Anti-Tumorigenic Activities of *Diodia Sarmentosa* Leaf Extract on Diethyl Nitrosamine-Induced Hepatocellular Carcinoma in Wistar Rats

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Abstract: This study is an integral part of the search for an alternative pharmacognostic solution for hepatocellular carcinoma. This is the first *in vivo* study on the anti-cancer activities of *Diodia sarmentosa leaves*. Therefore, it reveals the possibility of incorporating the leaf extracts into existing natural remedies or its application as a stand-alone therapy. It evaluated the biochemical, antioxidant, and anti-tumour potentials of the *Diodia sarmentosa* leaf extract on diethylnitrosamine-induced hepatocellular carcinoma in adult albino wistar rats. Liver cancer was successfully induced in the experimental animals through oral administration of diethylnitrosamine (20 mg/kg body weight) dissolved in normal saline (0.9%), 5 times a week for 6 weeks. Biochemical and histopathological evaluations were made to determine the impact of diethylnitrosamine in the serum and on the tissues of the adult wistar rats. In the treated group, significant (P<0.05) improvements were recorded in the serum levels of glutathione peroxidase, superoxide dismutase, catalase, vitamin C, glutathione, and total antioxidant capacity compared to the positive control. In the positive control, serum levels of the electrolytes (Na^+, Cl^-, K^+, and HCO_3^-), gamma-glutamyl transferase, alanine aminotransferase, and aspartate aminotransferase were significantly ($P<0.05$) altered compared to the normal control. The photomicrographs of the treated group show diffused necrosis and atrophy of tumour nodules, with one-cell-thick hepatocellular plates separated by sinusoids with a normal nucleus. The development of liver cancer in the experimental animals affected not only the liver but also the kidney. These are evidenced by the significant alterations in some biochemical parameters associated with kidney damage and/or disease. Through this study *Diodia sarmentosa* leaf extract shows evidence-based assertions as an anti-cancer agent and can be useful in the search for a natural and more effective remedy for cancer.

Keywords: Antioxidant, Liver cancer, *Diodia sarmentosa*, Histopathology, Albino rat, medicinal plant.

INTRODUCTION

Liver cancer, also referred to as hepatocellular carcinoma (HCC), is the third most prevalent type of cancer mortality, the sixth most usually diagnosed disease [1],and the second most frequent cause of cancer-related premature mortality [2]. Globally, 830,200 persons lost their lives to liver cancer in 2020, according to estimates of 905,700 new cases [3]. While the most significant exogenous risk factors for primary liver cancer are infections with hepatitis B (HBV) and hepatitis C (HCV), excessive alcohol use and the associated metabolic syndrome conditions (type 2 diabetes, obesity, and non-alcoholic fatty liver disease) have also emerged as major causes of primary liver cancer[4, 5].Research indicates that HBV accounts for 56% of liver cancer cases, while HCV accounts for 20% of cases [6]. A further 18% of liver cancer cases may be linked to tobacco use [7], and 17% of cases worldwide due to alcohol consumption [8]. Apoptosis evasion, abnormal angiogenesis, altered cell cycle control, and lack of intrinsic mechanisms to regulate cell proliferation are the main mechanisms implicated in the formation and progression of HCC [9]. The increased vascularity of the tumour is one of the distinctive pathological characteristics of HCC [10].

The only medication for advanced HCC approved by the US federal drug regulator is sorafenib, which increases life expectancy by three months. In the biosafety cabinet (BSC) context, sorafenib increases overall survival in patients with advanced HCC when compared to individuals given a placebo [11]. Some other drugs with similar mechanisms of action such as brivanib, erotinib, and orantinib (TSU-68) have also been clinically investigated for advanced HCC patients. Single doses of sorafenib [12], sunitinib [13, 14], brivanib [15], erlotinib [16], and TSU-68 [17] resulted in response rates (RRs) of 2.3 – 3.3%, 2.7 – 2.9%, 5.0%, 9.0%, and 8.6%, in that order. Nivolumab was discovered to be useful in the treatment of metastatic HCC in 2014 [18]. It was also found from the same study that nivolumab led to a significant shrinkage of tumours in about 20% of patients treated with the drug.

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Approximately 70% of patients who received nivolumab were still alive at one year, compared with the historical tumour response rate for sorafenib of only 2% to 3% and the one-year survival rate of 30%. Severe adverse effects of nivolumab included elevated levels of lipase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) [19]. Two patients had their tumours completely treated while others who responded had responses lasting more than nine months.

To complement the numerous studies on synthetic drugs for liver cancer treatment, there is also a dire need to search for alternative natural remedies with fewer adverse effects and increased response rates. It is given this, that we embarked on this study, with a focus on *Diodia sarmentosa* leaf's medicinal impact. *Diodia sarmentosa* is traditionally used in treating local ailments, bruises, minor cuts, swellings [20], and dysentery [21, 22]. The antiulcer [23], anti-inflammatory, analgesic [20], antioxidant [24, 25], and anti-diabetic [26] properties of *Diodia sarmentosa* in albino rats have been reported. However, there is no report on the anti-cancer properties of *Diodia sarmentosa* leaf extract. Hence, this study has filled that knowledge gap.

This is the first *in vivo* study exploring the anti-cancer potentials of *Diodia sarmentosa* leaves. Sequel to the *in vitro* studies [24] confirming the antioxidant activities of *Diodia sarmentosa* leaves, this study aimed to consolidate these findings *in vivo* and determine the possibilities of combating oxidative stress which is one of the key players in cancer pathogenesis. The *in vitro* studies on *Diodia sarmentosa* leaves had earlier revealed the presence of certain phenolic compounds such as flavonoid (320.15 ± 1.83 mg/100g), tannins (64.68 ± 1.08 mg/100 g), and total phenolics (1121.02 ± 5.67 mg/100 g) [24] which are considered the chief compounds in anti-cancer drugs [27]. Considering this, it was expedient to investigate the anti-cancer potentials of *Diodia sarmentosa* leaves. The biochemical and antioxidant implications of the leaf extract in the induced disease condition were also assessed. These are embellished with a histopathology analysis to determine the combating effect of the leaf extract on the affected liver tissue.

MATERIALS AND METHODS

Collection of Plant Materials and Extraction

Fresh samples of *Diodia sarmentosa* leaves were sourced from nearby vegetation within the environs of the Federal University of Technology, Owerri and were identified by the Department of Crop Science. The leaves were washed with distilled water, air-dried, and oven-dried (digital TT 9083, Techmel & Techmel USA) at 40°C. The dried plant material was ground into powder and a portion (800 g) was weighed and soaked separately in 4 L of ethanol for 48hours on an orbital shaker. The extracts were obtained using a What man No. 1 filter paper and a Buckner funnel, then concentrated to dryness using a rotary evaporator at 40 °C.

