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Investigation of Physiological and Immunohistochemical Changes in Rat Treated with 5-Fluorouracil

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Abstract | 5-fluorouracil is a drug given as an injection to treat cancers of the breast, colon, rectum, stomach, and pancreas and as a cream to treat actinic keratosis and certain types of basal cell skin cancer. It is used under the brand names Carac, Tolak, Efudex, and Fluoroplex to treat actinic keratosis. It stops cells from making DNA and may kill cancer cells. 5-fluorouracil is a type of antimetabolite. A protein that helps control whether a cell lives or dies by blocking cell death called apoptosis. The gene for BCL2 is found on chromosome 18, and transfer of the BCL2 gene to a different chromosome is seen in many B-cell leukemias and lymphomas. This study aims to show the effect of 5-fluorouracil on hematological assessments (RBCs, WBCs and HB), and on bcl-2 immunoreactivity which has an important place in the apoptotic mechanism in kidney and liver tissue. A total of two groups including control and treated experimental groups (intraperitoneal injection of 5-FU (60 mg\kg body weight) were formed in the study. After intraperitoneal injection of 5-FU on days 0, 5, 10, 15, all rats were scarified at day 21 and blood collected for hematological kidney and liver tissues taken from the rats and fixed in 10% formaldehyde solution for histological and immunohistochemical analyses. The results showed that 60 mg/kg of 5-FU drug significantly decreased RBCs and WBCs count in all rats in comparison , furthermore results referred that HB concentration was significantly decreased in 5-FU group compared to the control group The histopathological examinations revealed that while the liver and kidney tissue had a normal structure in the control group, glomerular degeneration increase in the capsular area, glomerular atrophy in kidney tissue and showing vacuolated cells and severe congestion in central vein determined in the 5-FU groups. In the kidney tissue, Bcl-2 immunoreactivity was observed in glomerular cells, sinusoidal epithelium, and proximal and distal tubule cells. The 5-FU treatment caused pathological changes in the kidney this effect also affect the expression of bcl-2 genes, which are biomarkers in the apoptotic mechanism. 5-FU accumulates in tissues, it the anti-apoptotic bcl-2 expression.

Keywords | 5-Fluorouracil, Blood parameterers, DWBcs

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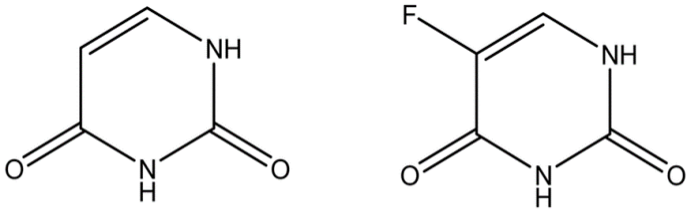
INTRODUCTION

Cytotoxic chemotherapeutic agents were affecting directly to the highly mitotic basal epithelial cells, injured additional epithelial cells leading to many dysfunction Longley et al. (2003). Furthermore, the anti-neoplastic agent's not just impact the cancer cells but also the normal cells of the body and induced cell death

by apoptosis (Casale et al., 2004). Uracil considered as privileged structures in drug discovery with a wide array of biological activities and synthetic accessibility. Uracil derivatives such as 5-fluorouracil or 5-chlorouracil were the first pharmacological active derivatives to be generated. Poor selectivity limits its therapeutic application, resulting in high incidences of gastrointestinal tract or central nervous toxicity (Walko and Lindley, 2005) (Figure 1).



Uracil 5-Fluorouracil

Figure 1: Chemical structure of uracil and 5-fluorouracil.

