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Ameliorating Role of Jambolan of Capecitabine Induced Hepato-and Nephrotoxicities in male rats

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A B S T R A C T

Capecitabine (Xeloda, Roche) is a pro- anticancer drug, that is enzymatically converted to 5-fluorouracil in the body. In the present work, we evaluate the ameliorated role of the Jambolan fruit-extract against the hepato-& nephrotoxicity of the mentioned drug. Forty male Wistar albino rats (*Rattus norvegicus*) weighing approximately 120 g were used. Animals were divided into four groups; control (saline-treated), Jambolan-treatment (400mg/kg, of fruit components), capicitabine-treatment (40 mg /Kg body weight for 30 days) and capicitabine and Jambolan-treatment. Daily oral treatment was carried out for 30 days. At the end of treatment, the animals were sacrificed and their livers and kidney were incised and processed for histopathological investigation as well as flow cytometric analysis for apoptosis. The present findings revealed that the pro-anticancer drug capecitabine-treatment possessed dramatic hepato- and nephrotoxicity. The hepatotoxicity characterized by widespread of pyknotic nuclei, focal leukocytic infiltration. The nephrotoxicity categorized by lobulated glomeruli, interstitial inflammatory cell infiltration and swollen of tubules associated with degeneration of their epithelial lining cells. Flowcytometric analysis confirmed the pathological aberrations by a marked increase of M1 (subG1 apoptsis) in liver and renal tissues. Jambolan-treatment showed a sticking amelioration of the pathological picture and apoptosis. Finally, the authors concluded that the Jambolan-treatment with its higher content of antioxidants resoluted the toxicological aspects of the anticancer drugs.

Introduction

Capecitabine (Xeloda, Roche) is an orally-administered chemotherapeutic agent used in the treatment of numerous cancers (Rossi, 2013). It is a prodrug, that is enzymatically converted to 5-fluorouracil in the body (Joint Formulary Committee, 2013). Capecitabine is an orally administered precursor of 5-fluorouracil (5-FU), a fluoropyrimidine antimetabolite. It is converted to 5-FU preferentially in tumor

tissue, and also in the liver, by way of a three-step enzymatic cascade (Miwa *et al.,* 1998). Capecitabine is a relatively new agent, with FDA approval in 2001 for use as an alternative to the Mayo Clinic 5-FU/folinic acid regimen for metastatic colon cancer. It has since been approved for use in the adjuvant treatment of colon cancer, as well as for metastatic breast cancer.

Hepatic steatosis, a mild manifestation of nonalcoholic fatty liver disease (NAFLD), may occur after treatment with 5-FU. This has become a more recognized complication in the era of hepatic surgery for colorectal liver metastases, where hepatic steatosis is associated with increased post-operative morbidity (Zorzi *et al.,* 2007). Peppercorn *et al.* (1998) found that 47% of patients with colorectal liver metastases treated with systemic 5-FU and folinic acid had computed tomography (CT) findings consistent with fatty change. Another report described laboratory abnormalities consistent with hepatic toxicity in 40% of patients who received adjuvant therapy with 5-FU and levamisole after undergoing surgical resection for Stage II or III colon cancer, with CT and biopsy evidence of steatosis in a few cases (Moertel *et al.,* 1993).

Chin *et al.* (2010) reported a case of a 74-year-old Armenian woman who received capecitabine as adjuvant treatment for colon cancer and subsequently developed abnormal liver biochemical tests and radiographic findings in keeping with hepatic steatosis.

On the other hand, nephrotoxicity is one of most common side effects of the anticancer drugs for malignancy. Antimetabolites, alkylating agents and anthracyclines developed nephrotoxicity (Kintzel, 2001; Erkurt *et al.,* 2008). Renal tubular damage is a well-known renal complication induced by anticancer drugs (Kakihara *et al.,* 2003). However, there are several studies about anticancer inducing glomerular dysfunction (Ikarashi *et al.,* 2004). Cisplation one of the most commonly used anticancer drugs have been associated with increased oxidative stress via free radical formation which led to impairment in tubular dysfunction and development of deformation of micrivilli and vesicuolation of rough endoplasmic reticulum (Ravindra *et al.,* 2010). The nephrotoxic potential of most anticancer agents dramatically increases in the presence of borderline or overt pre-existing chronic kidney disease and measurement of renal function is therefore of utmost importance in the cancer patient before any treatment is initiated (Lameire *et al.,* 2011).