Chemicals

Diethylnitrosamine was obtained from Sigma-Aldrich company, Germany. Other chemicals used for the experiment were of analytical grade.

Experimental Design

Twenty-one adult male Wistar albino rats weighing 100-110 g were conditioned in an animal house for 10 days with a 12-hour natural light-dark cycle, at 25 – 27 °C and a relative humidity of 40 – 65%. Then they were classified into three groups containing 7 animals each.

They were fed with commercial rat pellets and water *ad libitum* according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments. The animals were grouped as follows:

Group A: This is the normal control (NC) for the experiment. They were without any induction, provided with commercially available rat chew and water *ad libitum*.

Group B: This is the positive control (PC) for the experiment. The rats were induced with liver cancer followingan oral gavage introduction of 20mg/kg of DEN dissolved in normal saline (0.9%). They were without any treatment.

Group C: This is the treated group (TG) for the experiment. After successful cancer induction, the animals were treated with 400mg/kg body weight (b.w.) of ethanol extracts of *Diodia sarmentosa*leaves from weeks 7 to9 after tumour induction. A fresh stock solution of the plant material was prepared every other day by dissolving 8g of the dried extract in 50ml of distilled water.

Tumour Induction

Liver cancer was induced through an oral gavage of 20 mg/kg b.w. of DEN dissolved in 0.9% normal saline and administered five times a week for six weeks,

according to the method of Darwish and El-Boghdady with little modifications [28]. After tumour induction, representative liver samples were harvested from the three experimental groups and processed using histological techniques and routine haematoxylin and eosin (H&E) staining.

This was done to confirm the successful induction of the tumour before commencing treatment. An abnormal liver architecture revealed a solid growth pattern with large tumour nodules separated by indistinct fibrous bands, and the presence of more than 2-3 cell-thick hepatocellular plates confirmed the successful induction of liver cancer.

Acute Toxicity Test of Plant Extract and Administration

Lorke's method [29] was used to determine the average lethal dose (LD_{50}) of the plant extract. Nine (09) rats weighing 100 – 110g, were divided into 3 treatment groups (1600, 2900, and 5000mg/kg) of the extract. The experimental rats were monitored for 24 hours, while signs of toxicity and death were recorded.

Blood Collection and Histopathology Analysis

At the end of the experiment, the rats were fasted for 12 hours and then sacrificed. Blood was drawn from the rats' orbital sinuses using 0.5cm^3 syringes. From each of the three treatment groups, representative liver samples were taken and stored in 10% neutral buffered formalin. To prepare the samples for histopathological analyses, standard protocol H&E stain was used for processing the samples [30].

Biochemical Assays

Sood's spectrometric approach [31] was used to determine the serum electrolytes (HCO^{3-}, Na^+, Cl^-, K^+). Berthelot's method was used to evaluate urea, and the alkaline picrate method was used to analyse serum creatinine [32]. The enzymatic approach outlined by Fôssati and Precsipe [33] was used to assess uric acid. A colorimetric technique based on the Biuret reaction was used to measure total protein [34]. The International Federation of Clinical Chemistry's approach, as outlined by Bergmeyer and Horden [35], was followed while assaying ALT. A kit from JASTH (JAS Diagnostics Incorporated, Miami, Florida) was used to assay gamma-glutamyl transferase (GGT). The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [36] states that the JASTM method is predicated on the kinetic photometric test. The International Federation of Clinical Chemistry's suggested approach for performing AST was followed [37]. The Accup Bind ELISA Micro wells immunoenzymometric assay (Type 3) (Monobind Inc., Lake Forest, CA 92630, USA) was utilized to measure alpha-fetoprotein (AFP).

Antioxidant Assay

The modified Hadwan method was used to determine the amount of catalase [38]. Ellman's reagent and hydrogen peroxide were used to measure serum glutathione peroxidase [39]. The thiobarbituric acid (TBA) technique was used to detect lipid peroxidation [40]. The activity of superoxide dismutase was measured using the pyrogallol test [41]. The Rel Assay Diagnostics Total Antioxidant Status kit (Relassay, Turkey) [42], with minor adjustments, was used to measure total antioxidant capacity. The Jargar *et al*. method [43] was used to calculate the vitamin E content. The technique of Glustarini *et al*. [44] was used to reduce glutathione. Using Kalambe *et al*.'s approach [45], ascorbic acid (vitamin C) was measured.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 23 was used to analyse the data. The information was provided as mean ± standard deviation. A one-way analysis of variance and the least significant difference test were used to establish statistical significance. $P<0.05$ was the threshold for statistical significance [25].

RESULTS

Extract Toxicity Test Analysis

After a full day, there were two recorded deaths in the dose groups with the highest concentrations (5000 mg/kg) and the medium dose groups (2900 mg/kg), but not in the lowest dose group (1600 mg/kg). A safe dosage of 400 mg/kg of the plant extract was therefore determined for the treatment.

Biochemical Parameters

Serum levels of the electrolytes (Na^+, Cl^-, K^+, and HCO^{3-}) indicate significant ($P<0.05$) differences between the PC and NC (Figure **1**). There is a considerable ($P<0.05$) increase in blood levels of Na^+, Cl^-, and HCO^{3-} in the PC compared to the NC, but a significant ($P<0.05$) decrease in serum levels of K^+. Serum creatinine, urea, and uric acid levels show

comparable substantial (P<0.05) increases in the PC group compared to the NC group. Serum ALT, GGT, and AST levels are significantly (P<0.05) higher in the PC than in the NC (Figure **1**). Very high percentage increases; 117%, 85%, and 77.81% were recorded in serum AFP, urea, and GGT respectively. There was also a noteworthy (P<0.05) decrease in serum total protein in the PC as compared to the NC. However, the TG showed a 26.8% increase in serum total protein compared to PC, and this was also statistically significant. All biochemical markers measured in the experiment showed a substantial (P<0.05) reversal in the TG, except for serum creatinine, and uric acid which did not show any significant (P<0.05) change. Serum AFP showed the highest percentage (58.30%) reduction in TG compared to PC while the least reduction was recorded in serum urea, with an 11.82 % reduction in TG compared to its initial 85% rise in PC. Other significant reductions were observed in serum bicarbonate, with a 28% reduction in TG compared to PC; GGT, with a 33.68% reduction in TG compared to PC; and ALT, with about 20% reduction in TG compared to PC.

