ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com
Journal DOI:10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

INFERRING THE EFFICACY AND SAFETY OF GREEN TEA WATER EXTRACTAFTER PROLONGED CONSUMPTION AGAINST EPIRUBICIN-INDUCED HEPATOTOXICITY IN MICE: A HISTOLOGICAL STUDY.

Mona Ramadan Alshathly.
Biology Department, King Abdulaziz University, Jeddah, Saudi Arabia.

Manuscript Info

Manuscript History:

Received: 15 May 2016
Final Accepted: 22 June 2016
Published Online: July 2016

Key words:
Epirubicin, Green Tea, Mice, Hepatotoxicity, Histology.

Corresponding Author

Mona Ramadan Alshathly.

Abstract

Hepatotoxicity is a well-known complication of anticancer agent epirubicin, as a result of oxidative stress. One of the most commonly consumed herbal extracts with antioxidant properties is green tea.The objective of this study was to evaluate the effectiveness and safety of prolonged consumption of green tea water extract against epirubicin-induced hepatotoxicity in mice. One hundred adult female mice were divided into control (GI), treated with access to water (GIIa), treated with access to green tea water extract (GIIb) and untreated with access to green tea water extract only (GIII).Epirubicin was administrated every three weeks for eight cycles. Livers from five mice were taken from each group after one, three, four, six and eight cycles. The GIIa group showed hydropic degeneration, apoptosis and pyknotic nuclei from early cycles of administration and continued to be observed until marked necrosis and haemorrhage were observed after the 6^{th} cycle. In contrast, the GIIb group showed good protective profile against epirubicin-induced histological changes observed in GIIa group until the 4^{th} cycle where frequentpresence of changes such as bi-nucleated cells and inflammations were observed. By the end of the 6^{th} cycle, severe histological changes were observed including marked necrosis followed by exponential increased of mortalities. The GIII group started showing histological changes after the 6^{th} cycle. Although consumption of green tea ameliorated the epirubicin-induced changes, prolonged consumption introduced a potential pro-oxidant property when combined with epirubicin. This suggests that green tea anti-oxidant and pro-oxidant properties are interchangeable depending on the duration of consumption.

Introduction:-

Epirubicin (EPI) is the 4'-epimer of doxorubicin (DOX), both well-known and studied anticancer drugs from the anthracycline family.They have very similar pharmacologic profiles being metabolized in the liver and eliminated through the bile. Nevertheless, at similar doses, EPI appears to have a better side-effect profile than DOX (Mouridsen et al., 1990). The main mechanism of EPI antitumor effect is through intercalation of DNA and generation of free radical including reactive oxygen species (ROS)(Gianni et al., 1983). This could infer the more favourable toxicity profile of EPI compared to DOX.

Epirubicin has been commonly used solely and in combination against various types of cancer, especially parenchymal organs cancer in vivo such as breast, gastric, lymphoma, cardiac, liver(Di-Wen et al., 2016; Jänicke, 2000; Lopez et al., 1984; Sasu et al., 2015; Sha et al., 2012; Štěrba et al., 2012)and to a certain extent small-cell lung cancer(Jacot et al., 2012).The most common side effects are alopecia, nausea/vomiting, cardiotoxicity, hepatotoxicity, leukopenia, and stomatitis(Bonadonna et al., 1993; Cersosimo and Hong, 1986). However, side effects post-EPI treatment are considered less compared to other anthracyclines (Robert, 1993).

A recent study suggests that EPI-loadedliver-targeted drug delivery system could effectively inhibit the growth of liver tumours*in situ* and potentially reduce the systemic side effects(Di-Wen et al., 2016). Alas, EPI is known to be an irritant drug thatis giving mainly by intravenous injection (IV) causing extensive tissue damage and blistering if escapes from the vein (Doellman et al., 2009).

Due to the intense toxicity profile, limited dosage and accompanying side effects, alternative and herbal treatment of cancer have been widely researched recently. The focus-concerned herbal extracts with antioxidant activity to ameliorate the drug's effect. One of the most commonly consumed beverages is green tea (GT), prepared from the dried leaves of the plant *Camellia sinensis*, has been studied extensively for its cancer preventive effects in many different experimental systems, including animal models (Clark and You, 2006; Hou et al., 2004; Ju et al., 2007; Khan et al., 2006).A study showed that powdered GT extract reconstituted in distilled water offers protection against DOX-induced cardiotoxicity via reduction of oxidative stress (Patil and Balaraman, 2011). This protection property is attributed to green tea catechins (GTC), the main metabolites in GT(Quiles et al., 2002).Theypossess outstanding antioxidant and free radical scavenging properties (Scott et al., 1993; Toda, 2011) and include (-)-epigallocatechin-3-gallate (EGCG) which composes more than 50% of the mass of all GTCs(Nagle et al., 2006). Furthermore, EGCG has been implicated with chemoprevention and anticancer properties (Azam et al., 2004) alas, high dosage of EGCG was reported to cause toxicity of rat liver mitochondria and hepatocytes (Kucera et al., 2015) as well as severe hepatic necrosis due to increased oxidative stress and lipid peroxidation in mice (Lambert et al., 2010).

The present study aimed to shed the light on the possible protective effect of GT water extract against EPI-induced hepatotoxicity in mice and evaluate its safety upon prolonged consumption in uncontrolled dose using histopathological evaluation of liver parenchyma.

Material and Methods:-

Materials:-

Ellence®Pharmorubicin (the trade name of EPI) was purchased in the form of powder package 10 mg from Pfizer Company (Shanghai, China). Dried leaves of GT was purchased from local market in Jeddah, Saudi Arabia. All other chemicals were purchased from Sigma–Aldrich (St. Louis, Missouri, United States).

Epirubicin preparation:-

For IV injections, packagesof 10 mg epirubicin was dissolved in 5 ml of normal saline for a final concentration of 2mg/ml according to manufacturer's instructions.

Green tea preparation:-

The GT water extract was prepared by adding 100 ml boiling water to 2 g of GT leaves, cooled down to room temperature, filtered and put in drinking bottle for mice(Jiang et al., 2001). Adjusted daily water intake for mice was reportedto be 5.7±0.2 ml/30 g body weight (Bachmanov et al., 2002).

