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The potential effect of Annona muricata and Cisplatin as antioxidant and antitumor in rat with liver cancer by induction of apoptosis through P13K \ AKT signaling pathway

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ABSTRACT

Objective: The aim of this study was to investigate the effects of Cisplatin and Annona muricata's efficacy, safety, and tolerability in treating liver cancer in rats. **Method:** Animals: 56 male albino rats, 3–4 months old, weighing 180–200 g on average, were purchased and brought to the animal house at the Department of Biochemistry Faculty of Science, Zagazig University. Rats used in the experiment were split into 4 groups, as follows: 14 healthy rats in Group (1) are left untreated (Negative Control). 42 healthy rats were separated into three equal groups, after receiving an IP dose of CCl4 (1 ml/kg/ twice a week for 8 weeks) to induce HCC. Group (2) consists of 14 rats with liver cancer which were not treated (Positive control). Group (3) has 14 liver cancer-afflicted rats, received IP dose of Cisplatin (6 mg/kg/week for 4 weeks). Group (4) has 14 liver cancer-afflicted rats that were given oral dose of Annona muricata (300 mg/kg every day for 4 weeks). **Results**: At the end of experiment blood samples were taken from all rats in all groups to preserve the serum for estimation of serum liver enzymes (AST, ALT and ALP), Serum proteins (total protein, albumin, globulin and A/G ratio), serum kidney function tests (urea and creatinine) and inflammatory marker (TNF-α). Liver tissues were collected for gene expression determination of PI3K and Akt levels and histopathological study. **Conclusion**: The present study indicates that Annona muricata gives significant results in treatment of Albino rats with liver cancer, due to its antioxidant, anti-inflammatory and anticancer effects beside improvements of hepatorenal cellular, so we should interest in research in the same field with conducting experiments using Annona muricata as a protective agent for the liver against cancer.

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INTRODUCTION

Cisplatin is a platinum drug has anticancer effect treatment of solid tumors and lung cancer. Cisplatin is a potent anticancer agent but therapeutic use is limited due to its nephrotoxic effects [1]. It's effective against cancer but induces cell damage and has inhibition role on immune system [2]. Cisplatin has cytotoxic effect on immune cells [3].

Medicinal plants contain phytochemical components; tannins, alkaloid terpenoids, and phenolic compounds [4]. Natural antioxidant has protective effects against toxicity such as drug toxicity, pollutants, and pathogenic factors [5]. Medicinal plants contain many phytochemicals [6]. Medicinal plants are considered an important source of many active principles used for the development of new drugs for the treatment of different body disorders [7]. Among these medicinal plants, Annona muricata is a valuable one used in traditional remedies in European and Mediterranean countries. Annona muricata is belonging to the family Annonaceae identified in tropical regions to traditionally treat diverse conditions ranging from fever to diabetes and cancer [8]. Annona muricata is belongs to the Annonaceae family [9]. Annona muricata contain more than 200 phytochemical compounds; alkaloids, phenols, and acetogenins [10]. Phytochemicals present in Annona muricata were characterized by antioxidant, antimicrobial, anti-inflammatory, insecticidal, larvicidal, and cytotoxic to cancer cells [11]. Annona muricata has cytotoxic effects on breast cancer, human liver cancer, colon carcinoma and Pancreatic cancer [12].

Hence, the present work was designed to test the curative efficacy of Cisplatin and Annona muricata against CCl_4-induced HCC in the rat model through measuring P13K \ AKT signaling pathway markers, serum liver enzymes (AST, ALT and ALP), Serum proteins (total protein, albumin, globulin and A/G ratio), serum kidney function tests (urea and creatinine), inflammatory marker (TNF-α) and liver histopathological studies.

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MATERIAL AND METHODS

Drugs:-

Cisplatin: a clear, colourless to pale yellow aqueous solution that is sterile and sold by Sigma Chemical Co. in vials of 50 ml concentrate for solution for infusion containing 50 mg of Cisplatin.

Annona muricata (Graviola): dry grey tablet, 500 mg, based on graviola, soluble in water, obtained from ORIGINI NATURALI Company.

Animals:-

56 male albino rats, 3–4 months old, weighing 180–200 g on average, were purchased and brought to the animal house at the Department of Biochemistry Faculty of Science, Zagazig University. Before beginning the tests, rats were first acclimated with a regular diet of barley, powder milk, and clean water for two weeks in clean, disinfected cages at room temperature with a 12-hour light-dark cycle.

Experimental design:

Rats used in the experiment were split into 4 groups, as follows:

14 healthy rats in **Group (1)** are left untreated (Negative Control).

After receiving an IP injection of CCl_4 at a dose of 1 ml/kg/ twice a week for 8 weeks in order to induce HCC, 42 healthy rats were separated into three equal groups, each with 14 rats.