Oxidative Stress and Antioxidant Parameters

The PC has significantly (P<0.05) higher serum levels of the oxidative stress marker malondialdehyde (MDA) than the NC. Several antioxidant indices are significantly (P<0.05) reduced in the PC than in the NC, including total antioxidant capacity (TAC), reduced glutathione (GSH), catalase (CAT), SOD, glutathione peroxidase (GPx), vitamin E, and reduced glutathione (GSH) (Figure **2**). The PC group showed an 80% reduction in GSH compared to NC, and this was also observed to be the highest significant reduction among all other antioxidants. The least percentage reduction was recorded in SOD with a 4.23% reduction in PC

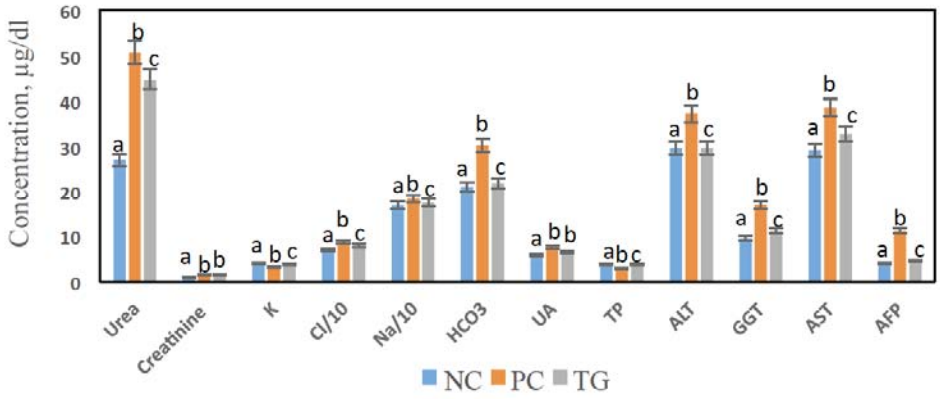


Figure 1: Biochemical parameters.

Values expressed as Mean ± SD (n = 4). Uric acid (UA), Total protein (TP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST) AFP: alpha-fetoprotein.

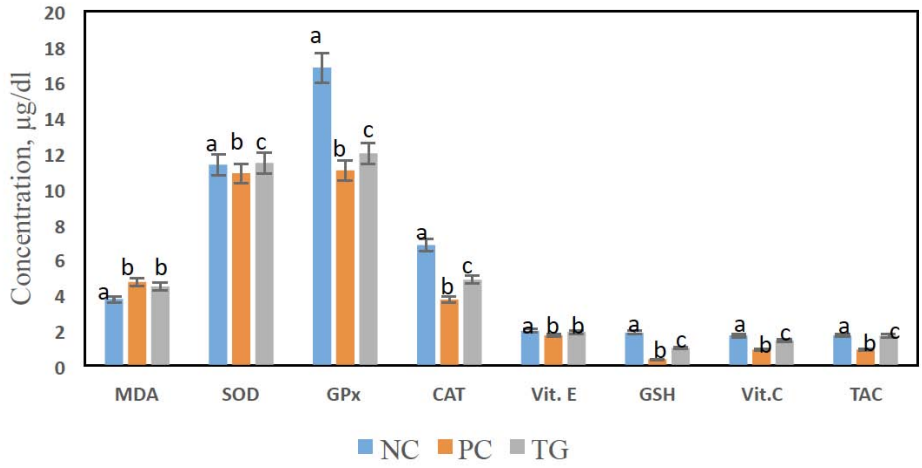


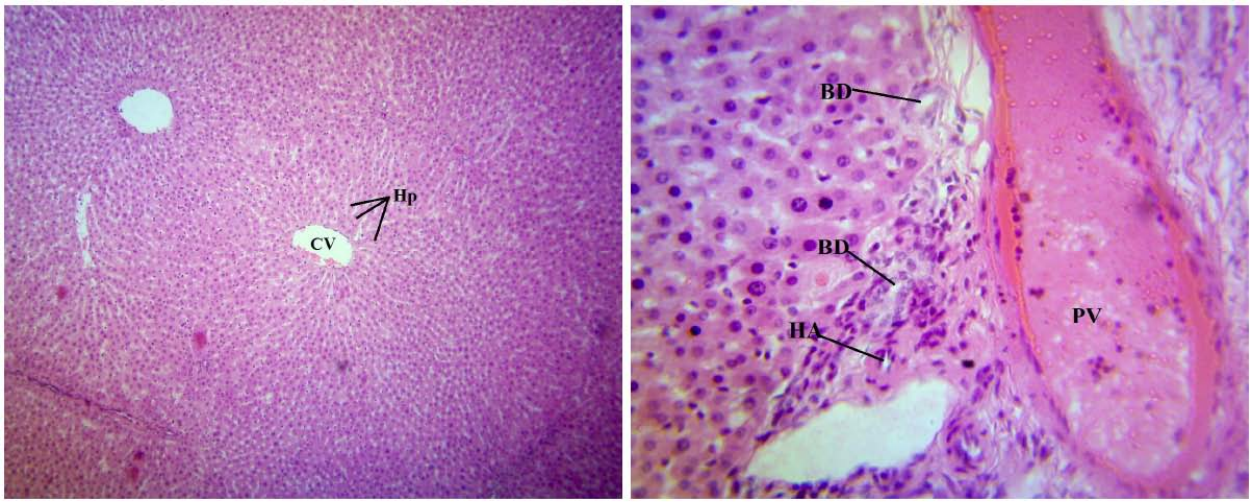
Figure 2: Antioxidant parameters

The values are presented as mean ± SD (n = 4). Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT), Vitamin E (Vit. E), Vitamin C (vit. C), total antioxidant capacity (TAC).

compared to NC. Comparing the TG to the PC, there are no (P<0.05) improvements in the serum levels of SOD, GPx, CAT, GSH, vitamin C, and TAC. TG group showed a 58.01% improvement in vitamin C, 85.64% in TAC, 175.69% in GSH, 30.73% in CAT, 8.84% in GPx, and 5.34% in SOD compared to PC. However, there was no discernible (P<0.05) variation observed in the MDA and vitamin E serum levels between the TG and NC (Figure **2**).