Jambolan (*Syzgium cumini* Linn (Family Myrtaceae) tree cultivated in the gardens of Mansoura Universiy. The tree flourished their fruits during September to November which is oval-brownish colour of good taste. The fruits is rich in compounds containing anthocyanins, glucoside, elagic acid, isoquercetin, kamferol or antimellin which halts the diastatic conversion of starch into sugar (Ayyanar Subash-Babu, 2012). The fruits are rich in raffinose, glucose, fructose, citric acid, mallic acid (Lewis *et al.,* 1956), gallic acid, anthrocyanin (Jain and Seshadri, 1975), cyaniding diglycoside, petunidin and malvidin (Venkateswarlu, 1952), crude fiber, magnesium, calcium, phosphorus, iron, potassium, cupper, vitamin A, thiamine, riboflavin, niacin, folic acid, ascorbic acid, choline (Ayyanar Subash-Babu, 2012).

There are several works using the phytotherapy to ameliorate the hepato-and nephrotoxicity of chemotherapeutic drugs. Seeds and oil of Nigella sativa was found to ameliorate the gentamicin (GM) induced

nephrotoxicity by the generation of oxygen free radicals (Ali, 2004). Grape seed extract was found to ameliorate gentamicin-induced nephrotoxicity (El-Ashmawy *et al.*, 2006). Quercetin and arginine were found to ameliorate nano zinc oxide-induced nephrotoxicity in rats (Faddah *et al.*, 2012). Gemfibrozil and/or silymarin were efficiently attenuated cisplatin-induced nephrotoxicity evidenced by significant decrease of renal function associated with reduction of the necrotic damage (Kabel *et al.*, 2013).

Materials and Methods

Capecitabine and applied dose-treatment

Capecitabine (Xeloda, Roche) is an orally-administered chemotherapeutic agent used in the treatment of numerous cancers. It is a pro-drug, that is enzymatically converted to 5-fluorouracil in the body. The therapeutic dose (40 mg/kg body weight in 0.4 mL saline solution orally administered for one month) of this drug for rat was calculated according to Paget and Barnes (1964). The chosen dose was nearly comparable to the human effective therapeutic dose (ETD). The applied dose emulsified in saline solution and orally administered daily for one month.

Phytotherapy-treatment with Jambolan-fruit

The jambolan is fast-growing tree belong to Myrtaceae. It is cultivated in India and Oceania and Florida and recently cultivated in the gardens of the mansoura university gardens. The tree reached up to 35 or 40 ft (110-111 m). Their fruits are oval-shaped dark-brown colour and edible in taste and become flourished annually during August-November. In the present study we take the fruits and homogenated in saline solution and applied oral doses of 400mg/kg, body weight (n=10). Jambolan tree cultivated in the gardens of Mansoura University, Egypt.

Experimental work

Forty male Wistar albino rats (*Rattus norvegicus*) weighing approximately 120 g were used in the present study. All rats were kept under good ventilation and aerated room. Excess standard diet was supplied *ad libitum* during the experimental period. They were allowed free access to water. Animals were divided into four groups. The first served as control, the second received Jambolan-treatment (400mg/kg, of fruit homogenized with saline solution) (n=10). In the third group, capicitabine-treatment (40mg/kg body weight). The fourth group received daily oral doses of jambolan equal to 400 mg /Kg body weight for 30 days. At the end of treatment, the animals were sacrificed and their liver and kidney were incised. Part of the tissues were kept in refrigerator and the other ones were fixed in fixed in 10% phosphate buffered formalin (pH 7.4) for routine histological investigation.

Histological investigations: Liver and kidney of control and experimental groups were incised immediately, fixed in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending grades of ethyl alcohol, cleared in xylol, and mounted in molten pararplast 58−62 °C. Serial 5 µm thick sections were cut and stained with ematoxylin and eosin (H&E), examined under bright field light microscopy, and photographed.