Group (2) consists of 14 rats with liver cancer which were not treated (Positive control).

Group (3) has 14 liver cancer-afflicted rats, received IP injections of Cisplatin at a dose of 6 mg/kg/week for 4 weeks [13].

Group (4) has 14 liver cancer-afflicted rats that were given oral Annona muricata at a dose of 300 mg/kg every day for 4 weeks [14].

Ethical approval:

Animal ethics committee at Zagazig University approved this work with approval number ZU-IACUC/1/F/37/2019.

Samples collection:

All rats in all groups had blood drawn from the orbital plexus on the first day following the ending of the experiment using a heparinized micro-hematocrit tube. The blood was then placed in a clean, dry centrifuge tube, allowed to clot at 25 °C, and centrifuged at 3000 rpm/20 min to preserve

the serum for further examination. The acquired clear serum was transferred to clean, dry, and sterilised plastic eppendorf tubes with a good stopper and kept at -20oC until determination of the biochemical investigation; serum liver enzymes [AST, ALT [15] and ALP [16]] , Serum protein picture [total protein [17], albumin [18], globulin was determined by subtraction obtained albumin level from the level of total proteins [19], A/G ratio [20]], serum kidney function tests [urea [21] and creatinine [22]] and TNF-α.

After that the rats were euthanized via thiopental sodium anesthesia; the livers were harvested and divided into two-part:

1st part each group's collected tissue livers were frozen at -20°C for subsequent analysis of genes expression (PI3K and Akt). Utilizing the quantitative real time-polymerase chain reaction (qRT-PCR) Analyse, Primers sequence of the studied genes **Table (1).**

2nd part for histopathological analysis, liver tissue from each group was collected, fixed in 10% buffer formalin solution, embedded in paraffin sections with a 5 micron thickness and stained by haematoxylin and eosin. These sections were then microscopically seen [23].

Statistical analysis:-

Results were reported in means ± SEM (Standard Error of Mean) for continuous variables. The value of $P < 0.05$ was used to indicate statistical significance. Post hoc testing was performed for inter group comparisons using the least Significant Differences (Duncan) test Differences between groups were determined by one–way analysis of variance (ANOVA) [24].

RESULTS

Biochemical results; Table (2&3):

In the current study, it was discovered that, when compared to negative control rats, rats with HCC displayed significantly elevated levels of serum ALT, AST, ALP, urea, creatinine, TNF-α and PI3K & Akt gene expression, along with significantly decreased levels of serum total protein, albumin, and globulin coupled with a non-significant reduction in A/G ratio.

In comparison to positive control rats, Cisplatin-treated HCC rats showed non-significant decreases in serum ALT, AST, ALP, urea, creatinine, and TNF-α levels as well as significant decreases in PI3K and AKT gene expression and non-significant increases in serum total protein, albumin, globulin, and A/G ratio levels.

Comparing HCC-affected rats to positive control rats, the Annona muricata-treated rats showed significantly lower levels of AST, ALT, ALP, Urea, creatinine, and TNF-α as well as PI3K and AKT gene expression, significantly higher levels of serum total protein, albumin, and globulin, and a non-significant increase in the A/G Ratio.

Histopathological results:

Our histopathological results of all experimental groups were exhibited several mild to Severe lesions based on the treatment each group received and those lesions were showed as following:

Livers of rats of negative control group were almost normal end exhibited normal tissue architecture and cellular details. The liver parenchyma is composed of small lobules of a roughly hexagonal shape with portal tracts at the apices. Inside the lobules, the hepatocytes are arranged as cords of cells connecting the portal tracts in the periphery to the central veins (terminal branch of hepatic veins) **(Fig.1).**

Livers of rats of positive control group showed severe lesions, malignant neoplasm (Cancer) were the most characteristic lesion appeared in most rats' livers of this group. Hepatocellular carcinoma was the most detected form of this hepatic neoplasm with ranged grade from II to IV. The main criteria of Cancer were exhibited in rats with HCC as loss of both tissue architecture and Cellular details with cellular and nuclear pleomorphism and missed cellular polarity those represented in **(Fig.2A)**. Many types of hepatocellular Carcinomas were also exhibited the previously mentioned criteria in addition to other characteristic lesions as massive fibroblasts infiltrated the hepatic parenchyma (fibrosis) which is given fibro lamellar type of mainly grade II **(Fig.2B)**. Other cases showed the basic criteria of cancer of acinar type (pseudoglandular) which formed in Pseudo or false acini by polymorphic hepatocytes rearrangement **(Fig.2C)**.