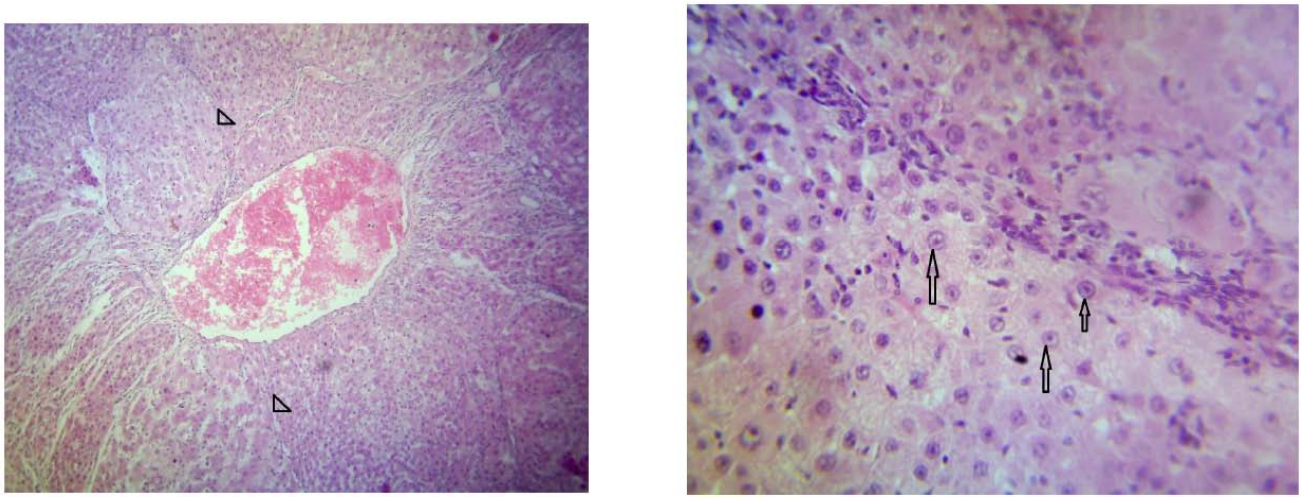
Histopathology

A section of the NC group's liver is shown in a photomicrograph at magnification (X100), which has normal histologic architecture, exposing the central vein and hepatocytes arranged in single-cell-thick plates or cords and separated by sinusoids (Figure **3A**). At a greater magnification (X400) (Figure **3B**), the hepatic artery and portal vein branches, the bile duct, lymphatic arteries, and nerves are all carried by a connective tissue septum. Photomicrographs of the liver of the PC at low (X100) and high (X400) magnifications (Figure **4A** and **4B**) show a solid growth pattern with large tumour nodules separated by indistinct fibrous bands (two arrowheads). Additionally, there are hepatocellular plates or cords that are thicker than two to three cells. Tumour cells that bear a strong similarity to hepatocytes are observed to exhibit nuclear atypia, which is responsible for their enlarged nuclei (high N/C ratio) and conspicuous nucleoli (arrows) (Figure **4A** and **4B**).



A **B**

Figure 3: A and B: Photomicrographs of a section of the NC group's liver in X100 and X400, respectively, demonstrating normal histologic architecture with HA (hepatic artery), PV (portal vein), BD (bile duct), and CV (central vein).



A **B**

Figure 4: A and B: A photomicrograph displaying aberrant liver architecture in X100 and X400, respectively, of a liver section from the positive control group.

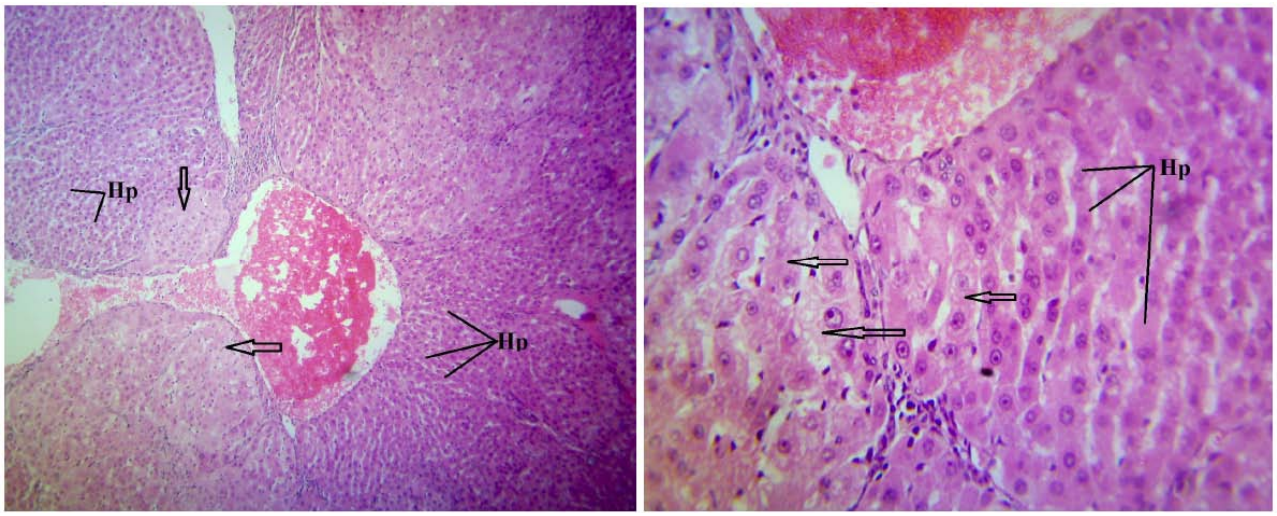


Figure 5: A and B: Photomicrographs of the treated group in X100 and X400, respectively. Hp: hepatocellular plates.

Photomicrograph of portions of the liver of the TG at low (X100) and high (X400) magnification respectively, show diffused necrosis and atrophy (arrows) of tumour nodules, and the presence of one cell thick hepatocellular plates is separated by sinusoids with a normal nucleus, cytoplasmic ratio, and the absence of atypia nucleoli (Figure **5A** and **5B**).

DISCUSSION

Figure **1** shows that DEN-induced HCC in the albino rats is indicated by a considerable (P<0.05) increase in blood levels of the transaminases (ALT, AST, and GGT) in the PC compared to the NC. Similar increases in the activity of serum AST and ALT with DEN treatment have been documented in several investigations [46]. These increases in transaminases are thought to be the most sensitive indicators of hepatocellular damage, and when the membrane's functional integrity is lost, the cell's membrane architecture is disrupted and the contents leak into the serum, where their concentration increases [47, 48].Additionally, cholestasis and bile duct necrosis are revealed by increased serum levels of GGT. Since GGT is found on the outer membrane of hepatic cells, which indicates damage to the cell membrane owing to carcinogenesis, its considerable spike in serum can be linked to its escape from the plasma membrane into circulation [48]. Following DEN-induced lipid peroxidation of hepatocyte membranes and the ensuing increase in the leakage of enzymes from liver tissues by the generation of reactive oxygen and nitrogen species, the reported rise in liver function enzyme markers by DEN may be a subsequent event. Hepatic carcinogenesis is initiated by DEN, and DEN activity is a major factor in this process [47].