Flow cytometric analysis of cell cycle apoptosis

DNA ploidy and apoptosis were analyzed using fluorescence activated cell sorting

(FACS) flow cytometer (Becton Dickinson, Sunnyvale, CA) equipped with a 15 mW air-cooled 488 nm argon-ion laser. FL1 (FITC) signals were detected through a 530/30 nm band-pass filter; FL2 (PI) signals were detected through a 585/42 nm band-pass filter. A total of 20 000 events were recorded in list mode and analyzed using the Cell Quest Pro software (Becton Dickinson) at Mansoura University Hospital. The cell populations were gated assuming the linear forward scatter (FSC) and side scatter (SSC) properties. Biopsies from liver and kidney of studied animals were taken, and cell suspension was prepared with Tris-EDTA buffer (pH 74) (Sigma-Aldrich Co.). Cell suspension was fixed in ice-cold 96−100% ethanol (Sigma) at 4 °C overnight, centrifuged at 1500 rpm for 10 min, and then resuspended in PBS containing 50 µg/mL propidium iodide (PI) (Sigma-Aldrich Co.). The cells were incubated at 37 °C for 30 min before analysis by flow cytometry. PI fluorescence excitation at 512 nm, with a relatively large Stokes shift, emits at a maximum wavelength of 617 nm. Apoptosis was indicated by the percentage of cells in G0/G1, S, and G2/M phases of the cell cycle.

Results

Histopathological observations

Liver

In the control and phytotherapy-treatment with jambolan fruit, the hepatic tissue showed normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells (Fig. 1 A & B).

Capecitabine-treatment possessed dissolution of hepatic cords, which appeared as empty vacuoles aligned by strands of necrotic hepatocytes. The hepatic tissues showed the presence of dense focal inflammatory cells or necrotic tissues associated with bile duct obliteration, congestion of blood vessel with perivascular leukocyte infiltration. May of the hepatocytes showed clumped chromatin materials manifested pyknotic degeneration (Fig.1 C&D).

Jambolan-treatment of capecitabine-intoxicated rats revealed marked amelioration and improvements of the histologic picture of hepatic tissue (Fig. 1E&F).

Kidney

In control and Jambolan-treatment, the control exhibited normal cortex having renal corpuscles, each of which consists of an outer envelope of simple squamous epithelium (Bowman's capsule) surrounding a fluid-filled space (Bowman's space) within which is suspended a glomerulus which contains many cell identities such as endothelial cells, podocytes, or mesangial cells. The bulk of the cortex consists of convoluted tubules. Cells comprising proximal tubules stain more intensely eosinophilic than those comprising distal tubules. The lumens of distal tubules) commonly appear more open and clear than those of proximal tubules (Fig.3 A&B).

In experimental group treated with the anticancer drugs, the renal tissue exhibited marked dilation of the proximal convoluted tubules with slogging of almost entire epithelium due to desquamation of tubular epithelium, intense glomerular hypercellularity and diffuse thickening of the glomerular basement membrane giving characteristic of nodular glomerulosclerosis. Also, there were detected interstitial hemorrhages, glomerular congestion, tubular

congestion and atrophy with mild mesangial expansion and proliferation in the glomeruli (Fig.4 A&B).

In anticancer-treated group and received Jambolan phytotherapy-treatment, there was a marked amelioration of the histologic picture except mild interstitial haemorrgae (Fig.4 C & D).

Flow cytometry of cell cycle

From Tables 1 & 2, and Figs 2 & 5, there was a considerable increase of M1 (subG1 apoptsis) in capicitacin-treated liver and kidney and a decrease in the other cell cycle phases (M2, M3, and M4) comparing with the control and jambolan-treatment. On the other hand, capicitacin intoxication and jambolan-treatment exhibited marked amelioration by decrease the incidence of apoptosis of M1.