Livers of rats with HCC treated with

Cisplatin Showed no evidence of hepatic cancer; only some lesions which could be reversible were detected as severe changes of hepatic blood vessels which fibrosis of portal area which infiltrated with mononuclear cells infiltration in most cases **(Fig.3A).** Other case exhibited focal to diffuse congestion of hepatic blood vessels and sinusoids with diffuse coagulative necrosis of hepatocytes represented in pyknosis (small nucleus with condensed chromatin which represents the first stage of hepatocyte necrosis **(Fig.3B)**. Some other rats exhibited dissociation of hepatocytes with loss of cellular polarity **(Fig.3C)**.

Livers of rats with HCC treated with Annona muricata diminished the previously neoplasms and lesions were limited to some changes with moderate non advanced picture of complete carcinogenicity as demonstrated before. High biliary epithelial proliferation with periductal cellular nuclear pleomorphism with nuclear proliferation was seemed **(Fig.4A)**. Mild to severe fibrosis with or without hemorrhage and nuclear pleomorphic were detected in some rats **(Fig.4B)**. Lesion of this group in addition to some limited criteria of malignancy with moderate fibrosis was seen in other rats **(Fig.4C)**.

DISCUSSION

In the current work, it has been found that; rats with HCC showed significant elevation in serum ALT, AST, ALP, urea and creatinine in comparison with negative control rats.

The same findings were recorded that the treatment with CCl_4 had adverse side effects on liver and kidney of mice, were it induces liver and kidney damage in mice These effects were evidenced by a significant increase in serum hepatic enzymes (ALT, AST and ALP) and renal function markers (blood urea and creatinine) [25]**.** Keeping with this line CCl_4 resulted in an increase in the AST, ALT, ALP, urea and creatinine. CCl_4 induced profound elevation of free radical generation and oxidative stress, as evidenced by increasing lipid peroxidation and reducing catalase, superoxide dismutase and glutathione peroxidase activities in liver and kidney [26]**.**

Rats with HCC treated with Cisplatin displayed non-significant reduction in serum ALT, AST, ALP, urea and creatinine in comparison with positive control rats.

Cisplatin significantly elevated markers of liver function [AST, ALT activity, total cholesterol and triglyceride] and increased liver weight in comparison with normal ones [27]**.** Our result was supported by another results that Cisplatin induce hepatorenal damage, liver damage due to improvement in liver function, oxidative stress, and histological alteration and non-significant decrease in AST, ALT, ALP, urea and creatinine in rats with HCC [28]**.** Cisplatin-induced renal damage, increased renal biomarkers (urea and creatinine) [29]**.** Interventional ultrasound injection of Cisplatin in the treatment of HCC has a definite effect. It can effectively relieve liver damage, by decreasing AST, ALT and ALP levels reduce adverse reactions and improve serum tumor marker levels [30]**.** Cisplatin can bind to DNA and cause cross-reaction and damage DNA functions and further inhibits cancer cells from mitosis; Injecting Cisplatin into the tumor can cause cell protein degeneration, coagulation, necrosis, fixation and dehydration of the lesion tissue. The destruction of local endothelial cells in the lesion can cause thromboembolism, thereby blocking the blood supply to the tumor, leading to cell death. Cisplatin caused the hepatoxicity and renal toxicity in rats and could decrease in Urea and creatinine in comparison with CCl4 -induced hepatotoxicity and nephrotoxicity rats [31]**.**

Our result showed rats with HCC treated with Annona muricata displayed significant decrease in AST, ALT, ALP, Urea and creatinine in comparison with positive control rats.

A Data study clearly demonstrated that Annona muricata supplementation could at least partly overcome CCl_4 -induced hepatotoxicity and nephrotoxicity by decrease in serum AST, ALT ,ALP, urea and creatinine in diseased albino rats [32]**.** Rats suffering from hepatorenal damage and received Annona muricata show non-significant decrease in serum liver enzymes, urea and creatinine [33]**.** Rats suffering from kidney damage treated with Annona muricata showed non-significant decrease in urea and creatinine [34]**.** Our results were agreed with that Extracts of Annona muricata leaves induced a protective effect against CCl_4 toxicity and improved

hepatorenal function by lowering CCl_4-elevated serum enzyme markers like ALT, AST, urea and creatinine, The findings in the present study indicates that pretreatment with Annona muricata showed protective effect in vivo in CCl4 compromised liver and kidneys [35].

Our findings revealed that rats with HCC showed a significant reduction in serum total protein, albumin, and globulin level besides a non-significant reduction in A/G ratio in comparison with negative control rats.