AFP is a glycoprotein that is usually generated by the yolk sac, hepatocytes, and, to a lesser degree, the gastrointestinal system during fetal development. It is a biomarker that shows whether the liver is malignant [49]. A significant (P<0.05) elevation in the blood AFP level between the PC and NC (Figure **1**) may result from DEN poisoning leading to hepatocyte necrosis [50]. The defining characteristic that controls the activity of AFP production at the cellular level is the location of the hepatocytes, whether they are inside or outside the liver plate [51].The liver is where proteins are synthesized, and this is a significant process that occurs both in healthy and malignant environments. Cancer has a significant impact on the highest rate of synthesis of tissue proteins and key protein masses [52]. The development of DEN-induced hepatic lesions (Figure **4A** and **4B**) in the current investigation that impaired protein synthesis can be attribu to a significant (P<0.05) drop in serum total protein in the DEN-treated group, which is consistent with previous findings [53, 54]. In another report, Serum protein levels were lower in the DEN group when adult male Wistar albino rats were given 0.01 percent DEN in drinking water *ad libitum* for 13 weeks [53]. Serum uric acid levels were significantly (P<0.05) higher in PC than in NC (Figures **1** and **4**), indicating that a rise in uric acid may be a risk factor for the development of chronic liver disease. This is consistent with the research conducted by Benerji *et al*. [55], who found new links between blood uric acid levels and the frequency of hospitalizations linked to cirrhosis.

Diodia sarmentosa extract treatment of the caused cancer, however, does not significantly ($P<0.05$) affect the raised UA level since there is no significant ($P<0.05$) difference between the TG and PC groups. The relationship between serum uric acid and cancer in both cancer patients and healthy individuals has also been the subject of some research [56]. A significant prospective study involving over 28,000 old Austrian women discovered a correlation ($P<0.0001$) between high blood uric acid levels (>5.41 ml/dl) and occurrences of fetal cancer [57]. Equivalent results were also validated by Strasak *et al*. [57] in a male group spanning a broad age range. A dose-response to baseline blood uric acid, a time-dependent risk factor for cancer incidence, was also shown by them. The elevated hepatic enzymes (AFP, GGT, AST, and ALT) were significantly ($P<0.05$) reduced after the administration of *Diodia sarmentosa* leaf extract (Figure **1**), thereby re-establishing the levels of these enzymes in the rat model. This corresponds with the results of Singh *et al*. [58] who reported reduced levels of these liver enzymes in DEN-induced hepatocellular carcinoma in rat models, after the administration of *Carissa carandas* embedded silver nanoparticles (CCAgNPs). In another study, Dar *et al*. [59] also reported the reduction in elevated serum transaminases (ALT, AST), after the administration of extract of *B. ciliate* rhizome in a DEN-induced HCC mice model. The reduction in the levels of these liver enzymes could be attributed to the ability of the metabolites in *Diodia sarmentosa* leaves to ensure membrane integrity and reduction in enzymatic leakage [58].

The serum levels of urea, creatinine, Cl^-, Na^+, and HCO^{3-} showed a substantial ($P<0.05$) rise in PC compared to NC, and serum K^+ levels subsequently decreased, suggesting a potential renal injury in the DEN-induced group (Figure **1**). These data are in consonance with earlier findings by Bucsics and Krones [60], which associated kidney injury with liver diseases. The treatment with *Diodia sarmentosa* significantly reduced serum levels of urea, Cl^-, Na^+, and HCO_3^-, as well as improved K^+ levels.

When albino Wistar rats are given DEN, it causes major detrimental alterations in their antioxidant state as well as the production of reactive oxygen species. Figure **2** shows a substantial ($P<0.05$) rise in MDA serum levels and a significant ($P<0.05$) drop in antioxidants, including SOD, GPx, vitamin E (alpha-tocopherol), catalase, decreased GSH, vitamin C, and TAC, in the PC group when compared to the NC group. This indicates an impaired antioxidant system in the PC group of the experiment, which can be dueto a disease condition or oxidative stress. Lipid peroxidation state and changes in some endogenous radical scavenger levels are considered direct indicators of oxidative stress [61]. The enzymatic anti-oxidative defence system against reactive oxygen species is made up of SOD, CAT, and GPx, which interact with one another [62]. This study's findings indicate that the enzymes' overuse in neutralizing the free radicals produced by DEN metabolism is the cause of the decline in the enzymes' activity. An increase in lipid peroxidation levels provides more evidence for this.

The antioxidants (vitamins C, E, and GSH) significantly ($P<0.05$) decreased in serum levels after DEN delivery; this may have been caused by these vitamins being overused to scavenge free radicals generated during DEN metabolism. Vitamins C and E, for example, work in tandem as non-enzymatic antioxidants to scavenge free radicals produced inside the biological system [63]. Together with vitamin E and GSH, vitamin C also scavenges and eliminates free radicals [64]. These outcomes are consistent with previous research showing compromised antioxidant systems in Wistar albino rats given DEN [65].The antioxidant defence system was enhanced by treatment with *Diodia sarmentosa* leaf extract, as demonstrated by the considerable ($P<0.05$) rise in blood levels of SOD, GSH, GPx, vitamin C, CAT, and TAC in the TG relative to the PC (Figure **2**). Furthermore, it preserves the GSH homeostasis within the system, as demonstrated by the significant ($P<0.05$) rise in antioxidants in the TG in contrast to the PC (Figure **2**). The recycling of other antioxidants, including vitamin C and E, is facilitated by an increase in GSH levels [66]. This is also seen in the TG's elevated vitamin C activity (Figure **2**). This result concurs with earlier findings on the effect of *Diodia sarmentosa* leaf extract on altered antioxidant systems *in vivo* [25]. Ezejiofor and Okoroafor [25] reported a significant reduction of elevated antioxidant enzymes in monosodium glutamate-induced uterine leiomyoma in rat models. Furthermore, there was no statistically significant ($P<0.05$) variation in the serum levels of MDA and vitamin E between the PC and TG. The histological abnormalities in the PC, such as the nuclear atypia of tumour cells with obvious resemblance to hepatocytes and enlarged nuclei, were reduced in the TG (Figure **5**). This is evidenced by the presence of one-cell-thick hepatocellular plates or cords separated by sinusoids with a normal nucleus and cytoplasmic ratio and the absence of atypia nucleoli in the TG.