Discussion

From the present findings, treatment with the pro-anticancer drug capecitabine-treatment possessed dramatic hepatotoxicity. The hepatic tissue showed widespread of pyknotic nuclei, focal leukocytic infiltration. Similar hepatic damage was reported by Chin *et al.* (2010) in a case of a 74-year-old Armenian woman who received capecitabine as adjuvant treatment for colon cancer. Abou-Zeid (2014) reported hepatotoxicity post 5-flurouracil-treatment. 5-Fluorouracil (5-FU) is a potent antineoplastic agent commonly used for the treatment of various malignancies. It has diverse adverse effects such as nephrotoxicity and hepatotoxicity which restrict its wide and extensive clinical usage. It causes marked organ toxicity coupled with increased oxidative stress and apoptosis. The possible mechanism of 5-FU induced renal toxicity is the induction of oxidative stress; activation of apoptotic pathway by upregulation of p53, bax, caspase-3 and down regulating Bcl-2 (Rashid *et al.,* 2014). Also, the used anticancer drugs exerted nephrotoxicity categorized by lobulated glomeruli, interstitial inflammatory cell infiltration and swollen of tubules associated with degeneration of their epithelial lining cells. Similar glomerulosclerosis was reported after doxorubicin (Harmon *et al.*, 1979) and nitrosoureas-treatment (Alpers and Cotran, 1986). The present finding supported by marked increase of M1 (subG1 apoptosis) in capicitacin-treated liver and kidney. Our findings supported the work of many authors. Many anticancer drugs such as cisplatin, doxorubicin and camptothecin exerted DNA damage. Drugs such as Cisplatin induces DNA damage was carried out through caspase activation in enucleated cells (cytoplasts lacking a cell nucleus) as well as associated with rapid induction of cellular reactive oxygen (Havelka *et al.*,2007). Other mechanism involved DNA damage cell cycle arrest (Swift and Golsteyn, 2014).

On the other hand, Jambolan-treatment of capecitabine-intoxicated rats revealed marked amelioration and improvements of the histologic picture of hepatic and renal tissues associated with a marked reduction of DNA damage. The amelioration of the histologic picture as a result of jambolan fruits-extract treatment attributed to the antioxidant capacity including alpha-glucosidase and alpha-amylase inhibitory activities as well as Cyanidin, quercetin, ellagic acid (EA), proanthocyanidins, phenolic content catechin and also high-ascorbic acid (Correia *et al.*, 2012). Similar amelioration of hepato-and nephrotoxicity induced by cypermethrin were reported after aqueous extract of *Trigonella foenum graecum* (fenugreek) (Sushma and Devasena, 2010).

Fig.1(A-F) Photomicrographs of histological sections of liver; A&B. Control showing normal arrangement of hepatic cord, central vein and hepatocytes with characteristic nuclear chromatin; C&D. Capecitabine-treatment showing congested blood vessel (CBV), perivascular round cell infiltration (RCI) and increased average of pyknotic nuclei; E&F Capecitabine and Jambolan-treatment showing marked amelioration of hepatic picture. HX-E