The concentration of serum albumin and hepatic protein synthesis decrease post treatment with $CCl_4$for 8 weeks as compared to control group. CCl_4-significantly altered serum total protein, albumin, globulin, oxidative stress markers and lipid profiles [36]. It was thought that the inhibition of protein synthesis may be involved in cell injury or death mediated by free radicals. Our results agree with that Toxic effect of $CCl_4$was accompanied by a decline in the serum total protein, albumin, globulin, and prothrombin [25]. A remarkable increase in hepatic DNA strand breakages and histopathological distortion in liver and kidney specimens were observed in CCl_4-intoxicated groups. In addition, CCl_4 induced profound elevation of free radical generation and oxidative stress [26].

Rats with HCC treated with Cisplatin displayed non-significant elevation on total protein, albumin, globulin and A/G Ratio in comparison with positive control rats.

Same results reported that rats received Cisplatin showed reduction in total protein, albumin and globulin in comparison with normal ones due to cytotoxic effect of Cisplatin on liver and immune competent cells as B lymphocytes and plasma cells [27]. Close similarity was seen between the finding and those Cisplatin, one of the most effective anticancer drugs, is known to cause undesirable adverse effects, including immunotoxicity [37].

Rats with HCC treated with Annona muricata displayed significant increase in total protein, albumin and globulin coupled with non-significant increase in A/G Ratio in comparison with positive control rats.

The levels of total protein, albumin and globulin were significantly increased in the Annona muricata-treated groups compared to the positive control.This may also be due to the protective properties of the Annona muricata extract in hepatocyte regeneration, necrosis healing and anti-inflammatory actions, which may have enabled the liver to regain its functions [38]. Annona muricata induce insignificant elevate in total protein, albumin and globulin in rats suffering from liver damage [33]. Also, Annona muricata leaves showed protective effect against CCl_4 toxicity by elevating CCl_4-lowered serum in total protein, albumin and globulin [35]. Annona muricata act as antitumor activity regarded to the bio-production of secondary metabolites like alkaloids, phenols, flavonoids, and most unique group of compounds, namely, annonaceous acetogenins which induce improved in total protein, albumin and globulin [39].

Our study revealed that rats with HCC showed significant increase in serum TNF-α in comparison with negative control rats.

The obtained data are in agreeing with those CCl_4 induced hepatic damage and increase TNF-α [40]. Elevation in TNF-α post injection of CCl_4 may due to hepatic damage [41]. CCl_4-induced hepatotoxicity was manifested by an increase in the levels of TNF-α [42].

Rats with HCC treated with Cisplatin revealed non-significant decrease in serum TNF-α in comparison with positive control rats.

Cisplatin has been widely used in chemotherapy and transarterial chemoembolization in treatment for HCC, Cisplatin can overcome on resistance by decreasing TNF-α level [43]. Cisplatin induced significant decreases in TNF-α concentrations caused by CCl_4 [44]. When compared to the HCC group, Cisplatin caused a significant improvement in liver function tests, as well as a significant decrease in TNF-α levels, Cisplatin has been shown to have a synergistic anticancer impact [45].

Rats with HCC treated with Annona muricata showed significant decrease in serum TNF-α in comparison with positive control rats.

The in vitro study revealed that Annona muricata leaf extract possessed anti-inflammatory activity as it inhibited the inflammatory mediators, TNF-α, compared to untreated cells [46]. Statistical analyses showed non-significant decreased levels of TNF-α in Group with Annona muricata, compared with other Group [47]. Our results were comparable with these results; Graviola leaves enhance

health by deactivation of the production of TNF-α comparing to rats with HCC [48].

Our study revealed that rats with HCC showed significant increase in PI3K and Akt level in comparison with negative control rats.

CCl_4 induced hepatic apoptosis beside significant increase in the activation of the PI3K/Akt pathway in the liver [49]. In addition, CCl_4 induce toxicity in the hepatocytes of hepatic fibrosis rats, and significantly up-regulate the expression of PI3K and Akt [50]. The novel findings of this study suggested that the liver fibrosis, which is induced by CCl_4 in vivo associated with the up-regulation of PI3K/AKT/mTOR signaling pathways [51]. CCl_4 activated PI3K and Akt, so Inhibition of PI3K/Akt signaling pathway can reduce the deposition of extracellular matrix, inhibit the proliferation of hepatic stellate cells and promote its apoptosis to achieve the purpose of therapy [52].

Rats with HCC treated with Cisplatin revealed significant decrease in PI3K and AKT in comparison with positive control rats.

Mitochondria are important targets of Cisplatin in HCC cells. Cisplatin-induced mitophagy and lysosomal biogenesis constitute mitochondrial-lysosomal crosstalk, which is a crucial mechanism by which HCC cells overcome the cytotoxicity of Cisplatin. Cisplatin resistance of HCC cells by a PI3K/mTOR inhibitor to increase the sensitivity of HCC cells to Cisplatin [53]. The usage of Cisplatin in HCC cells cause deactivating PI3K/AKT signaling pathway in comparison with HCC group [54]. We all know that cisplatin has anti-cancer effect. cisplatin inhibits the PI3K/Akt signaling and induces cell apoptosis of HCC [55].