Animals:-

One hundred adult Albino female mice (BALB/C) strain were purchased from animal unit of King Fahd Medical Research CentreatKing Abdulaziz University, Jeddah, Saudi Arabia. Animal weight ranged between 18-22 g.Each five mice were put in a plastic cage and left one week for accommodation (Temperature 22 °C, Humidly35-50 % and light/dark cycle of 12:12 hours). Animal care and treatment were carried accord to rules provided by the ethical committee for research.

Animal grouping and dosage:-

Dosage was chosen according to previous studies (Meir et al., 2003; Pujol et al., 1997; Viens et al., 2001) and adjusted for mice (Paget and Barnes, 1964). Animals were divided into four groups as follows: GI (n=25) served as control, were given 0.9% normal saline IV via tail vein, and had food and Ad libitum access towater. GII (n=50)were injected with EPI via tail vein by standard dose of 90mg/m^2 which equal 11.7mg/kgbody weight. The dose was given every three weeks through eight cycles ("A prospective randomized phase III trial comparing combination chemotherapy with cyclophosphamide, fluorouracil, and either doxorubicin or epirubicin. French Epirubicin Study Group," 1988; Eksborg et al., 1992). Those animals were further divided into two sub-groups, GIIa (n=25) were allowed Ad libitum access towater and GIIb (n=25) were allowed Ad libitumaccess toGT water extract. GIII (n= 25) were untreated animals withAd libitumaccess to GTwater extract only.

Five animals from each group were euthanized after one, three, four, six and eight cycles. Heart was perfused with normal saline followed by 10% neutral buffered formalin to insure perfect organ fixation. The liver was dissected carefully, cut into small pieces ($1cm^2$) then re-fixed in 10% neutral buffered formalin for subsequent paraffin processing. Five micron thick sections were stained by Haematoxylin and Eosin (H&E) (Bancroft and Layton, 2008).Stained sections were examined using light microscope connected to digital camera (OLYMPUS, United States). Photographs from all groups were compared in regards to hepatocytes and sinusoidal changes in both central vein (CV) and portal area regions.

Results:-

Histological structure of control mice liver:-

The control mice liver showed histological structure similar to what was previously described in literature. Hepatic lobules are ill-defined and can be only marked by the presence of CV and the peripherally located portal regions containing branches of portal vein (PV), hepatic artery and bile duct while surrounded by scanty connective tissue. Hepatocytes are arranged in regular cords radiating from the CV, have slightly basophilic cytoplasm, and rounded vesicular nuclei of uniform size. Few cells are bi-nucleated and separated by thin walled blood sinusoids lined by flat endothelial cells. Von Kupffer cell nuclei are occasionally seen projecting into sinusoidal lumina (Fig. 1).

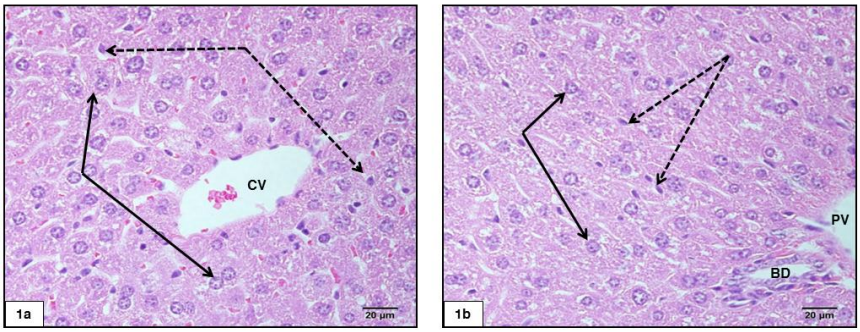

Fig. 1:- Sections of mice liver from the control group (GI). a. Central vein (CV) region with regular hepatocytes cord (black arrows) separated by thin walled blood sinusoids lined by flat endothelial cells (dotted arrows). b. Portal region showing bile duct (BD) and part of portal vein (PV) with hepatocytescord (black arrows) and blood sinusoids (dotted arrows) (H&E; x10).

Effect of EPI administration in different cycles on histological features in mice liver (GIIa):-

Histological structure of mice liver after 1st cycle:-

One cycle post-EPI administration, the mice liver showed swollen hepatocytes compressing or obliterating the blood sinusoids. Loss of cellular out lines and hydropic degeneration (unstained vacuolated cytoplasm) were observed. Nuclei appeared deformed and pyknoticor even absent (Fig.2a). Somecells showed shrunken, deeply stained acidophilic cytoplasm and frequent bi-nucleated cell were observed (Fig. 2b).

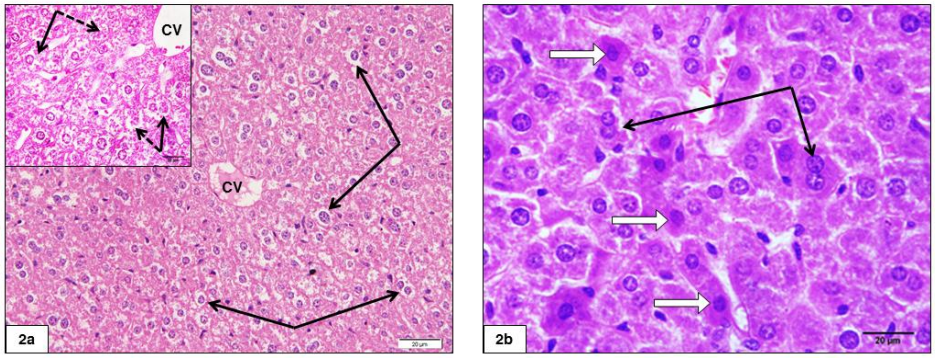

Fig. 2:- Sections of mice liver (GIIa) after 1st cycle of EPI administration. a. Swollen hepatocytes with unstained cytoplasmic regions (black arrows)andsome with deformed, decreased or karyolyticnuclei (dotted arrows). b.

Hepatocytes with shrunken cells with pyknoticnuclei (white arrows) and bi-nucleated cells (black arrows) (H&E; a (x10), b (x40)).