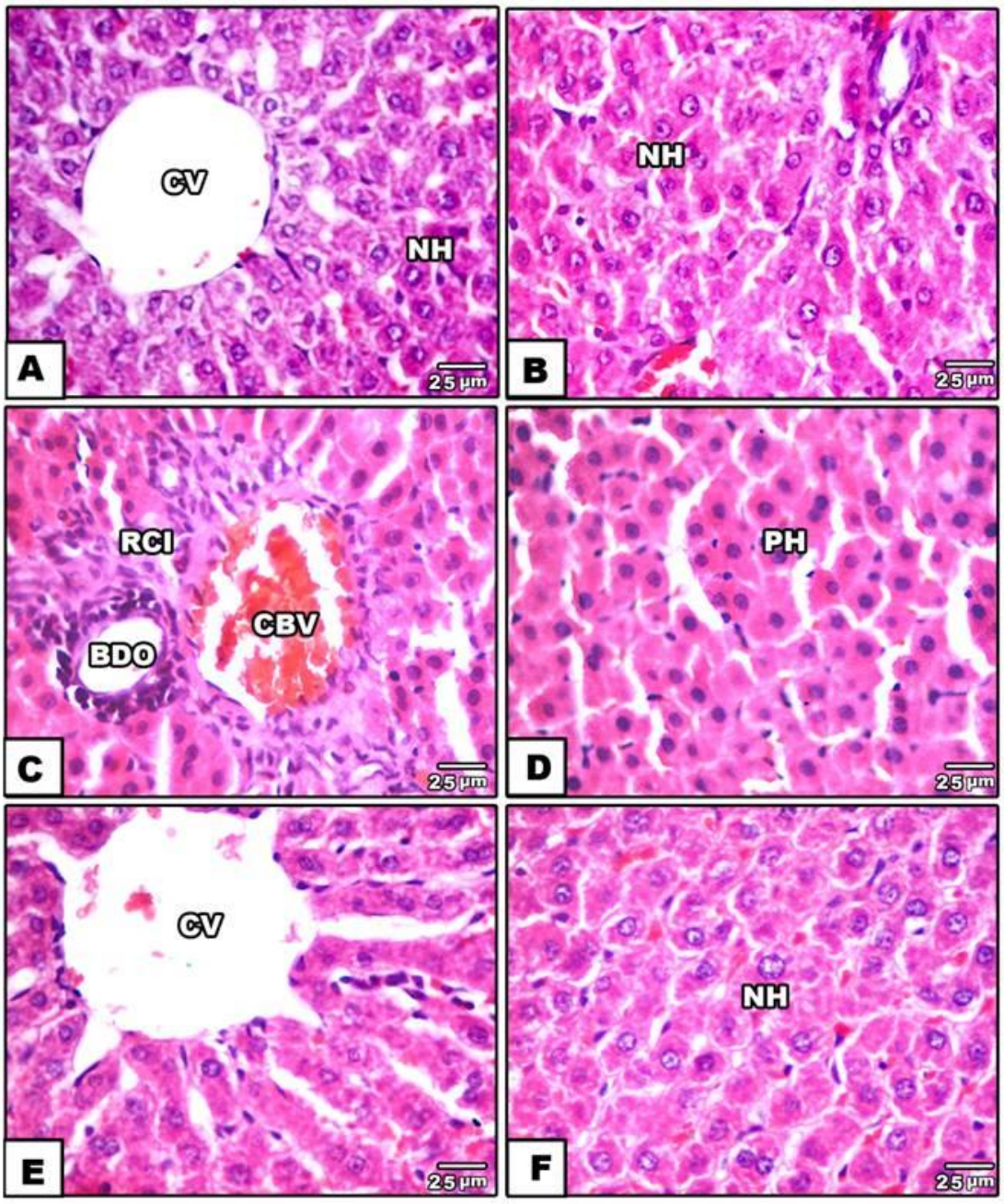


Fig.2 Flowcytometry of hepatocytes C&JB. Control and Jambolan-treatment showing normal cell cycle; CAP. Capecitabine-treatment showing increased average of apoptosis; JB&CAP. Combined capecitabine and Jambolan-treatment showing reduced cell damage

