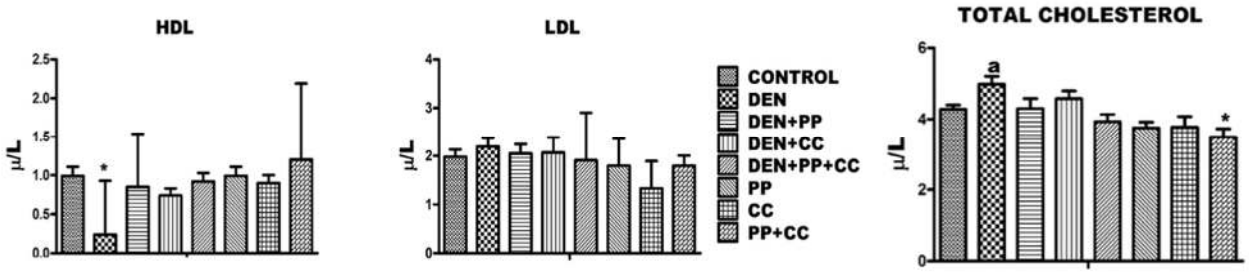

Figure 5:
Effect of diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*) on hepatic lipid profile in rats. Values are expressed as mean, ± S.D; significantly ($p< 0.05$) different compared to control [a]; significant (p < 0.01) compared to control*.

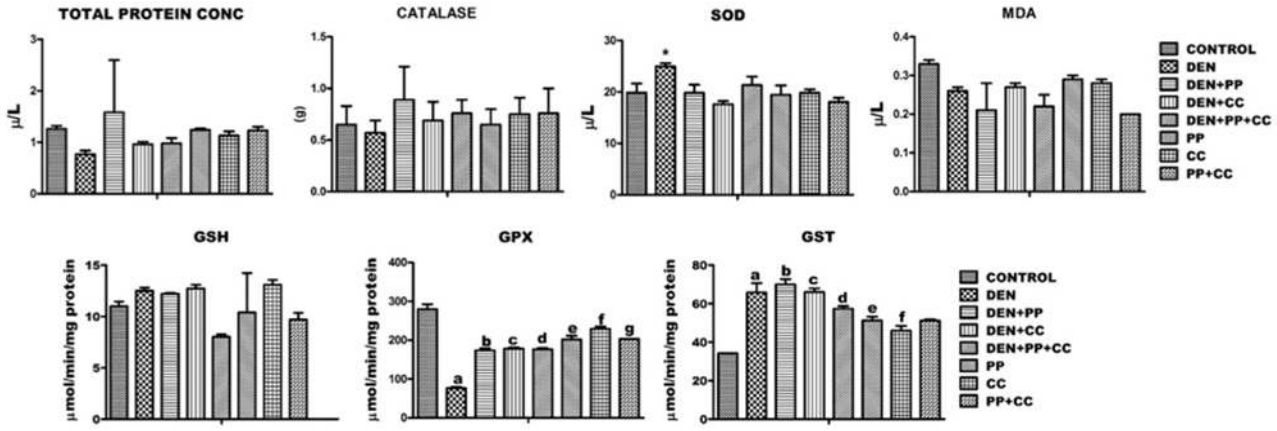

Figure 6:
Effect of diethylnitrosamine (DEN), *Pleiocarpa pycnantha (PP)* and *Cissampelos capensis (CC)* on total protein and hepatic antioxidant profile in rats.
Values are expressed as mean, ± S.D; values are significant (p<0.001) compared to control, DEN only[a], DEN+*PP*[b], DEN+CC [c], DEN+*PP*+*CC* [d]; *PP*[e], *CC* [f], *PP*+*CC* [g]. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione-s-transferase (GST), reduced glutathione (GSH), glutathione peroxidase (GPX).