Histological structure of mice liver after 3rdcycle:-

Continuous administration of EPI for the 3rdcycle resulted in marked ballooning of hepatocytes, which showed hydropic degeneration. The nuclei lost its vesicular appearance become small and deeply stained. Foci of cell necrosis with residual cell debris could be seen (Fig. 3).

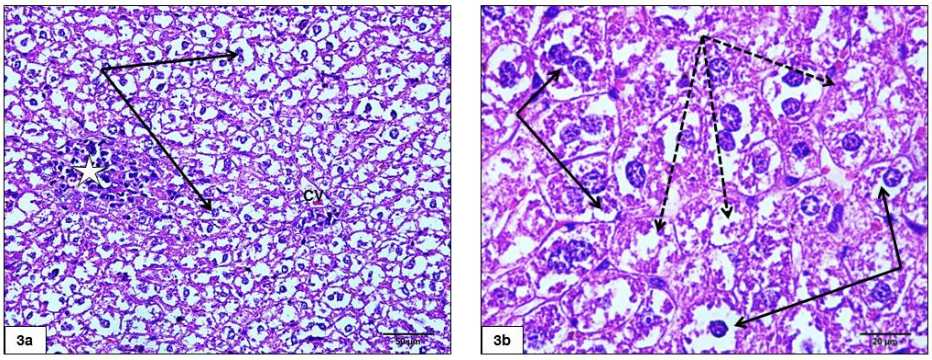

Fig. 3:- Sections of mice liver (GIIa) after 3rdcycle of EPI administration. a. Marked hydropic degeneration resulted in vacuolation or unstained cytoplasm (black arrow). Focal area of degenerated cells (star) is also present. b. Higher magnification showing hepatocytes with pyknoticnuclei and the vacuolated cytoplasm (black arrows) or with karyolytic nuclei (dotted arrows). CV, central vein (H&E; a (x10), b (x40)).

Histological structure of mice liver after 4thcycle:-

After four cycles of EPIadministration,showed marked swelling of hepatocyte around CV obliterating hepatic sinusoids (Fig 4). The cytoplasm of cells is unstained while the nuclei are small and dark. Hepatocytes near PV showed loss of normal hepatocyte arrangement andvacuolation of cytoplasm.Furthermore, giant cells and lymphocyte aggregation around PV were observed.In some cases, apoptosis and nuclear pleomorphism were observed.

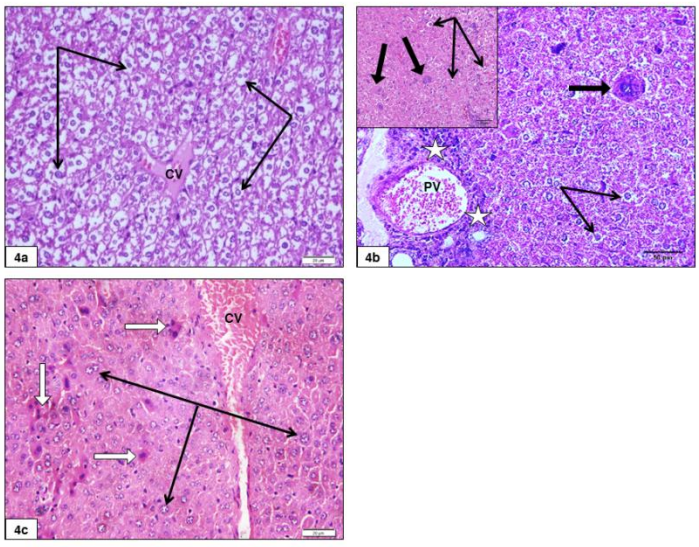

Fig. 4:-Sections of mice liver (GIIa) after 4thcycle of EPI administration. a. Marked swelling of hepatocytes (black arrows) around the central vein (CV) obliterating hepatic sinusoids. b. Hepatocytes near portal vein (PV) showing loss of normal hepatocytes arrangement andvacuolation of cytoplasm (black arrows), giant cells (Thick black arrows) and lymphocyte aggregation around PV (stars). c. Hepatocytes with apoptosis (white arrows) or nuclear pleomorphism (black arrows) (H&E; x10).

Histological Changes of mice liver after 6th cycle:-

Variationsin hepatocytes within this cycle was markedly observed. Some hepatocytes had marked loss of normal lobular architecture, ill-defined borders and pyknoticdegenerated nuclei withCV showed congestion. Few hepatocytes had nuclear pleomorphism and prominentVon Kupffer cell nuclei. Hepatocytes with unstained cytoplasmic regions, bi-nucleation nuclear inclusion and karyomegalywere also observed(Fig. 5).

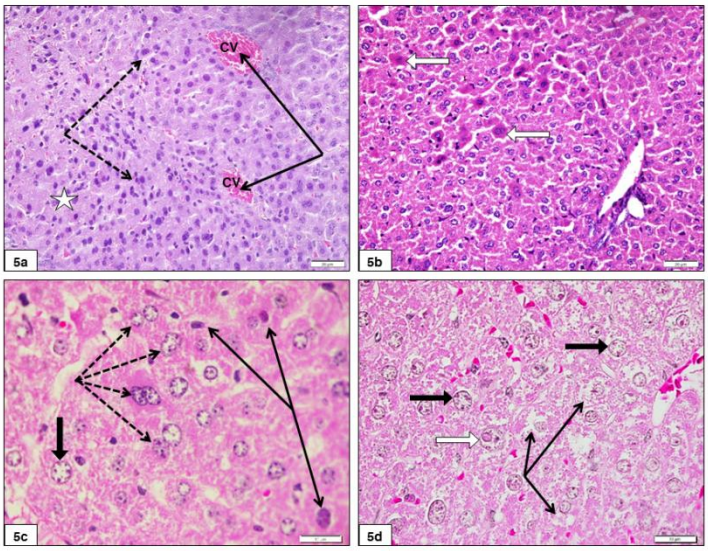

Fig. 5:- Sections of mice liver (GIIa) after 6th cycle of EPI administration. a. Most hepatocytes showing ill-defined borders (dotted arrows) and pyknoticnuclei (star). Central veins (CV) showed congestion (black arrows). b. Hepatocytes with dark cytoplasm and small pyknotic nuclei (white arrows). c. Hepatocytes with nuclear pleomorphismand bi-nucleated cells (dotted arrows) orkaryomegaly (thick black arrow). Von kuppfer cell nuclei were prominent (black arrows). d. Hepatocytes showing unstained cytoplasmic regions (thin black arrows),karyomegaly (thick black arrow) and nuclear inclusions (white arrow) (H&E; a-b (x10), c-d (x40)).