Rats with HCC treated with Annona muricata showed significant decrease in PI3K and AKT in comparison with positive control rats. Annonaceous acetogenins induce non-significant decrease of PI3K and Akt to inhibit the excessive deposition of collagen [56]. The same model investigated the effect of Annona muricata extract on the PI3K/Akt pathway, he revealed that, the extract significantly reduced the level of phospho-Akt. The inhibition of the PI3K/Akt pathway was more pronounced when the extract was administered as a post-treatment rather than a pre-treatment [57].

In the present study, rats with HCC showed hepatocellular carcinoma represented in loss of hepatic tissue architecture with cellular and nuclear pleomorphism and missed polarity, hepatocellular carcinoma of fibrolamellar type, loss of hepatic tissue architecture with pseudo or false acini formation.

CCl_4 induce distorted tissue architecture, submassive centrical necrosis, fatty changes, and inflammatory cell infiltration. CCl_4 induce necrosis and inflammatory reactions of liver sections [42]. CCl_4 induces hepatocytes' irregular nuclei, vacuolated cytoplasm, and distorted microorganelles. A remarkable increase in hepatic DNA strand breakages and histopathological distortion in liver and kidney specimens were observed in CCl_4-intoxicated groups [25].

Rats with HCC treated with Cisplatin displayed severe congestion of the hepatic blood vessel with fibrosis of the portal area which is infiltrated with mononuclear cells infiltration, diffuse congestion of hepatic vessels, and blood sinusoids with diffuse coagulate necrosis of hepatocytes represented in pyknotic nuclei, dissociation of hepatocytes with loss of cellular polarity.

Pathological lesions in internal organs may be due to the liberation of free radicals leading to degenerative diseases such as diabetes and cancer [58]. Same lesions that Cisplatin induces sinusoidal dilatation, venular fibrosis and centrilobular vein injury, periportal fibrosis, hepatic cord degeneration, and cystic lesions with demarcated margins [59]. Same pathological lesions in mice that received Cisplatin were confirmed by damage in liver and kidney tissues [60]. In addition, Cisplatin induced degeneration of hepatic cells and dilated blood sinusoids leading to apoptosis [61].

Rats with HCC treated with Annona muricata showed high biliary epithelial proliferation with periductal cellular and nuclear pleomorphism beside proliferative nuclei, severe fibrosis, and hemorrhage with mild nuclear pleomorphism, some criteria of hepatic malignancy with a focal area of fibrosis.

Same pathological changes were observed in rats received Annona muricata [62]. This result is supported by the findings that Annona muricata leaf extract could be hepatoprotective [63]. Pathological changes observed in liver post using Annona muricata were parallel to the result which stated that rats who received Annona muricata showed

normal hepatocytes arranged in cords separated by blood sinusoid [64]. In addition, Annona muricata induced regular cellular composition, no signs of injury, necrosis, clogging, fatty acid aggregation or hemorrhagic zones surrounding the central vein or liver sinusoids [39]. The hepatocytes organized in cords were quite clear. The liver revealed no lyses in blood cells or infiltration of neutrophils, lymphocytes or macrophages. Histopathologically, there was a marked reduction in cellularity, nuclear chromatin condensation, and a few normal cells in the group treated with chloroform fraction of methanolic extract of seeds of Annona muricata at a dose of 31mg/Kg [65].

CONCLUSION

The overall outcome of the current review suggests that A. muricata has a favourable safety and tolerability profile. Future studies investigating its use in people diagnosed with a range of cancers are warranted. Early studies with graviola have shown positive anti-cancer effects. However there are promising clinical effects. Further studies are needed to elucidate the mechanisms of action, dose, formulation and potential adverse effects and interactions to enable graviola to be used in cancer patients, either alone or part of a holistic, integrated treatment approach.

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Abbreviations:

HCC	Hepatocellular carcinoma
CCl_4	Carbone tetrachloride
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
TNF-α	Tumor necrosis factor alpha
PI3K	Phosphoinositide 3-kinase
Akt	Protein kinase B

Table (1): Primers sequence of the studied genes:

Gene symbol	**Primer sequence from 5′- 3′**
PI3K	F: TTAAACGCGAAGGCAACGA R: CAGTCTCCTCCTGCTGTCGAT
AKT	F: CAAGCCCAAGCACCGT R: GAATCACCTTCCCAAAGGTG
β-actin	F: GACGAGGCCCAGAGCAAGAGAGG R: GATCCACATCTGCTGGAAGGTGGAC