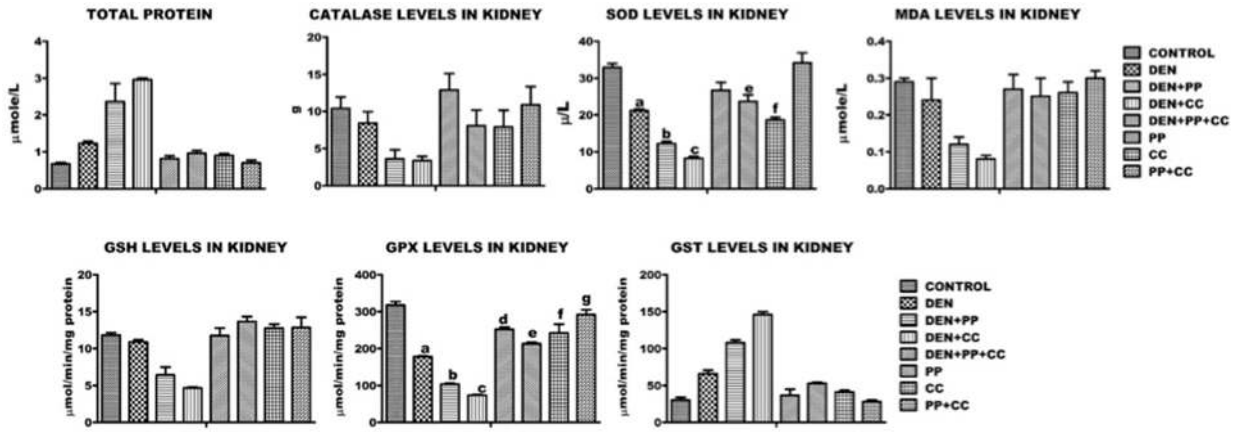

Figure 7:
Effect of diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*) on the antioxidant profile in kidney of treated rats.
Values are shown as means ± S.D; values are significant ($p< 0.001$) compared to control, DEN only [a], DEN+*PP* [b], DEN+C [c], DEN+*PP*+*CC* [d], *PP* [e], *CC* [f], *PP*+*CC* [g]. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione-s-transferase (GST), reduced glutathione (GSH), glutathione peroxidase(GPX).

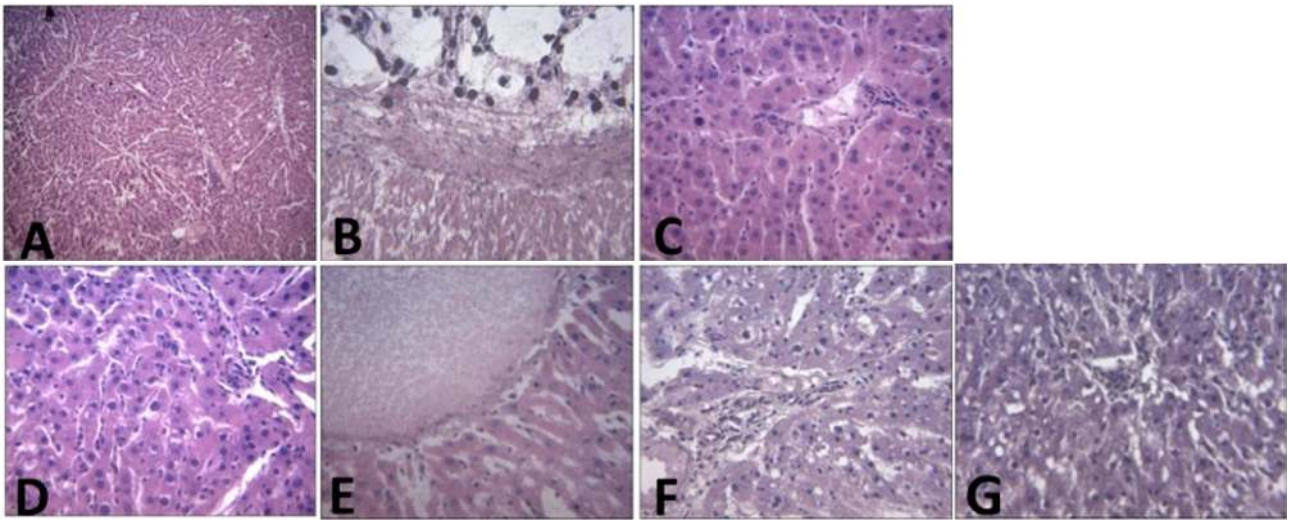

Plate 1:
Photomicrograph of liver sections from rats treated with Diethylnitrosamine (DEN), *Pleiocarpa pycnantha* (*PP*) and *Cissampelos capensis* (*CC*). (A) Control with no visible lesion seen; (B) DEN treatment characterized by severe portal cellular infiltration, congestion and necrotic stricture of hepatocytes with a characteristic periportal fibroplasias. (C) *PP* and *CC* only treated group, hepatocytes architecture appears similar to control but with clear vacuolar spaces and (D) DEN+*PP*+*CC* treatment with mild portal cellular infiltration and necrotic changes less severe compared with DEN only treated group (B). Limited histology of *PP* and *CC* treated groups (not pictured above) showed no visible lesions with limited portal congestion and cellular infiltration by mononuclear cells

Effect of DEN, *CC* and *PP* treatment on hepatic lipid profile: Figure 5 shows the effect of DEN, CC and *PP* treatment on cholesterol, triglyceride, HDL and LDL in serum, that was significantly ($p<0.001$) increased compared to control. Administration of CC and PP positively impacted the levels of cholesterol, triglycerides and HDL by further reducing them significantly ($p<0.05$), however, combination of CC and PP did not significantly reduced the levels of HDL levels.