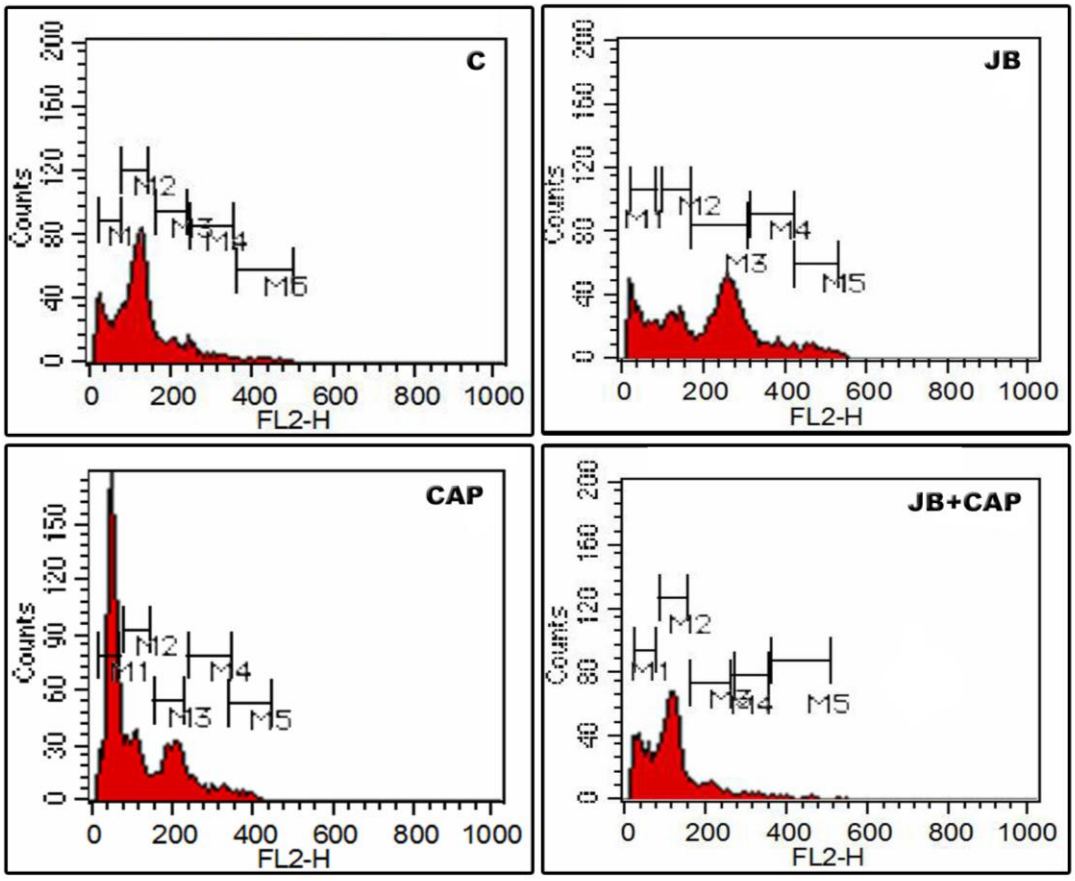


Table.1 Flowcytometry of hepatic and renal cell cycle following capecitabine-intoxication and phytotherapeutic-treatment with Jambolan fruit-extract

	Control		CAP-Treatment		JB-Treatment		CAP.& JB-Treatment	
	L	K	L	K	L	K	L	K
M_1 (sub-G0/G1 apoptosis)	12.8±1.7	11.2±2.1	58.3±4.1*	53.6±5.3*	18.9±1.3**	19.5±1.5*	29.1±2.4*	31.2±2.1*
M_2 (G0/1 phase)	31.1±2.8	30.5±3.7	17.8±1.9*	26.5±2.7**	17.8±1.5*	41.5±3.5**	14.6±1.4*	15.3±2.1*
M_3 (S phase)	6.5± 1.1	7.4±0.9	12.9±1.2*	18.9±2.1*	44.7±4.5*	30.3±2.7*	5.8±0.8**	6.6±0.5**
M_4 (G2/M phase)	2.7±0.6	3.8±0.4	4.1±0.3**	5.3±0.3**	8.9±1.1**	14.9±1.2*	2.3±0.2**	2.1±0.1**

Each replicate represent the M±SE. n=5. Significance at *P <0.05. Non signifcant **,.CAP, capecitabine, JB, Jambolan, L,Liver; K,Kidney.

Fig.3(A-B) Photomicrographs of histological sections of control kidney showing normal pattern of glomeruli (GL) and renal tubules (RT). HX-E