Considering the abundance of phenolic compounds in *Diodia sarmentosa* leaves, it may have exerted its anti-cancer properties by acting on molecular targets, especially by reducing the expression of a transcription factor regulating the expression of cytoprotective genes, reducing p53activation, decreasing Bcl-2 expression and mitochondrial membrane potential, suppressing the expression of HIF-1α, and increasing cellular apoptosis with the down regulation of p-Akt expression [67, 68].

CONCLUSIONS

The elimination of diethylnitrosamine-induced-hepatocellular carcinoma in albino rats with *Diodia sarmentosa* leaf extract was studied. It underpinned a correlation between liver cancer and oxidative stress. *Diodia sarmentosa* leaf extract significantly (P<0.05) combats the tumour, the oxidant system, and the altered biochemical parameters in the experimental rats. Thus, *Diodia sarmentosa* leaf extract possesses viable anticancer properties, which can be used alongside other anticancer remedies. Further study of the other parts of the *Diodia sarmentosa* plant can be explored in different solvent systems and, as well as increase the doses of the plant extracts administered to the experimental animal.

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Ethics Approval and Consent To Participate

The study was carried out according to the US National Institute of Health (NIH, 1978), standard laboratory principles for animal care and was approved by the ethical committee on the use of animals for research at the Department of Biotechnology, Federal University of Technology, Owerri, Nigeria.

CONFLICT OF INTEREST STATEMENT

The authors have no competing interest to declare.

REFERENCES

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A. Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J Clin 2021; 71(3): 209-249. https://doi.org/10.3322/caac.21660

[2] Bray F, Parkin DM. Cancer in sub-Saharan Africa in 2020: a review of current estimates of the national burden, data gaps, and future needs. Lancet Oncol 2022; 23(6): 719-728. https://doi.org/10.1016/S1470-2045(22)00270-4

[3] Rumgay H, Arnold M, Ferlay J, Lesi O, Cabasag CJ, Vignat J, Laversanne M, McGlynn KA Soerjomataram I. Global burden of primary liver cancer in 2020 and predictions to 2040. J Hepatol 2022; 77: 1598-1606. https://doi.org/10.1016/j.jhep.2022.08.021

[4] McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. Hepatol 2021; 73: 4-13. https://doi.org/10.1002/hep.31288

[5] Chuang SC, Vecchia CL, Boffetta P. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. Cancer Lett 2009; 286(1): 9-14. https://doi.org/10.1016/j.canlet.2008.10.040

[6] Maucort-Boulch D, de Martel C, Franceschi S, Plummer M. Fraction and incidence of liver cancer attribu to hepatitis B and C viruses worldwide. Intl J Cancer 2018; 142(12): 2471-2477. https://doi.org/10.1002/ijc.31280

[7] Murray CJL, Aravkin AY, Zheng P, Abbafati C, Abbas KM, Abbasi Kangevari M, *et al*. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the global burden of disease study 2019. Lancet 2020; 396(10258): 1223-1249. https://doi.org/10.1016/S0140-6736(20)30752-2

[8] Rumgay H, Shield K, Charvat H, Ferrari P, Sornpaisarn B, Obot I, Ialami F, Lemmens VEPP, Rehm J, Soerjomataram I. Global burden of cancer in 2020 attribu to alcohol consumption: a population-based study. Lancet Oncol 2021; 22(8): 1071–1080. https://doi.org/10.1016/S1470-2045(21)00279-5

[9] Zhang S, Yue M, Shu R, Cheng H, Hu P. Recent advances in the management of hepatocellular carcinoma: a review. JBUON2016; 21(2): 307-311.

[10] Meguro M, Mizuguchi T, Kawamoto M, Hirata K. The molecular pathogenesis and clinical implications of hepatocellular carcinoma. Intl J Hepatol 2011: 1-8. https://doi.org/10.4061/2011/818672

[11] Stock P, Monga D, Tan X, Micsenyi A, Loizos N, Monga SPS. Platelet-derived growth factor receptor-α: a novel therapeutic target in human hepatocellular cancer. Mol Cancer Therapeut 2007; 6(7): 1932-1941. https://doi.org/10.1158/1535-7163.MCT-06-0720

[12] Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, *et al*. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double blind, placebo-controlled trial. Lancet Oncol 2009; 10(1): 25-34. https://doi.org/10.1016/S1470-2045(08)70285-7

[13] Faivre S, Raymond E, Boucher E, Douillard J, Lim HY, Kim JS, Zappa M, Lanzalone S, Lin X, Deprimo S, Harmon C, Ruiz-Garcia A, Lechuga MJ, Cheng AL. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. Lancet Oncol 2009; 10(8): 794-800. https://doi.org/10.1016/S1470-2045(09)70171-8

[14] Zhu AX, Sahani DV, Duda DG, di Tomaso E, Ancukiewicz M, Catalano OA, *et al*. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. J Clin Oncol 2009; 27(18): 3027-3035. https://doi.org/10.1200/JCO.2008.20.9908

[15] Raoul JL, Finn RS, Kang YK, Park JW, Harris R, Coric V, Donica M, Walters I. An open-label phase II study of first- and second-line treatment with brivanib in patients with hepatocellular carcinoma (HCC). J Clin Oncol 2009; 27(15): 4577-4577. https://doi.org/10.1200/jco.2009.27.15_suppl.4577

[16] Philip PA, Mahoney MR, Allmer C, Thomas J, Pilot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C (2005) Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. J Clin Oncol 23(27): 6657- 6663. https://doi.org/10.1200/JCO.2005.14.696

[17] Kanai F, Yoshida H, Tateishi R, Sato S, KawabeT, Obi S, *et al*. A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma. Cancer Chemo Pharmacol 2011; 67(2): 315-324. https://doi.org/10.1007/s00280-010-1320-2

[18] Melero I, Crocenzi TS, Welling TH, Yau T, Yeo W, Chopra A, Grosso J, Lang L, Anderson J, Sangro B. Phase 1/11 safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-40. J Clin Oncol 2015; 33(18_suppl): LBA101. https://doi.org/10.1200/jco.2015.33.18_suppl.lba101

[19] American Society of Clinical Oncology. Clinical Cancer Advances: ASCO's Annual Report on Progress Against Cancer 2016. www.cancer.net/patient/Publications%20and%20Resourses/Clinical%20Cancer%20Advances/CCA_2016. Accessed October 30, 2016.

[20] Umoh UF, Ajibesin KK, Ubak NG. Preliminary anti-inflammatory and analgesic effects of *Diodia sarmentosa* leaf in rodents. World J Pharm Pharmaceut Sci 2016; 5(12): 203-212.

[21] Hemans M, Akoeginou A, Vander MJ. Medicinal plants used to treat malaria in Southern Benin: In Economic Botany. New York, Botanical Garden Press 2004; 5239-5252.