Effect of DEN, *CC* and *PP* treatment on Total proteins and antioxidant parameters of the liver: Figure 6 shows a significant ($p<0.001$) increase in the activity of hepatic protein level in DEN treated group compared to control animals. Similar trend were observed in DEN+*CC* and DEN+*PP* group. There was however a significant ($p<0.001$) decrease in animals treated with *CC* and *PP* compared to DEN only treated group. Lipid peroxidation (LPO) estimated by malondialdehyde (MDA) levels showed a significant ($p<0.001$) decrease in LPO in the DEN only treated group compared to control and in all other groups. Evaluation of Superoxide dismutase (SOD) showed a significant ($p<0.001$) decrease in hepatic activity of SOD and was similar in other groups. In the same vein, there was non-significant decrease in catalase activity in the DEN treated group compared to the control animals except in the group treated with a combination of *CC*+*PP*. Furthermore, glutathione–s-transferase (GST) activity was elevated in DEN treated animals significantly ($p<0.001$) compared to control, whereas reduced glutathione

(GSH) significantly (p<0.001) decreased in DEN only treated group compared to all other groups.

Effect of DEN, *CC* and *PP* treatment on Total proteins and antioxidant parameters of the kidney: Figure 7, there was a significantly (p<0.05) increase in the total renal protein levels in DEN only treated groups compared to control and in DEN+*CC* and DEN+*PP*. In addition, there was a decrease in the DEN+*CC*+*PP* and in *CC*+*PP* only treated animals. Markers of kidney lipid peroxidation (LPO) status estimated by malondialdehyde (MDA) levels showed a significant (p<0.05) decrease in LPO in the DEN only treated group compared to control and in all other groups.

Estimation of renal superoxide dismutase (SOD) levels showed a significant (p<0.05) decrease in renal activity of SOD and was similar in other groups. Similarly decrease in catalase activity observed in DEN treated group was not significant. Furthermore, glutathione –*s*-transferase (GST) activity was elevated in DEN treated animals significantly (p<0.05) compared to control, whereas reduced glutathione (GSH) significantly (p<0.05) decreased in DEN only treated group compared to all other groups. Renal catalase activity was decreased in DEN only treated animals compared to control animals and this trend was observed across all groups except in the *CC*+*PP* treated group.

Histopathological changes in liver of rats treated with DEN, CC and PP

The liver architecture appears normal in control animals, whereas in DEN treated rats there was sever portal cellular infiltration, congestion of hepatic sinusoidal areas and the appearance of tumor mass in the different lobes of the liver (Plate 1). Treatment with CC and PP, both singly and in combination did not impact on hepatic architecture adversely, although there were mild cellular infiltrations of mononuclear cells. In the presence of PP, diffuse vascular degeneration and necrotic features characterized DEN-induced damage.

DISCUSSION

Chemical carcinogens present in diet can transform normal cells into malignant ones or initiate the growth and spread of tumours in animals. These carcinogens include DEN (El-Shahat *et al*., 2012), and many dietary constituents can increase the risk of cancer incidence in humans. DEN is present in tobacco smoke, cured meat and fish, fried meals, agricultural chemicals etc., and proven to be a carcinogen in rats ((El-Shahat *et al*., 2012)), by altering deoxyribonucleic acid (DNA) structures and inducing chromosomal aberrations (Verna *et al*., 1996; Al-Rejaie *et al*., 2009) . DEN metabolism generates reactive oxygen species (ROS) induces oxidative stress and result in cell injury (Bartsch *et al*., 1989). that creates a permissive environment for further DNA damage to occur. Evidences from population as well as laboratory studies shows an inverse relationship between regular consumption of fruit (Ribeiro *et al*., 2014; Basu *et al*., 2014), nuts and vegetables (Park *et al*., 2013) rich in phytochemicals and the risk of specific cancers incidences (Luo *et al*., 2015; Linnewiel-Hermoni *et al*., 2015). Furthermore, there are continuing clinical trials on the use of nutritional supplements and modified diets (Rossi *et al*., 2014) to prevent cancer. It is conceivable that in the future, patients may only need to take formulations containing plants-derived phytochemical to prevent or delay cancer onset or other diseases (Ferrari *et al*., 2011) (17). Although, precise mechanistic assessment which phytochemicals are credited with (chemo- preventive and protective) need validation (Bode and Dong 2013), which include anti-carcinogenic and mutagenic properties. The great structural diversity of phytochemicals, preclude a define structure–activity relationships to deduce phytochemical mechanistic action (Chung *et al*., 2013)). It is suggested that a better approach is to analyse their effects on signal transduction pathways associated with cancer (Bode and Dong 2013). In addition, plants contain other substances that may be useful in cancer prevention, a broader understanding of the carcinogenic process revealed cellular events as targets of chemopreventive agents (Milner 2001). As earlier reported, a single chemopreventive phytochemical role in inhibiting tumour development should be seen in the light of a combination of several cellular effects, rather than a single biological response (Surh 2003). These include carcinogen detoxification, DNA damage and repair; cell-cycle progression and proliferation, differentiation and apoptosis; expression of oncogenes or tumour-suppressor genes; angiogenesis and metastasis; and hormonal and growth-factor activity (Surh 2003; Manson 2003).