Histological changes of mice liver after 8th cycle:-

Livers from this cycle showed marked lobular disruption with hydropic degeneration. Some showed massive necrosis andhaemorrhage. Inflammatory cell aggregation near portal area was also observed(Fig. 6).

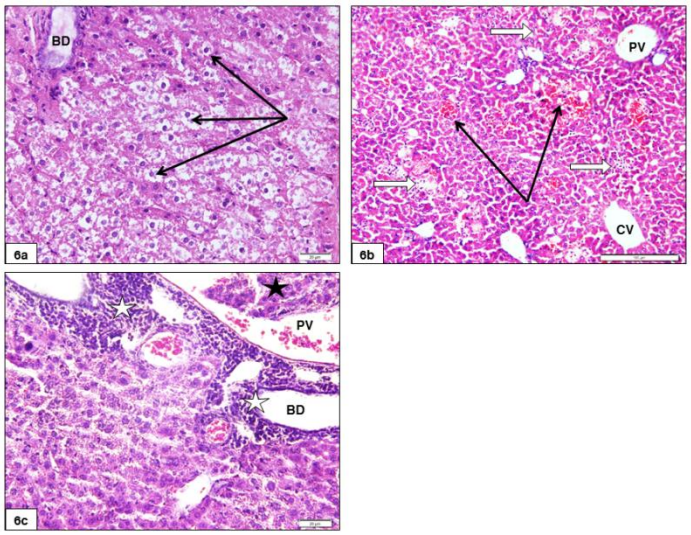

Fig. 6:- Sections of mice liver (GIIa) after 8th cycle of EPI administration. a. In the bile duct (BD) region, marked hydropic degeneration of hepatocytes (black arrows) were observed. b.Multiple regions of massive necrosis (white arrows) and haemorrhage (black arrows). c. Inflammatory cells (white stars) around dilated portal vein (PV) and bile duct (BD). Notice the desquamated cells (black star) within the PV. CV, central vein (H&E; x10).

Effect of GT consumption on mice liver post-EPIadministration (GIIb):-

After 1st cycle:-

Hydropic degeneration observed in EPI-treated group (GIIa) was markedly absent. Hepatocytes arrangement and histological features looked similar to control (Fig. 7).

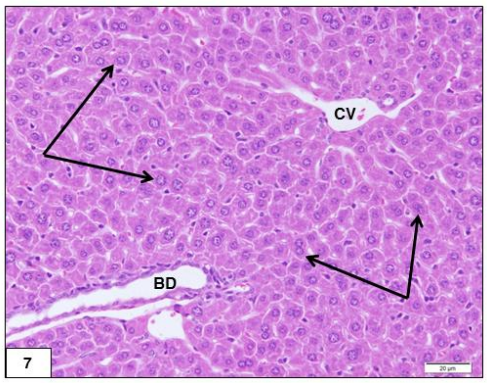

Fig.7:- Sections of mice liver (GIIb) after 1stcycle of EPI administrationand Ad libitum access of GT water extract. Central vein (CV) and elongated bile duct (BD) are shown with noticeable absence of hydropic degeneration. Hepatocytes arrangement and histology features seemed normal and comparable to control group (black arrows) (H&E; x10).

After 3rd cycle:-

The liver of this cycle showed continued histopathological protective profile with numerous presence of bi-nucleated hepatocytes(Fig. 8).

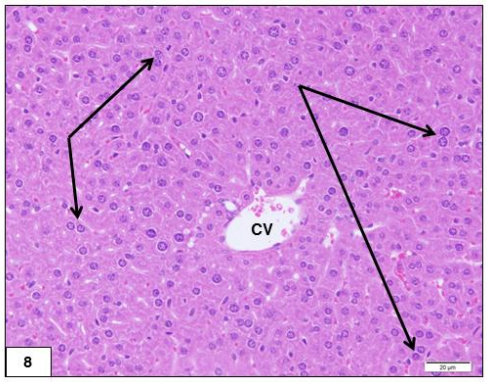

Fig.8:- Sections of mice liver (GIIb) after 3rdcycle of EPI administration and Ad libitum access of GT water extract. Central vein (CV) region showing protection profile from EPI-induced changes where hepatocytes arrangement and histological features looked similar to normal although, bi-nucleated cells are numerous (black arrows) (H&E; x10).

After 4th cycle:-

Green tea potential protection against EPI induced hepatic changes was still observed with less vacuolation of hepatocytes. In addition, inflammatory changes near portal triad and presence of giant cells were less frequent and few apoptotic cells were observed (Fig. 9).

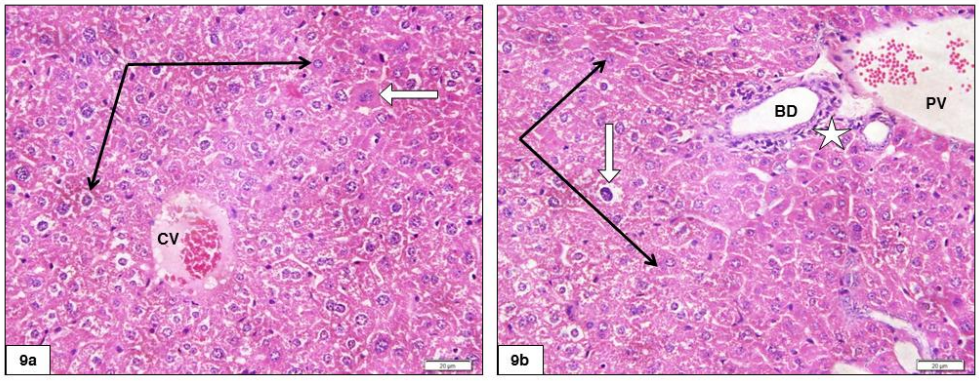

Fig. 9:- Sections of mice liver (GIIb) after 4thcycle of EPI administration and Ad libitum access of GT water extract.a. Moderate decrease in cell vacuolation (black arrows) althoughhepatocytes still appeared swollen compressing sinusoids and few apoptotic cells were observed (white arrow). b. Hepatocytes showing decreasedinflammatory cells (star) around bile duct (BD) and portal vein (PV). Hepatocytes looked less vacuolated (black arrows) while few degenerated giant cells were observed (white arrow). CV, central vein (H&E; x10).