Table (2): The result of serum liver enzymes (AST, ALT and ALP) and Serum protein picture (total protein, albumin, globulin and A/G ratio) from rats:

Groups	Liver enzymes (U/ml)			Protein picture (gm./dl)			
	AST	ALT	ALP	T. protein	Albumin	Globulin	A/G ratio
GP. 1 **(negative control)**	46.83 ± 1.52b	33.81 ± 1.87b	83.54 ± 1.34b	6.68 ± 0.8a	3.78 ± 0.51a	2.90 ± 0.49a	1.30 ± 0.21a
GP. 2 **(positive control)**	74.59 ± 1.62a	60.21 ± 1.34a	110.33 ± 1.92a	3.92 ± 0.49b	2.14 ± 0.17b	1.60 ± 0.18b	1.10 ± 0.32a
GP. 3 **(Cisplatin)**	72.21 ± 2.39a	58.81 ± 1.87a	106.42 ± 1.68a	4.08± 0.32b	2.32 ± 0.13b	1.94 ± 0.31b	1.45 ± 0.33a
GP. 4 **(Annona muricata)**	49.24 ± 2.44b	36.59 ± 1.55b	86.42 ± 1.44b	5.66 ± 0.89a	3.02 ± 0.68a	2.64 ± 0.68a	1.14 ± 0.32a

Means within the same column carrying different superscripts are significant different at P < 0.05

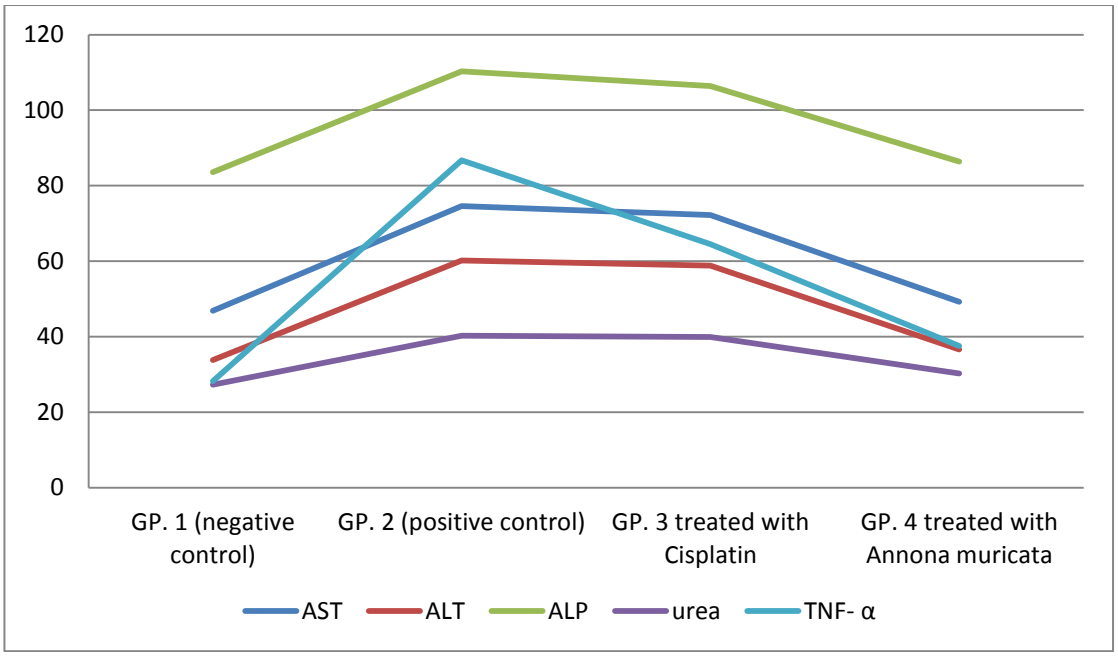
Means within the same column carrying same superscripts are non-significant different at P < 0.05