5-Flourouraci (5-FU) is an antimetabolite cytotoxic drug labor by blocking fundamental biosynthetic processes or by way being integrated in to macromolecules, including RNA and DNA (1). Sonis was considered that 5-FU the best chemotherapeutic agents associated with oxidative stress (Sonis, 2010). It is an effective antineoplastic drug used for treating diversity of malignancies, mainly of the breast, colon or rectum and in the remedy of gastric, pancreatic, uterine, ovarian and bladder carcinomas (Meulendijks et al., 2017). The mechanism action of its cytotoxicity has been attributed to the misincorporation of fluornucleotides in to RNA and DNA and to the suppression of thymidylate synthase enzyme, (a critical enzyme in nucleotide metabolism), both of which lead to cell death (Meulendijks et al., 2017; Barasch and Peterson, 2003). Furthermore, it is though that the progress of oxidative stress overproduction of reactive oxygen species (ROS) caused by chemotherapeutic agents are major actions in most ways leading to outcomes in the public health problems and enteric microbes, fungi and viruses and lead to elevated infection risk, especially through immune (Mescher, 2010). 5-FU availability for intracellular anabolism mainly depends on tissue drug catabolism. After administration, 5-FU follows different metabolic destinations: more than 80% of the dose is inactivated by biotransformation primarily in the liver, approximately 15–20% is eliminated in the urine and only a small fraction remains available to exert its anti-tumor action (Casale et al., 2004).

This to summarize recent developments in research to show whether treatment with 5FU is linked to the occurrence of histological and cellular changes in other organs such as the liver and kidneys. The expression of bcl2, and histological examination were chosen for the purpose of confirming the aforementioned purpose.

MATERIALS AND METHODS

Experimental animals

Twelve adult male rats (*Rattus norvegicus*) weighting 200-250 gram and aged 10-12 weeks were used. The rats were housed and breed in isolated plastic cages under strict clean, hygienic and standard managing conditions at temperature (20-25°C), controlled room on a hour light/dark cycle with the humidity rate was 50%. All animals were kept for ten days for acclimating with novel location before the beginning of the experiment, allowed palate common nutrition and drinking water *ad libitum*.

The animals were divided randomly two main experimental groups, with 6 males at each group as following: injected intraperitoneally (IP) with 0.5 ml normal saline as the vehicle on days (0, 5, 10, and 15).Group II injected intraperitoneally with 0.5 ml 5-FU on days (0, 5, 10, and 15).

Collection of blood samples

Blood sample (Casale et al., 2004) ml was collected after via cardiac puncture by using disposable syringe from each animal, each sample was separated into two portions, the blood were poured into plan tubes to be centrifuged at 3000 cycle\min for 15 min, to get the serum which then transferred into several Eppendorf tubes, serum may be used directly or may be stored at (-20°C) for biochemical analysis, while the blood of heparinized tubes was employed immediately for determination of hematological parameters.

Animals were sacrificed on days 21 under general anesthesia via cardiac puncture. Samples of Liver and Kidney tissues were taken and washed with normal saline and fixed in 10% neutral formalin fixative for histological analysis (Mescher, 2010) and immunohistochemistry detection anti-apoptosis antigen BCL-2 (Jackson and Blythe, 2013).

RESULTS AND DISCUSSION

The results showed that dose 60 mg/kg of 5-FU drug significantly decreased (RBCs) all comparison with control urthermore, results referred that HB concentration was significantly decreased at in 5-FU group compared to control. Our data revealed that WBCs count has significant decrease (P≤ 0.05) in rats treated with dose 60mg/kg of 5-FU especially after in comparison with control group as showing in Table 1.

Table 1: Estimation of concentration, blood cells and white blood cells count in experimental groups. Values represent as (mean±SD).

WBCS	RBCs	Hemoglobin	Groups
9.62±0.17 a	8.94 ± 0.042 a	10.92± 0.037 a	Control G
7.61±0.02 b	6.46± 0.038 b	9.13 ± 0.033 b	Treated G

Histological results

As seen in the samples of the control group shows normal form and organization, the kidney structure consists of renal tubules that are embedded in the parenchyma consisting of inter renal cells. It was observed that the glomerulus, in the form of a capillary glomerulus, forms the malpighian corpuscle that is externally surrounded by a connective tissue capsule (Figure 2).

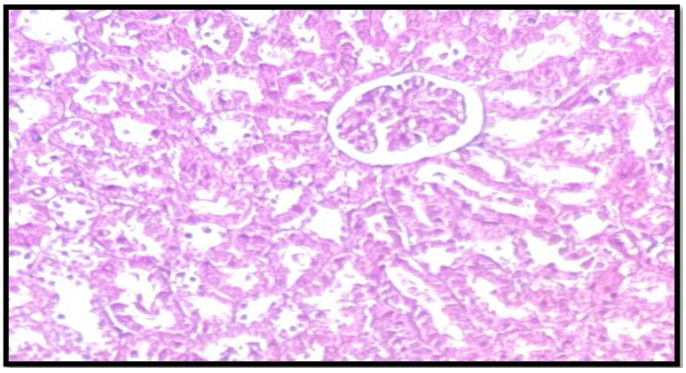


Figure 2: Histological structure of the kidney control group the kidney structure consists of renal tubules that are embedded in the the malpighian corpuscle that is externally surrounded by a connective tissue capsule H and E.