After 6th cycle:-

Continuous similarities with 4th cycle were observed in the 6th cycle as well. Potential protection was observed in liver of some animals where necrotic foci and haemorrhagic regions were less frequent. Apoptotic cells and cells with ill-defined borders were still observed.Congestion of central and portal vessels were still evident (Fig. 10).

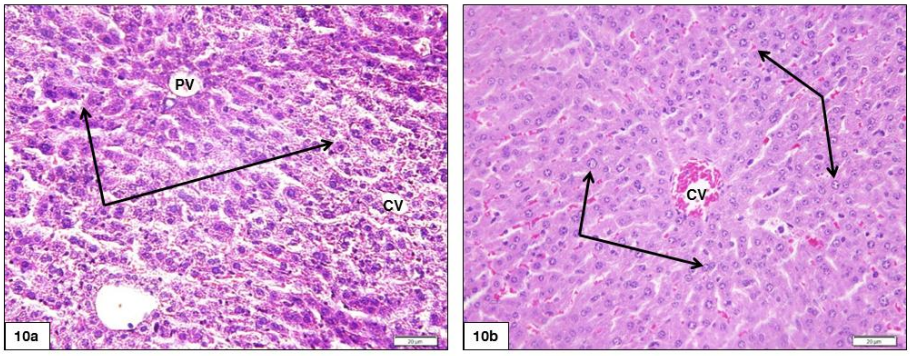

Fig. 10:-Sections of mice liver (GIIb) after 6th cycle of EPI administration and Ad libitum access of GT water extract. a. Hepatocytes still showing ill-defined outlines with pyknoticnuclei(black arrows). b. Hepatocytes showing decreased frequency of nuclear pleomorphism and nuclear inclusion (black arrows) and slight congestion of central vein (CV). PV, portal vein (H&E; x10).

Mortality in this group (GIIb)increased exponentially after the 7th cycle with all the animals dead before the 8th cycle. The liver showed marked necrosis and loss of normal texture (Fig. 11).

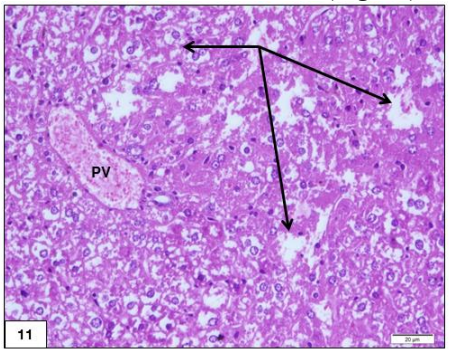

Fig.11:-Sections of mice liver (GIIb) after 7th cycle of EPI administration and Ad libitum access of GT water extract.Massive necrosis and loss of hepatocytes (black arrows) located near portal vein (PV) were observed (H&E; x10).

Effect of GTconsumption for eight cycles on histological changes in untreated mice liver (GIII):-
This group did not show significant changes on histological structure of mice liver until the 8th cycle. Bi-nucleated cells were frequent and nuclei with increased peripheral chromatin were observed (Fig. 12a, 12b,12c). Furthermore, slight vacuolation of hepatocyte cytoplasm, darkly stained small sized deformed nucleiwere observed (Fig. 12d).

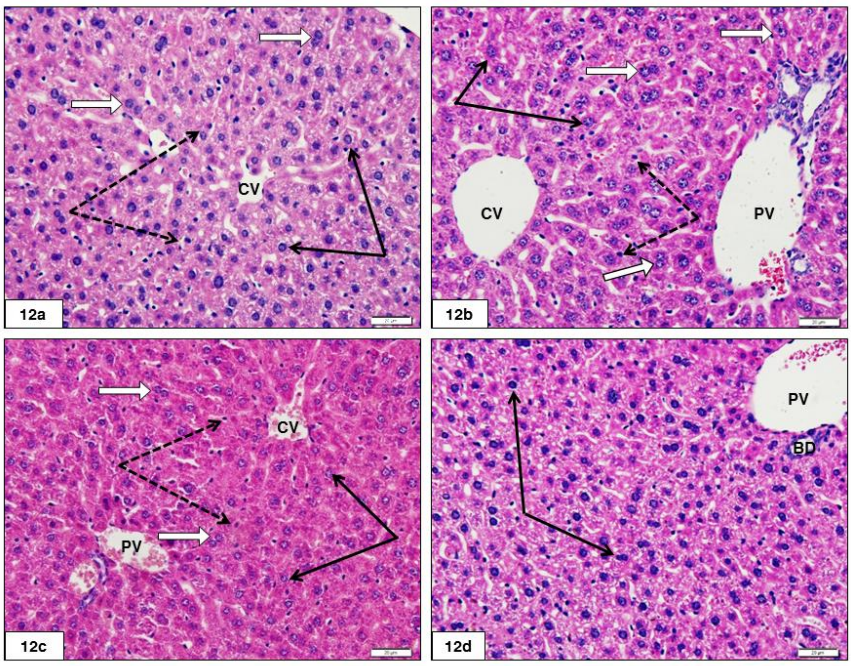

Fig.12:- Sections from mice liver (GIII) allowed Ad libitum access of GT water extract only. a. After one cycle, normal hepatocytescord (black arrows), basophilic cytoplasm, few bi-nucleated cells (white arrows) andsinusoidal nuclei (dotted arrows) were observed. b. After three cycles,similarities to normal hepatocyte architecture are still observed with normal hepatocytes cords (black arrows), basophilic cytoplasm, few bi-nucleated cells (white arrows) and sinusoidal nuclei (dotted arrows). c. After six cycles, normal hepatocytescord (black arrows), bi-nucleated cells (white arrows) and sinusoidal nuclei (dotted arrows)were still observed. d. After eight cycles, darkly stained, small sized, deformed nuclei and slight vacuolation of cytoplasm (black arrows) were observed. CV, central vein;PV, portal vein and BD, bile duct (H&E; x10).