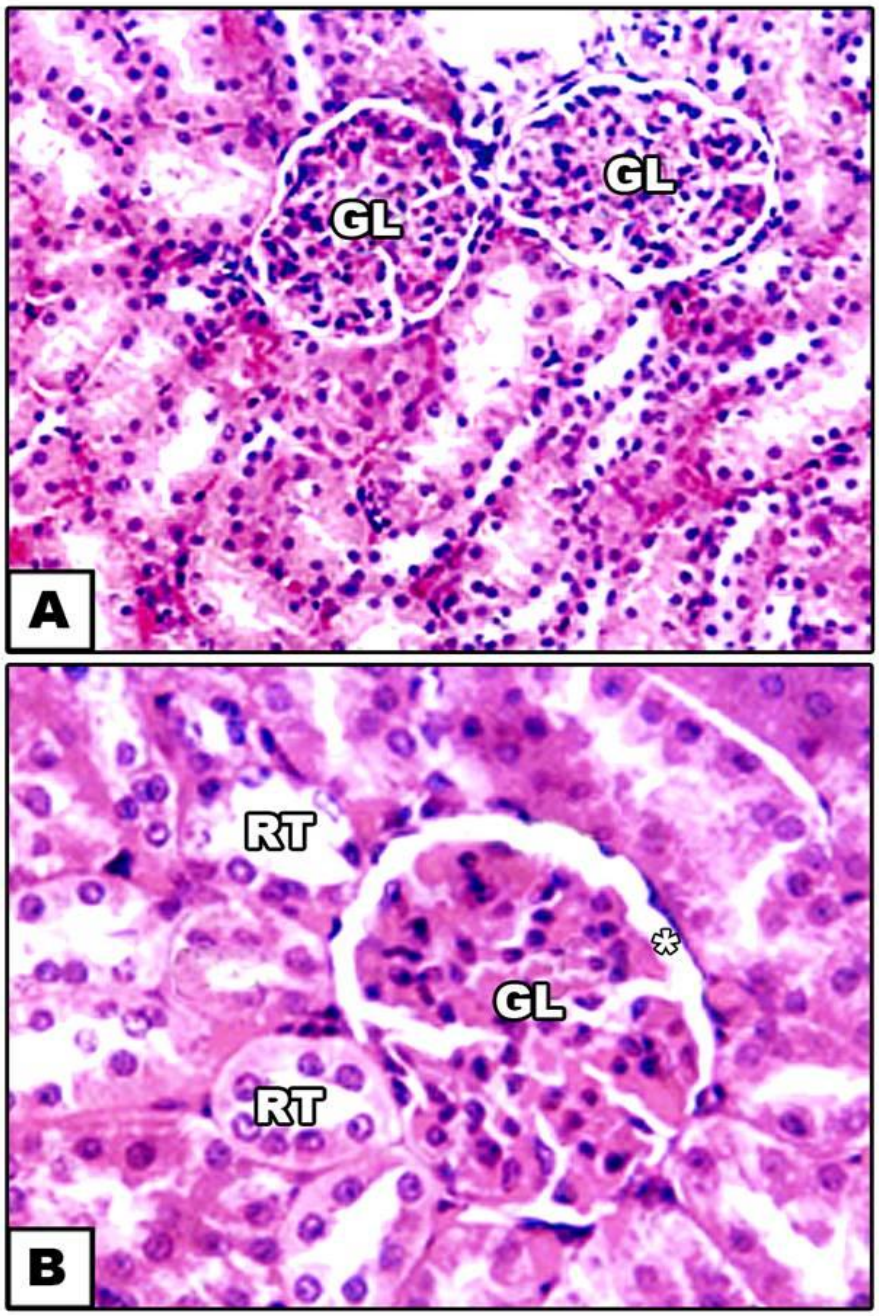


Fig.4(A-D) Photomicrographs of histological section of kidney; A&B Capecitabine-treatment showing clobulated glomeruli (LG), swollen renal tubules (ST) and degeneration of epithelial lining the tubules (DE); C&D Capecitabine and Jambolan-treated group showing amelioration of the renal picture. HX-E