Findings in the present study show increases in hepatic transaminases and bilirubin in serum of DEN-only treated rats compared to control, supporting earlier reports (Kim *et al*., 2008).. Elevated transaminase particularly GGT, may be permissive to an inflammatory microenvironment that promote cell proliferation and tumorigenesis He *et al*., 2013; Grimm *et al*., 2013). CC and PP significantly reduced hepatic transaminase to near baseline level synergistically indicating a phytoprotective role and abating DEN damaging effects. Furthermore creatinine and urea are useful in evaluating renal function. Renal dysfunction diminished the ability to filter creatinine resulting in elevated serum levels. The increased serum creatinine and urea observed is indicative of kidney function impairment (Abdel-Monein *et al*., 2016). Administration of CC and PP reversed these trends similarly to those observed in the liver.

Endogenous antioxidants, supplemented by non-enzymatic antioxidants (Liebler 1993) derived from a variety of sources play an important role in the defence of cellular macromolecule and integrity Birben *et al*., 2012) (70), against oxidative stress. The balance between ROS generated by cellular metabolism and the antioxidant defence, prevent damages and disease pathogenesis attributed to redox imbalance. DEN elevated ROS production in treated rats as evidenced by alterations in markers of oxidative stress. Conversely, the presence of CC and PP, alone and in combination ameliorated these effects attributable to antioxidant potentials of CC and PP. Lipid peroxidation (LPO) mediated by free radical is considered to be primarily responsible for cell membrane damage and implicated in a number of deleterious cellular effects (Phaniendra *et al*., 2015). Increased level of renal and hepatic LPO was observed

in DEN only treated rats, and were reversed in the presence of CC and PP, LPO level markedly, conferring phytoprotection to cellular lipids, membranes and enhanced overall cellular antioxidant capacity.

In contrast to control, DEN treatment reduced final body weight, increased liver, relative liver and kidney weights. Final blood glucose in DEN rats was also elevated, probably as a result of a switch to aerobic utilization of glucose preferred by transformed and cancerous cells (Devic 2016). The presence of CC and PP did not reverse these observed trends though blood glucose levels were increased in rats treated with CC+PP. Epidemiological studies have found obesity to be a risk factor in the development of certain cancers (Chen *et al*., 2016) , DEN, treatment elevated serum cholesterol, triglyceride, LDL and caused a reduction in HDL, compared to control. Whereas, the presence of CC and PP reversed these observed trends, combination of CC+PP did not significantly increased total cholesterol level.

As expected, gross examination of the liver revealed several tumors mass in all lobules of DEN treated rats (Xu *et al*., 2017) Although this trend was not completely abated in rats co-treated with CC and P.P, there was however a reduction in tumor burden in rats co-exposed to DEN, CC and PP. Histopathological assessment of liver sections characterized by marked cellular architectural disruption by tumors in the hepatic lobules in DEN treated rats, were partially reversed with CC and PP co-treatment without preventing tumor onset entirely. Serum levels of α-fetoprotein were also elevated in DEN only treated animals (Afzal *et al*., 2017) , a hallmark of carcinogenesis and was reduced in the presence of CC and PP treatment.

Taken together, exposure to DEN from a variety of sources can lead to cellular ROS generation that may perturb the delicate cellular and systemic antioxidant balance and induce oxidative stress. This imbalance has been implicated in a variety of disease pathogenesis including cancers of various types. Extracts of Cissampellos capensis and Pleiocarpa pycnantha containing bioactive secondary metabolites of plant origin, has exhibited chemoprotective capacity in attenuating the deleterious cellular damage associated with DEN exposure and metabolism due to oxidative damage in Albino Wistar rats.

Further studies leading to isolation and characterization of the bioactive principles in CC and PP and elucidation of any toxicological properties are required to ascertain their therapeutic efficacy and biochemical mechanism of its chemoprotective and chemopreventive activities is highly desirable.

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