Discussion:-

Throughout the eight cycles, the liver of the control group (GI) was similar to what was previously described in literature pertaining to normal histological structure. The uniformity of the nuclei, the basophilic cytoplasm and the regular arrangement of the hepatocytes close to CV were observed in all the samples. Although the negative control group (GIII) showed similar histological structure compared to GI, significant changes were observed after the 8th cycle. Particularly, the frequent presence of bi-nucleated cells and increased peripheral chromatin of the nuclei. This might infer that prolonged consumption of GT might affect the normal hepatocytes.

After one cycle of EPI administration, treated group (GIIa) started showingswollen hepatocytes, loss of cellular out lines and hydropic degeneration,all signs of hepatotoxicity introduction. Furthermore, signs of programmed cell death (apoptosis) were also observed in the form of deformed and pyknoticnuclei(small and darkly stained due to chromatin condensation).The mechanism of inducing apoptosis by EPI was previously reported in non-small cell lung cancer and hepatica G2 cells via productions of ROS and reduction of scavenging enzymes(Ozkan and Fişkin, 2004).The acidification of the cytoplasm was also observed which indicates disruption of cellular signalling and membrane transport in both the endocytic and exocytic directions(Cosson et al., 1989)as well as disturbance of

cellular homeostasis sincethe intracellular pH is an important parameter for cellular functions and membrane traffic. On the other hand, the treated group (GIIb) already started showing protectiveprofile compared to (GIIa). The absence of hydropic degeneration was markedly observed and hepatocytes are more comparable to the control group (GI).Similar protective profileafter DOX treatment was observed in a previous study where decreased lipid peroxidation induced by the drug increased the cellular concentration of glutathioneH which is needed to protect hepatocytes(Sugiyama and Sadzuka, 2016). Given that EPI is a derivative of DOX this mechanism could interpret the protective profile observed in this study.

After the 3rdand 4th cycles of continuous administration of EPI, the unceasing ballooning of hepatocytes in the treated group (GIIa)was still observed. The continuous chromatin condensation in the nuclei observed as darkening of the stain, which suggest progression of apoptosis in the cell appears to develop into cell necrosis with residual cell debris observed. Vacuolation of the cytoplasm, giant cells and lymphocyte aggregation around PV were also observed.This suggest that EPI induction of hepatotoxicity is efficient and increases with the continuous dose administration of EPI.

Compared to this treated group, continued histopathological protective profile was perceived in the treated group (GIIb) as well as the presence of some bi-nucleated hepatocytes. The decreased inflammatory changes near PV, which points toward GT being a good source for anti-oxidant herbal extract against EPI-induced hepatotoxicity.The 6th dose of EPI administration marked a cycle with numerous variations within each treated group. The (GIIa) showed continuous hepatotoxicity similar to the previous cycles with the addition of marked loss of normal lobular architecture, nuclear pleomorphism, bi-nucleation inclusions and karyomegaly(enlargement of the nuclei) observed. Nuclear pleomorphism, inclusions and karyomegaly were previously described in literature in cases of different forms of hepato- or nephrotoxicity (Baudrimont et al., 2001; Bokhari and Shaker Ali, 2008). Nuclear inclusion observed with EPI hepatotoxicity in the present study have been described previously with aging (Wenger et al., 2014), virus infection (Yang et al., 2011), alteration in cellular metabolism and exposure to toxic drugs (Takahashi et al., 2003; Uchihara et al., 2003).Though (GIIb) continuesto have a protective profile and similarities with the control group (GI), apoptotic cells as well as congestion of central and portal vessels were observed.

After the 8th EPI administration, necrosis, haemorrhages and marked lobular disruption of hepatocytes were present more frequently in the treated group (GIIa). Previous study showed that the use of chemotherapeutic agents might induce injury to endothelial cells lining the sinusoids, leading to subintimal thickening and extravasation of erythrocytes into the sub-endothelial space of Disse (perisinusoidal space) (Maor and Malnick, 2013). Similar mechanism may also cause the massive focal regions of necrosis and haemorrhage observed in the present study, which may result in ultimate liver failure and animal death.

On the contrary, mortalitiesin the treated group (GIIb) increased exponentiallyafter the 7th cycle with all the animals dead before the 8th cycle. Marked degenerative changes on liver of surviving animals includednecrosis and loss of normal texture.This suggests that the protective profile of GT was challenged by the prolonged consumption alongside the EPI administrations. The severity of the histological changes can be interoperated as the anti-oxidant properties of GT werereversed with pro-oxidant counter-response due to the prolonged consumption.Similar effect was observed in a previous study where GT increased DOX histological damage in tumours by one-to-seven folds compared to DOX treated only (Sadzuka et al., 1998). This enhanced activity of antitumor agents may provide possible new role of GT as a biochemical modulator.

A study have reported that EGCG from GT can interact with proteins, phospholipids in plasma and mitochondrial membranes resulting in severe oxidative stress that damages cellular integrity, disrupt nuclear and mitochondrial function and inducing cell death(Kim et al., 2000). Another study showed evidence that GTCs may be related to oxidative stress where such pro-oxidant effects appear to be responsible for the induction of apoptosis in tumour cells as a protective response against carcinogenic cells (Lambert and Elias, 2010). Such effect could be the cause of massive necrosis of mice liver parenchyma observed in the present study upon prolonged usage with subsequent liver failure and death of animals.

Conclusion:-

Green tea extract through its anti-oxidant effect could provide a protectiveprofile for mice liver from hepatic injury caused by EPI if given for a short duration and controlled dosage. Alas, prolonged consumption with continuous EPI administration may lead to a synergetic effect as anti-cancer and pro-oxidants will increase hepatotoxicity and

severe necrosis of liver parenchyma resulting in increased mortalities in animals. Further studies are needed to determine the anti-oxidant defence agents in the serum and liver homogenates of treated animals.