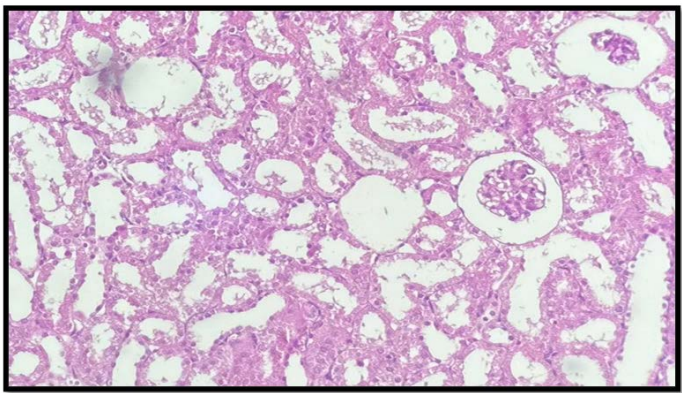


Figure 3: Histological structure of the kidney 5FU treated group showing degeneration, increase in bowman capsular area, hemorrhage, atrophy, necrosis (H and E).

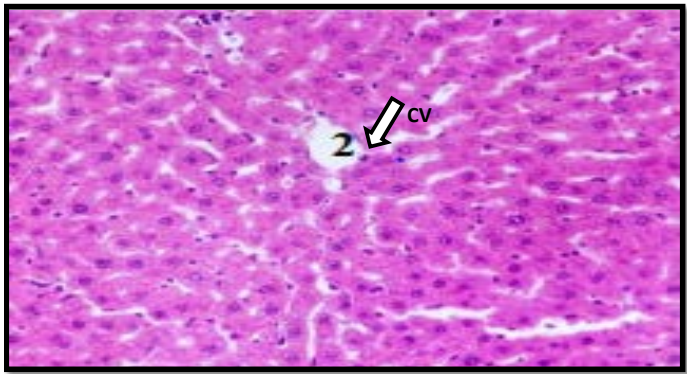


Figure 4: Liver tissues in control rat group stained by (H and E staining) showing the normal architecture of the liver with hepatic lobules around the central vein (CV) and each lobule consisting of hepatic strands of hepatocytes.

In the 5-FU treated group, it was observed that degeneration in the distal tubules increase and reached necrotic sizes in the kidney histology. Glomerular atrophy was observed from place to place in addition to the glomerular degeneration occurring in the form of changes in the glomerulus morphology. Upon the increase in the capsular area, the Bowman's capsule disappeared as a result of the adhesion of glomerulus to the pari*et al* leaves of the Bowman's capsule. Thus, it was found that glomerulus almost completely filled the Bowman's capsule, and an appearance with no bowman's capsule was observed (Figure 3). Liver of control rats showed a normal architecture, with hepatic lobules around the central vein and each lobule consisting of hepatic cords of hepatocytes (Figure 4). Liver of 5-FU rats showed severe congestion of central vein and necrosis with cytoplasmic vacuolization of Centro lobular hepatocytes (Figure 5).

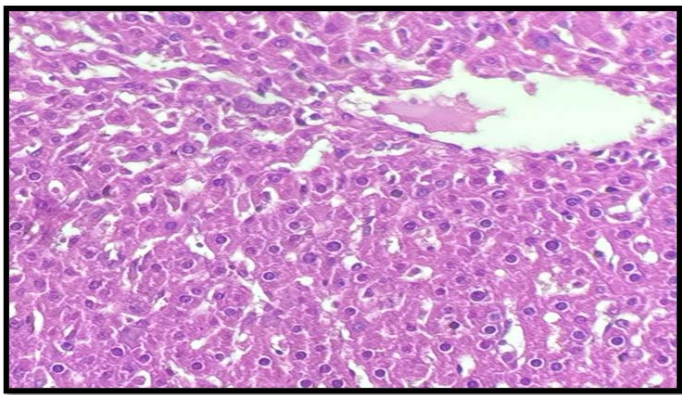


Figure 5: Liver tissues in 5FU treated group stained by (H and E staining). A5FU group showing the leukocytes infiltration, vacuolated cells and severe congestion in central vein (CV).