[22] Soladoye MO, Osipitan AA, Sonibara MA, Chukwuma EC. From vagabond to ethnobotanical relevance: weeds of the campus sites of Olabisi Onabanjo University, Awo-Iwoye, Nigeria. Ethnobotan Leaflets 2010; 14: 546-548.

[23] Akah PA, Orisakwe OE, Gamaniel KS, Shittu A. Evaluation of Nigerian traditional medicines: II. Effects of some Nigerian folk remedies on peptic ulcer. J Ethnopharmacol 1998; 62(2): 123-127. https://doi.org/10.1016/S0378-8741(98)00060-9

[24] Okoroafor HC, Awagu FE, Azeke EA. Phytochemical and antioxidant properties of Diodia sarmentosa swartz leaves. Mong J Chem 2020; 21(47): 27-32. https://doi.org/10.5564/mjc.v21i47.1430

[25] Ezejiofor TIN, Okoroafor CH. Effects of ethanol extracts of *Diodia sarmentosa* leaves on biochemical and histopathological indices of monosodium glutamate-induced uterine leiomyoma in rats. Biomed Res Ther 2022; 9(7): 5140-5148. https://doi.org/10.15419/bmrat.v9i7.749

[26] Elechi NA, Okezie-Okoye C, Abo KA. Anti-diabetic potentials of *Diodia sarmentosa* Sw (Rubiaceae) leaves of aloxan-induced diabetic rats. Saudi J Med Pharmaceut Sci 2020; 6(9): 622-626. https://doi.org/10.36348/sjmps.2020.v06i09.006

[27] Bakrim S, El Omari N, El Hachlafi N, Bakri Y, Lee LH, Bouyahya A. Dietary Phenolic Compounds as Anticancer Natural Drugs: Recent Update on Molecular Mechanisms and Clinical Trials. Foods 2022; 11(21): 3323. https://doi.org/10.3390/foods11213323

[28] Darwish HA, El-Boghdady NA. Possible involvement of oxidative stress in diethylnitrosamine-induced hepatocarcinogenesis: chemopreventive effect of curcumin. J Biochem 2011; 37(2013): 353-361. https://doi.org/10.1111/j.1745-4514.2011.00637.x

[29] Lorke D A new approach to practical acute toxicity testing. Arch Toxicol 1993; 54: 275-287. https://doi.org/10.1007/BF01234480

[30] Gurina TS, Simms L. Histology, staining. [updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-, Available from: https://www.ncbi.nlm.nih.gov/books/NBK557663/

[31] Sood R. Medical Laboratory Methods and Interpretations. New Delhi, India: Jaycee Brothers Medical Publishers 1990; 132-144.

[32] Tietz NW, Prude E. Tietz textbook of Clinical Chemistry. In London: W.B. Saunders Company 1994; 1354-1374.

[33] Fossati P, Precscipe LB. Use of 3, 5- dichloro-2-hydroxybenzene sulphuric acid 14- aminophenazone chromagenic' sy in direct enzymatic assay of uric acid insert and urine. Clin Chem 1980; 26(2): 227-230. https://doi.org/10.1093/clinchem/26.2.227

[34] Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular diagnostics. St. Louis, USA: WB Saunders Company, Elsevier 2012; 123-134.

[35] Bergmeyer HU, Horden M. International federation of clinical chemistry. Scientific committee. Expert panel on enzymes. IFCC document stage 2, draft 1; 1979-11-19 with a view to an IFCC recommendation. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1980; 18: 521-534.

[36] Shaw LM, Stromme JH, London JL, Theodorsen L. International Federation of Clinical Chemistry, (IFCC), Scientific Committee, Analytical Section. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4. IFCC method for gamma-glutamyltransferase [(gammaglutamyl)-peptide: amino acid gamma-glutamyltransferase, EC 2.3.2.2]. J Clin Chem Clin Biochem 1983; 21: 633-646.

[37] IFCC. Expert panel of enzymes of the International Federation of Clinical Chemistry. Clin Chem 1986; 24: 497-510.

[38] Hadwan MH. New method for assessment of serum catalase activity. Indian J Sci Technol 2016; 9(4): 1-5. https://doi.org/10.17485/ijst/2016/v9i4/80499

[39] Razygraev AV, Tumasova ZN, Pats KM, Pitukhina NN, Alexeeva PA, Baranova NI. Determination of glutathione peroxidase activity in serum of laboratory mice using hydrogen peroxide and Ellman's reagent. Butlerov Soobshch 2016; 45(2): 123- 130.

[40] Banjare J, Salunke M, Indapurkar K, Ghate U, Bhalerao S. Estimation of serum malondialdehyde as a marker of lipid peroxidation in medical students undergoing examination-induced psychological stress. J Sci Soc 2017; 44: 137-9. https://doi.org/10.4103/jss.JSS_13_17

[41] Engelbrecht I, Horna S, Giesyc JP, Pieters R. Determining superoxide dismutase content and catalase activity in mammalian cell lines. Methods X 2023; 11 (102395): 1-6. https://doi.org/10.1016/j.mex.2023.102395

[42] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more s ABTS radical cation. Clin Biochem 2004; 37: 277–285. https://doi.org/10.1016/j.clinbiochem.2003.11.015

[43] Jargar JG, Hattiwale SH, Das S, Dhundasi SA, Das KK. A modified simple method for determination of serum α - tocopherol (vitamin E). Journal of Basic Clinical. Physiological Pharmacol 2012; 223: 45-48. https://doi.org/10.1515/jbcpp-2011-0033

[44] Giustarini D, Tsikas D, Colombo G, Milzani A, Dalle-Donne I, Fanti P, Rossi R. Pitfalls in the analysis of the physiological antioxidant glutathione (GSH) and its disulfide (GSSG) in biological samples: an elephant in the room. J Chromatogr B: Anal Technol Biomed Life Sci 2016; 1019: 21e8. https://doi.org/10.1016/j.jchromb.2016.02.015

[45] Kalambe AS, Muhammad RC, Ayanniyi AA, Imam A. Serum Levels of Vitamin C among Patients with and Without Age-

Related Cataract at The University of Abuja Teaching Hospital, Abuja, Nigeria. Niger J Ophthalmol 2022; 30: 69-74. https://doi.org/10.4103/njo.njo_45_21

[46] Mansour DF, Abdallah HMI, Ibrahim BMM, Hegazy RR, Esmail RSE, Abdel-Salam LO. The carcinogenic agent diethylnitrosamine induces early oxidative stress, inflammation and proliferation in rat liver, stomach, and colon: protective effect of ginger extract. Asian Pac J Cancer Prev 2019; 20(8): 2551-2561. https://doi.org/10.31557/APJCP.2019.20.8.2551

[47] Sayed-Ahmed MM, Aleisa AM, Al-Rejaie SS, Al-Yahya AA, Al-Shabanah OA, Hafez MM, Nagi MN. Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signaling. Oxidative Med Cellular Longevity 2010; 3(4): 254-261. https://doi.org/10.4161/oxim.3.4.12714

[48] Hassan S, Mousa A, Eshak MG, Farrag ARH, Badwi AEM. Therapeutic and chemopreventive effects of nano curcumin against diethylnitrosamine induced hepatocellular carcinoma in rats. Int J Pharm Sci 2014; 6(3): 54-62.