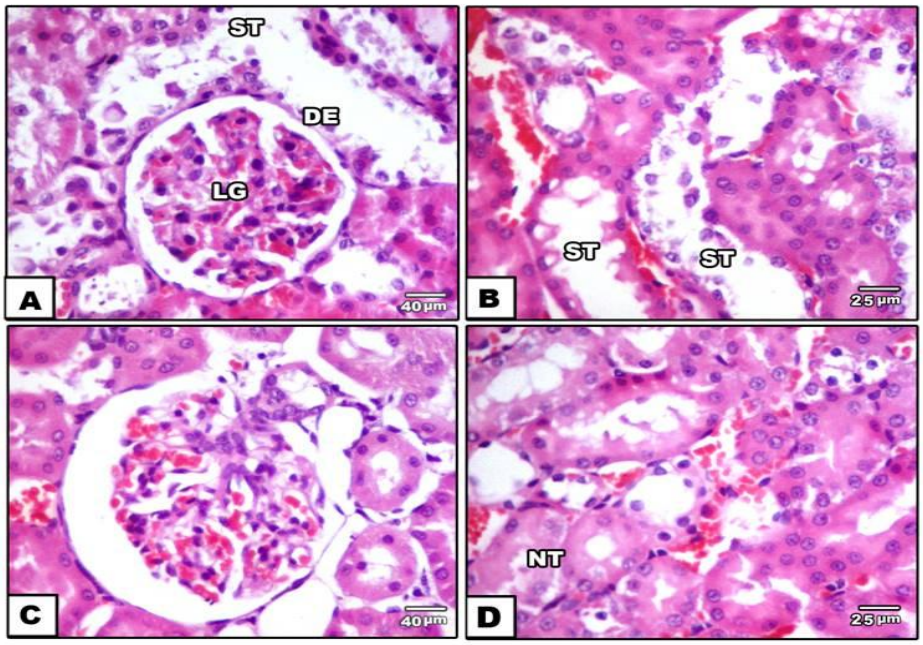
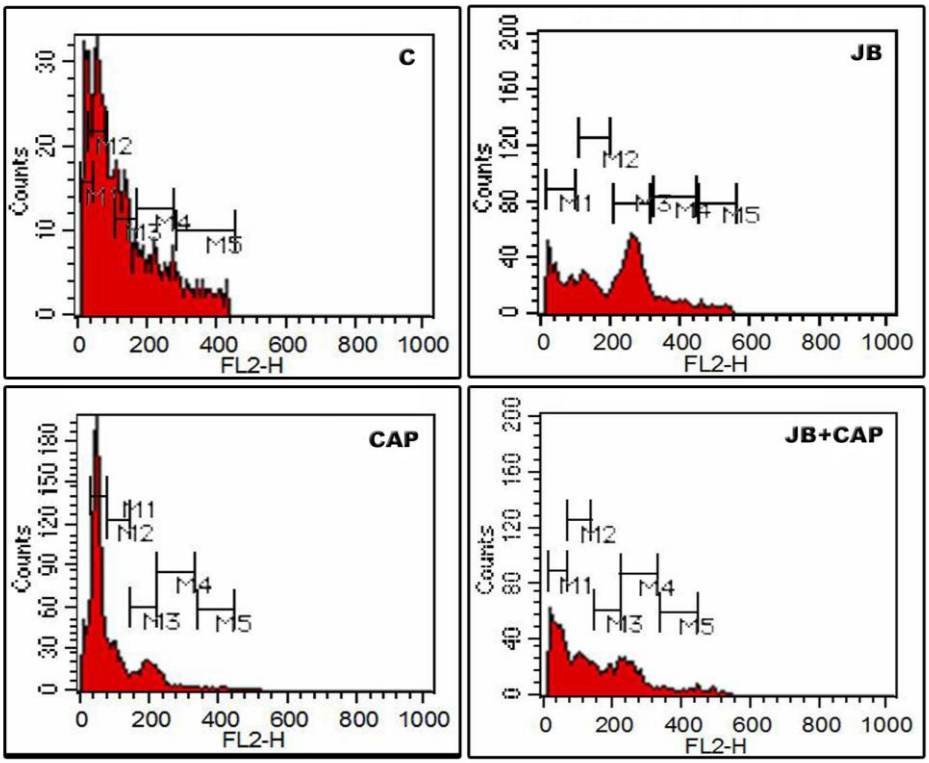


Fig.5 Flowcytometry of renal cells. C&JB. Control and Jambolan-treatment showing decreased average of apoptosis. CAP. Capecitabine-treatment showing increased average of apoptosis. JB&CAP. Combined Capecitabine and Jambolan-treatment showing reduced cell damage. HX-E



Oxidative stress occurring during chemotherapy through liberation of reactive oxygen species such as cisplatin-induced nephrotoxicity may be ameliorated by lycopene the major carotenoid present in tomatoes, which is a potent antioxidant (Sahin *et al.*, 2010).

Chinese herbal medicine ameliorating glomerulosclerosis and renal interstitial fibrosis during the progression of chronic renal failure (CRF) by improving glomerular hemodynamics turbulence, podocyte injury, transforming growth factor (TGF)-beta over-expression, hyperlipidemia, macrophage infiltration, tubular epithelial myofibroblast transdifferentiation, and nephrotoxicity of proteinuria (Feng *et al.*, 2011).

Finally the authors concluded that during chemotherapeutic treatment, the patient must administered fruits and vegetable containing antioxidants to ameliorate their hepatic and renal toxicities.

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