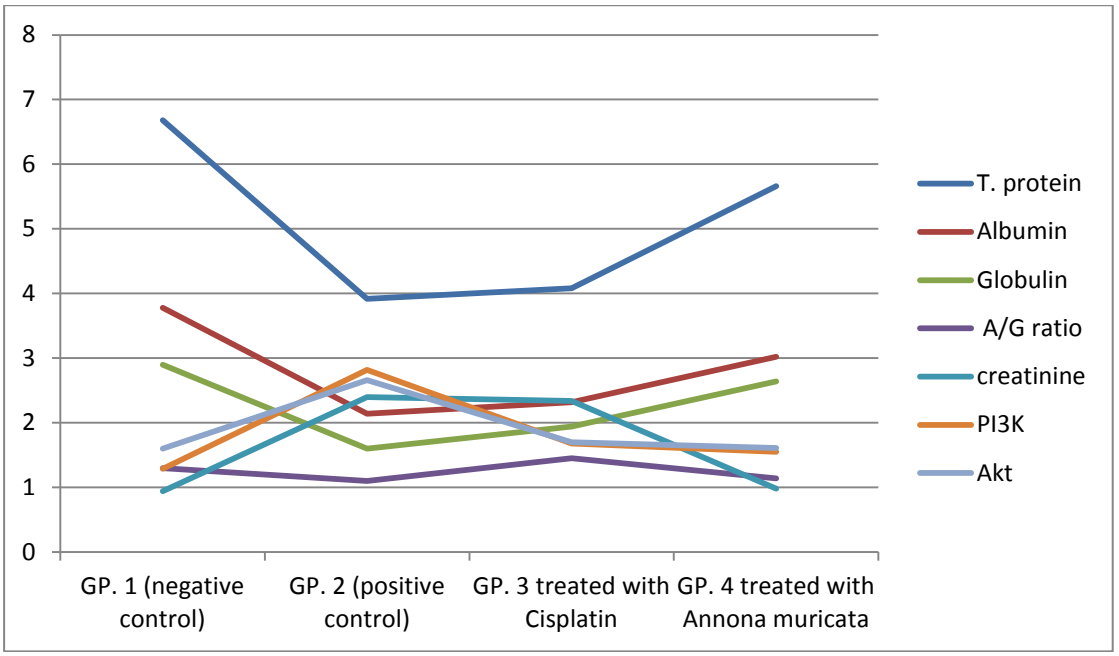
Table (3): The result of serum kidney function tests (urea and creatinine), TNF-α liver tissue gene expression PI3K and Akt from rats:

Groups	Kidney function (mg/ml)		TNF- α (pg/mL)	PI3K	Akt
	Urea	Creatinine			
GP. 1 **(negative control)**	27.25 ± 1.21c	0.94 ± 0.12b	28.2 ± 1.95c	1.29 ± 0.26b	1.60 ± 0.37b
GP. 2 **(positive control)**	40.28 ± 1.87a	2.40 ± 0.51a	86.7 ± 2.55a	2.82 ± 0.48a	2.66 ± 0.51a
GP. 3 **(Cisplatin)**	39.89 ± 1.63a	2.34 ± 0.47a	64.5 ± 3.44a	1.68 ± 0.27b	1.70 ± 0.28b
GP. 4 **(Annona muricata)**	30.21 ± 1.18b	0.98 ± 0.31b	37.5 ± 1.88b	1.55± 0.21b	1.61 ± 0.19b

Means within the same column carrying different superscripts are significant different at P < 0.05

Means within the same column carrying same superscripts are non-significant different at P < 0.05





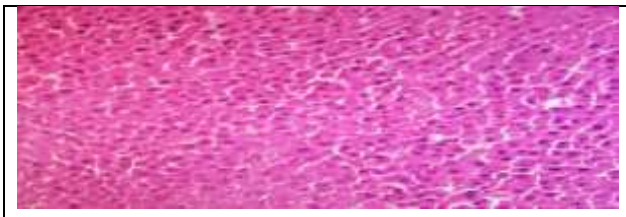


Fig. 1: Photomicrograph of rat's liver of Negative control group showing normal tissue architecture and cellular details, (H&E x400).

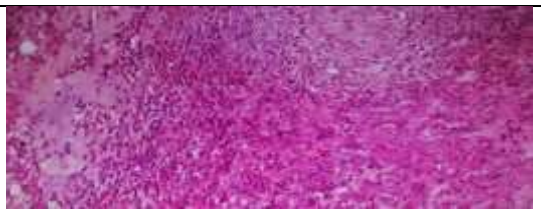


Fig. 2 A: Photomicrograph of rat's liver of Positive control group showing hepatocellular carcinoma grade IV represented in loss of hepatic tissue architecture with cellular and nuclear pleomorphism and missed polarity, (H&E x200).

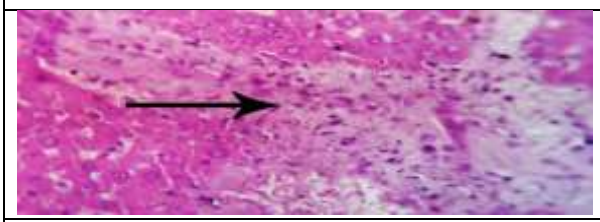


Fig. 2 B: Photomicrograph of rat's liver of Positive control group showing hepatocellular carcinoma grade II of fibro lamellar type (arrow), (H &E x400).