List of abbreviations:-

Bile duct (BD); Central vein (CV); Doxorubicin (DOX); (-)-epigallocatechin-3-gallate (EGCG); Epirubicin (EPI); Green tea (GT); Green tea catechins (GTC); Haematoxylin and Eosin (H&E); Intravenous injection (IV); Portal vein (PV); Reactive oxygen species (ROS)

Ethical approval:-

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest:-

The author declares no conflict of interest.

Acknowledgement:-

The author would like to thank professor Soad Shaker Ali, faculty of medicine at King Abdulaziz University in Jeddah for providing valuable comments and reviewing the manuscript.

References:-

1. A prospective randomized phase III trial comparing combination chemotherapy with cyclophosphamide, fluorouracil, and either doxorubicin or epirubicin. French Epirubicin Study Group, 1988. . J Clin Oncol 6, 679–688.
2. Azam, S., Hadi, N., Khan, N.U., Hadi, S.M., 2004. Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: Implications for anticancer properties. Toxicol Vitr. 18, 555–561.
3. Bachmanov, A.A., Reed, D.R., Beauchamp, G.K., Tordoff, M.G., 2002. Food intake, water intake, and drinking spout side preference of 28 mouse strains. Behav Genet 32, 435–443.
4. Bancroft, J.D., Layton, C., 2008. The hematoxylins and eosin, in: Suvarna, S.K., Layton, C., Bancroft, J.D. (Eds.), Bancroft's Theory and Practice of Histological Techniques. Elsevier Health Sciences, pp. 173–186.
5. Baudrimont, I., Sostaric, B., Yenot, C., Betbeder, A.M., Dano-Djedje, S., Sanni, A., Steyn, P.S., Creppy, E.E., 2001. Aspartame prevents the karyomegaly induced by ochratoxin A in rat kidney. Arch Toxicol 75, 176–183.
6. Bokhari, F., Shaker Ali, S., 2008. Effects of ochratoxin a contaminated Arabian coffee seeds on liver and kidney functions and structure in mice: Protective role of roasting and vitamin C. Adv Biol Res 2, 17–25.
7. Bonadonna, G., Gianni, L., Santoro, A., Bonfante, V., Bidoli, P., Casali, P., Demicheli, R., Valagussa, P., 1993. Drugs ten years later: Epirubicin. Ann. Oncol. 4 , 359–369.
8. Cersosimo, R.J., Hong, W.K., 1986. Epirubicin: A review of the pharmacology, clinical activity, and adverse effects of an adriamycin analogue. J Clin Oncol 4, 425–439.
9. Clark, J., You, M., 2006. Chemoprevention of lung cancer by tea. Mol. Nutr. Food Res. 50, 144–151.
10. Cosson, P., de Curtis, I., Pouysségur, J., Griffiths, G., Davoust, J., 1989. Low cytoplasmic pH inhibits endocytosis and transport from the trans-Golgi network to the cell surface. J. Cell Biol. 108, 377–387.
11. Di-Wen, S., Pan, G.-Z., Hao, L., Zhang, J., Xue, Q.-Z., Wang, P., Yuan, Q.-Z., 2016. Improved antitumor activity of epirubicin-loaded CXCR4-targeted polymeric nanoparticles in liver cancers. Int. J. Pharm. 500, 54–61.
12. Doellman, D., Hadaway, L., Bowe-Geddes, L.A., Franklin, M., LeDonne, J., Papke-O'Donnell, L., Pettit, J., Schulmeister, L., Stranz, M., 2009. Infiltration and extravasation: Update on prevention and management. J Infus Nurs 32, 203–211.
13. Eksborg, S., Hardell, L., Bengtsson, N.O., Sjödin, M., Elfsson, B., 1992. Epirubicin as a single agent therapy for the treatment of breast cancer — A pharmacokinetic and clinical study. Med Oncol Tumor Pharmacother 9, 75–80.
14. Gianni, L., Corden, B.J., Myers, C.E., 1983. The biochemical basis of anthracycline toxicity and antitumor action, in: Hodgson, E., Bend, J.R., Philpot, R.M. (Eds.), Reviews in Biochemical Toxicology 5. Elsevier Biomedical, New York, Amsterdam, Oxford, pp. 1–82.
15. Hou, Z., Lambert, J.D., Chin, K.-V., Yang, C.S., 2004. Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention. Mutat. Res. Mol. Mech. Mutagen. 555, 3–19.
16. Jacot, W., Pujol, J.-L., Chakra, M., Molinier, O., Bozonnat, M.-C., Gervais, R., Quantin, X., 2012. Epirubicin and ifosfamide in relapsed or refractory small cell lung cancer patients. Lung Cancer 75, 213–216.