[49] Roy SR, Gadad PC. Effect of β -asarone on diethylnitrosamine-induced hepatocellular carcinoma in rats. Indian J Health Sci 2016; 9: 82-88. https://doi.org/10.4103/2349-5006.183687

[50] Fathy AH, Bashandy MA, Bashandy SAE, Mansour AM, Elsadek B. Sequential analysis and staging of a diethylnitrosamine induced hepatocellular carcinoma in male Wistar albino rat model. Canadian J Physiol Pharmacol 2017; 95(12): 1462-1472. https://doi.org/10.1139/cjpp-2017-0413

[51] Motalleb G, Hanachi P, Fauziah O, Asmah R. Effect of berberis vulgaris fruit extract on alpha-fetoprotein gene expression and chemical carcinogen metabolizing enzymes activities in hepatocarcinogenesis rats. Iran J Cancer Prev 2008; 1: 33-44.

[52] Kumar RS, Kumar SV, Rajkapoor B, Pravin N, Mahendiran D. Chemopreventive effect of Indigofera linnaei extract against diethylnitrosamine induced hepatocarcinogenesis in rats. J Appl Pharmaceut Sci 2016; 6(11): 199-209. https://doi.org/10.7324/JAPS.2016.601131

[53] Ha WS, Kim C, Song S, Kang C. Study on mechanism of multistep hepatotumorigenesis in rat: Dev hepatotumorigenesis. J Vet Sci 2001; 2: 53-58. https://doi.org/10.4142/jvs.2001.2.1.53

[54] Ahmed OM, Mohamed BA, Hanaa IF, Ayman MM, Noha AA. Preventive effect of spirulina versicolor and enteromorpha flexuosa ethanolic extracts against diethylnitrosamine/benzo (a) pyrene-induced hapatocarcinogencity in rats. J Intl Acad Res Multidiscipl 2014; 2(7): 633-650.

[55] Benerji GV, Babu MF, Rekha KD, Saha A. Comparative study of ALT, AST, GGT,anduric acid levels in liver diseases. J Dental Med Sci 2013; 7(5): 72-75. https://doi.org/10.9790/0853-0757275

[56] Yiwei L, Pengfei S, Yaping X, Shenxian Q. Serum uric acid level is a prognostic indicator and improves the predictive ability of the IPI score in diffuse large B-cell lymphoma. Int J Clin Exp Med 2018; 11(3): 2223-2231.

[57] Strasak AM, Rapp K, Hilbe W, Oberaigner W, Ruttmann E, Concin H, Diem G, Pfeiffer KP, Ulmer H, VHM & PP Study Group. The role of serum uric acid as an antioxidant protecting against cancer: prospective study in more than 28 000 older Austrian women. Ann Oncol 2007; 18: 1893-1897. https://doi.org/10.1093/annonc/mdm338

[58] Singh D, Singh M, Yadav E, Falls N, Dangi DS, Kumar V, Ramteke, PW, Verma A.Attenuation of diethylnitrosamine (DEN) – Induced hepatic cancer in experimental model of Wistar rats by Carissa carandas embedded silver nanoparticles. Biomedicine & Pharmacotherapy 2018; 108: 757-76. https://doi.org/10.1016/j.biopha.2018.09.066

[59] Dar KK, Ali S, Ejaz M, Nasreen S, Ashraf N, Gillani SF, Shafi N, Safeer S, Khanc MA,Andleeb S, and Mughal TA. *In vivo* induction of hepatocellular carcinoma by diethylnitrosoamine and pharmacological intervention in Balb C mice using *Bergenia ciliata* extracts. Braz J Biol 2019; 79(4): 629-638. https://doi.org/10.1590/1519-6984.186565

[60] Bucsics T, Krones E. Renal dysfunction in cirrhosis: acute kidney injury and the hepatorenal syndrome. Gastroenterol Rep 2017; 5(2): 127-137. https://doi.org/10.1093/gastro/gox009

[61] Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. Antioxidants 2019; 8(3): 72. https://doi.org/10.3390/antiox8030072

[62] Bhattacharjee R, Sil P. The protein fraction of *Phyllanthus niruri* plays a protective role against acetaminophen-induced hepatic disorder via its antioxidant properties. Phytother Res 2006; 20: 595-601. https://doi.org/10.1002/ptr.1933

[63] De la Fuente M, Sánchez C, Vallejo C, Díaz-Del Cerro E, Arnalich F, Hernanz Á. Vitamin C and vitamin C plus E improve the immune function in the elderly. Exp Gerontol 2020; 142: 111118. https://doi.org/10.1016/j.exger.2020.111118

[64] George J. Ascorbic acid concentrations in diethylnitrosamine-induced hepatic fibrosis in rats. Clin Chim Acta 2003; 335(1-2): 39-47. https://doi.org/10.1016/S0009-8981(03)00285-7

[65] Singh D, Chaudhary D, Kumar V, Verma A. Amelioration of diethylnitrosamine induced renal oxidative stress and inflammation by Carissa carandas embedded silver nanoparticles in rodents. Toxicol Rep 2021; 8: 636-645. https://doi.org/10.1016/j.toxrep.2021.03.014

[66] Exner R, Wessner B, Manhart N, Roth E. Therapeutic potential of glutathione. Wien Klin Wochenschr 2000; 112: 610-616.

[67] Mottaghi S, Abbaszadeh H. Natural Lignans Honokiol and Magnolol as Potential Anticarcinogenic and Anticancer Agents. A Comprehensive Mechanistic Review. Nutr Cancer 2022; 74: 761-778. https://doi.org/10.1080/01635581.2021.1931364

[68] Yoganathan S, Alagaratnam A, Acharekar N, Kong J. Ellagic Acid and Schisandrins: Natural Biaryl Polyphenols with Therapeutic Potential to Overcome Multidrug Resistance in Cancer. Cells 2021; 10: 458. https://doi.org/10.3390/cells10020458

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