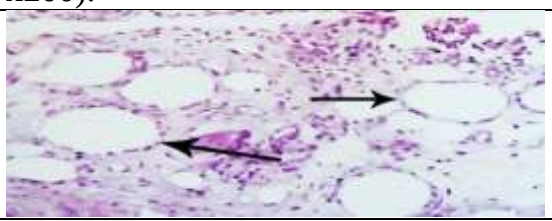


Fig. 2 C: Photomicrograph of rat's liver of Positive control group showing hepatocellular carcinoma grade IV of acinar type (pseudo glandular) represented in loss of hepatic tissue architecture with pseudo or false acini formation (arrows), (H&E x200).

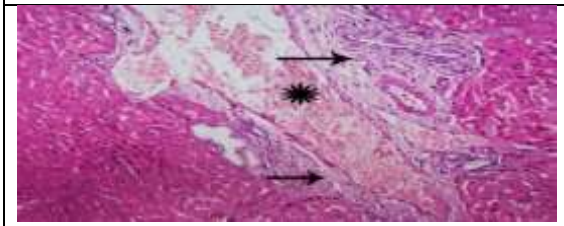


Fig. 3 A: Photomicrograph of livers of

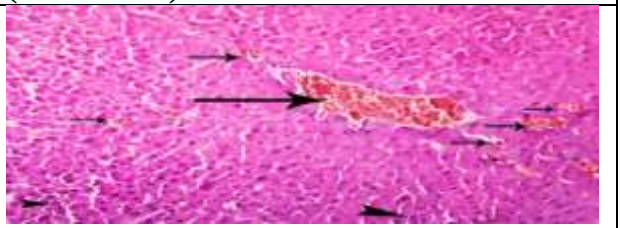


Fig. 3 B: Photomicrograph of livers of

rats with HCC treated with Cisplatin showing severe congestion of the hepatic blood vessel (star) with fibrosis of the portal area which infiltrated with mononuclear cells infiltration (arrows), (H&E x200).	rats with HCC treated with Cisplatin showing diffuse congestion of hepatic vessels and blood sinusoids (arrows) with diffuse coagulative necrosis of hepatocytes represented in pyknotic nuclei (arrowhead), (H&E x200).

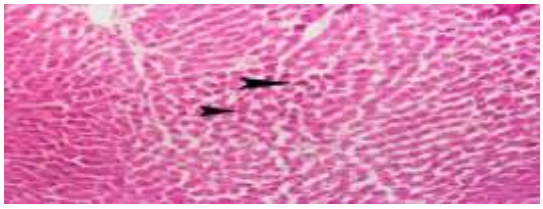
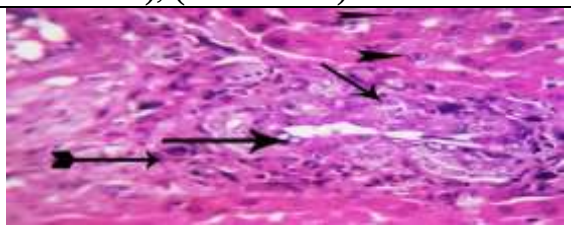

	

Fig. 3 C: Photomicrograph of livers of rats with HCC treated with Cisplatin showing dissociation of hepatocytes (arrowheads) with loss of cellular polarity, (H&E x200).	**Fig. 4 A:** Photomicrograph of livers of rats with HCC treated with Annona muricata showing high biliary epithelial proliferation (arrows) with periductal cellular and nuclear pleomorphism (tailed arrow) in addition to proliferative nuclei (arrowhead), (H&E x400).

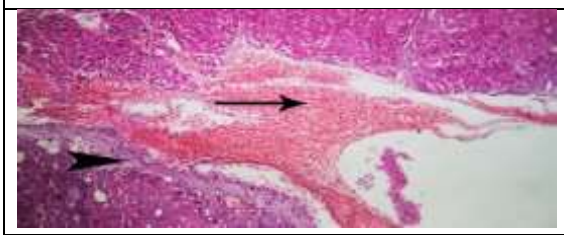
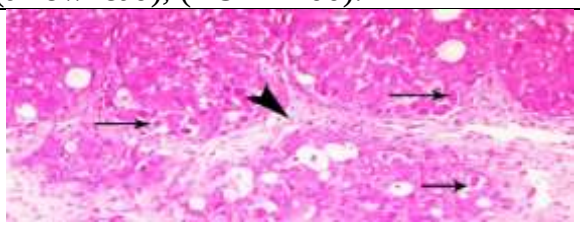

	

Fig. 4 B: Photomicrograph of livers of rats with HCC treated with Annona muricata showing severe fibrosis (arrowhead) and hemorrhage (arrow) with mild nuclear pleomorphism, (H&E x200).	**Fig. 4 C:** Photomicrograph of livers of rats with HCC treated with Annona muricata showing some criteria of hepatic malignancy (increase N/C ratio) (arrows) with a focal area of fibrosis (arrowhead), (H&E x200).