17. Jänicke, F., 2000. Selection criteria for epirubicin-based adjuvant chemotherapy in node-negative breast cancer. Clin Breast Cancer 1, S57–S61.
18. Jiang, T., Glickman, B.W., de Boer, J.G., 2001. Protective effect of green tea against benzo[a]pyrene-induced mutations in the liver of Big Blue® transgenic mice. Mutat Res-Fund Mol M 480–481, 147–151.
19. Ju, J., Lu, G., Lambert, J.D., Yang, C.S., 2007. Inhibition of carcinogenesis by tea constituents. Semin. Cancer Biol. 17, 395–402.
20. Khan, N., Afaq, F., Saleem, M., Ahmad, N., Mukhtar, H., 2006. Targeting multiple signaling pathways by green tea polyphenol (−)-epigallocatechin-3-gallate. Cancer Res. 66, 2500–2505.
21. Kim, S., Lee, M.J., Hong, J., Li, C., Smith, T.J., Yang, G.Y., Seril, D.N., Yang, C.S., 2000. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. Nutr Cancer 37, 41–48.
22. Kucera, O., Mezera, V., Moravcova, A., Endlicher, R., Lotkova, H., Drahota, Z., Cervinkova, Z., 2015. In vitro toxicity of epigallocatechin gallate in rat liver mitochondria and Hepatocytes. Oxid Med Cell Longev Article ID 476180.
23. Lambert, J.D., Elias, R.J., 2010. The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. Arch. Biochem. Biophys. 501, 65–72.
24. Lambert, J.D., Kennett, M.J., Sang, S., Reuhl, K.R., Ju, J., Yang, C.S., 2010. Hepatotoxicity of high oral dose (−)-epigallocatechin-3-gallate in mice. Food Chem Toxicol 48, 409–416.
25. Lopez, M., Di Lauro, L., Papaldo, P., Lazzaro, B., 1984. Therapeutic activity of epirubicin in malignant melanoma and non-Hodgkin's lymphoma. Clin. Ter. 109, 31–35.
26. Maor, Y., Malnick, S., 2013. Liver injury induced by anticancer chemotherapy and radiation therapy. Int J Hepatol Article ID 815105.
27. Meir, H., Ashour, W., El-Balawi, I., Talaat, E., 2003. Assessment of response of locally advanced breast carcinoma to docetaxel and epirubicin neoadjuvant combination regiman: A prospective phase II study. Med J Cairo Univ 71, 249–258.
28. Mouridsen, H.T., Alfthan, C., Bastholt, L., Bergh, J., Dalmark, M., Eksborg, S., Hellsten, S., Kjaer, M., Peterson, C., Skovsgård, T., Sørensen, J.B., Tropé, C., Aabo, K., 1990. Current status of epirubicin (Farmorubicin) in the treatment of solid tumours. Acta Oncol. (Madr). 29, 257–285.
29. Nagle, D.G., Ferreira, D., Zhou, Y.D., 2006. Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. Phytochemistry 67, 1849–1855.
30. Ozkan, A., Fişkin, K., 2004. Epirubicin HCl toxicity in human-liver derived hepatoma G2 cells. Pol. J. Pharmacol. 56, 435–444.
31. Paget, G.E., Barnes, J.M., 1964. Toxicity Tests, in: Laurence, D.R., Bacharach, A.L. (Eds.), Evaluation of Drug Activities: Pharmacometrics. Academic Press, pp. 135–166.
32. Patil, L., Balaraman, R., 2011. Effect of green tea extract on doxorubicin induced cardiovascular abnormalities: antioxidant action. Iran J Pharm Res 10, 89–96.
33. Pujol, M., Muñoz, M., Prat, J., Girona, V., De Bolós, J., 1997. Stability study of epirubicin in NaCl 0.9% injection. Ann Pharmacother 31, 992–995.
34. Quiles, J.L., Huertas, J.R., Battino, M., Mataix, J., Ramírez-Tortosa, M.C., 2002. Antioxidant nutrients and adriamycin toxicity. Toxicology 180, 79–95.
35. Robert, J., 1993. Epirubicin. Drugs 45, 20–30.
36. Sadzuka, Y., Sugiyama, T., Hirota, S., 1998. Modulation of cancer chemotherapy by green tea. Clin Cancer Res 4, 153–156.
37. Sasu, A., Herman, H., Mariasiu, T., Rosu, M., Balta, C., Anghel, N., Miutescu, E., Cotoraci, C., Hermenean, A., 2015. Protective effects of silymarin on epirubicin-induced mucosal barrier injury of the gastrointestinal tract. Drug Chem Toxicol 1–10.
38. Scott, B.C., Butler, J., Halliwell, B., Aruoma, O.I., 1993. Evaluation of the antioxidant actions of ferulic acid and catechins. Free Radic. Res. Commun. 19, 241–253.
39. Sha, X., Guo, J., Chen, Y., Fang, X., 2012. Effect of phospholipid composition on pharmacokinetics and biodistribution of epirubicin liposomes. J. Liposome Res. 22, 80–88.
40. Štěrba, M., Popelová, O., Vávrová, A., Jirkovský, E., Kovaříková, P., Geršl, V., Šimůnek, T., 2012. Oxidative stress, redox signaling, and metal chelation in anthracycline cardiotoxicity and pharmacological cardioprotection. Antioxidants Redox Signal. 18, 899–929.
41. Sugiyama, T., Sadzuka, Y., 2016. Theanine, a specific glutamate derivative in green tea, reduces the adverse reactions of doxorubicin by changing the glutathione level. Cancer Lett. 212, 177–184.
42. Takahashi, J., Fujigasaki, H., Iwabuchi, K., Bruni, A.C., Uchihara, T., El Hachimi, K.H., Stevanin, G., Dürr, A.,

Lebre, A.S., Trottier, Y., de Thé, H., Tanaka, J., Hauw, J.J., Duyckaerts, C., Brice, A., 2003. PML nuclear bodies and neuronal intranuclear inclusion in polyglutamine diseases. Neurobiol Dis 13, 230–237.
43. Toda, S., 2011. Polyphenol content and antioxidant effects in herb teas. Chin Med 2, 29–31.
44. Uchihara, T., Tanaka, J., Funata, N., Arai, K., Hattori, T., 2003. Influences of intranuclear inclusion on nuclear size – morphometric study on pontine neurons of neuronal intranuclear inclusion disease cases. Acta Neuropathol 105, 103–108.
45. Viens, P., Roché, H., Kerbrat, P., Fumoleau, P., Guastalla, J.P., Delozier, T., 2001. Epirubicin--docetaxel combination in first-line chemotherapy for patients with metastatic breast cancer: Final results of a dose-finding and efficacy study. Am J Clin Oncol 24, 328–335.
46. Wenger, B., Schwegler, M., Brunner, M., Daniel, C., Schmidt, M., Fietkau, R., Distel, L. V, 2014. PML-nuclear bodies decrease with age and their stress response is impaired in aged individuals. BMC Geriatr 14, 1–8.
47. Yang, Z., Zhang, L., Zhang, Y., Zhang, T., Feng, Y., Lu, X., Lan, W., Wang, J., Wu, H., Cao, C., Wang, X., 2011. Highly efficient production of soluble proteins from insoluble inclusion bodies by a two-step-denaturing and refolding method. PLoS One 6, e22981.