Immunohistochemical findings

As a result of the immunohistochemical evaluation, kidney bcl-2 immunoreactivity was observed in glomerular, sinusoidal epithelium, distal, and proximal tubule cells. In the kidney tissue obtained from the control groups, bcl-2 expression showed moderate immunoreactivity than the 5-FU group (Figures 6, 7). In the kidney tissue obtained from the 5-FU group bcl-2 immunoreactivity was higher in the cortex region of the kidney than in the medulla part.

Immunohistochemical staining of control group liver with Bcl2 showing cellular distribution of immunoreactive Bcl2 protein as indicated by brown, Control group showing strong positive reaction, while 5FU treated liver showing weak reaction.

Our study showed that RBCs count and Hb concentration were decreased significantly (P≤0.05) in 5-FU treated group, this can be referred to few eating and drinking of rats leads to dehydration and loss of weight. Also, the

chemotherapeutic agents and inflammatory infections caused suppression of bone marrow can result to (Singh et al., 2008).

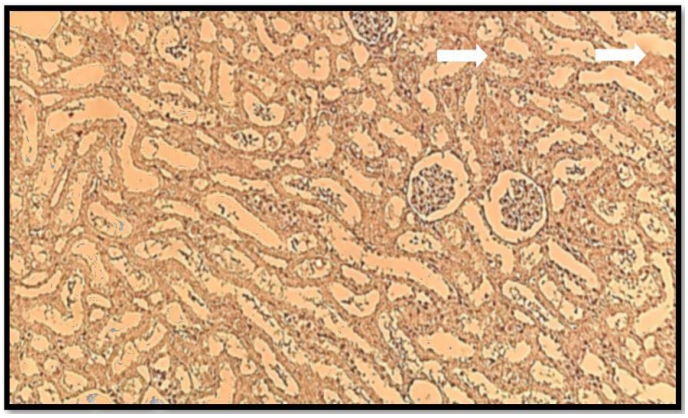


Figure 6: Immunohistochemical staining of control group kidney with bcl2, A control group showing moderate immunoreactivity in the cortex region of the kidney.

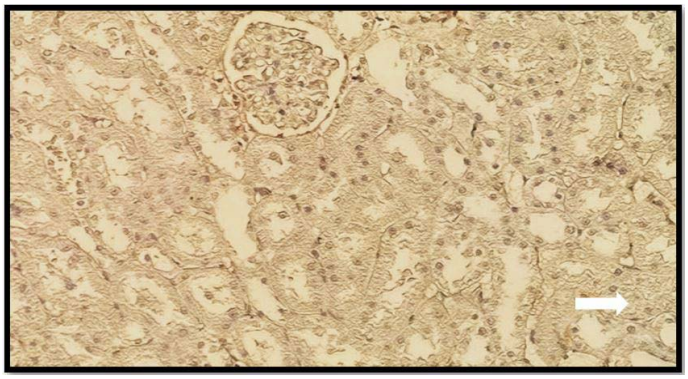


Figure 7: Immunohistochemical staining of 5FU treated group kidney with Bcl2. A 5FU group showing weak immunoreactivity in the kidney section.

In addition, WBCs count was significant decreased in 5-FU treated in comparison with normal control, this may be regarded to the cytotoxicity caused by this drug and to suppression of immune response. These results consisted with these of (Medeiros et al., 2011) who they have observed leukopenia in chemotherapy treatment animals and the danger of myelosuppression is raised with chemotherapeutic agents are offered, leukopenia already happened because of the toxic effect of 5-FU on myeloid in the bone marrow, hence fluorouracil remedy was consider as immune-suppressive drug.

The 5-fluorouracil has an effect not only on cancer cells, but also on healthy cells, leading to dose dependent toxicities. The toxicity of 5-fluorouracil has been reported to be focused on rapidly dividing cell lines leading to a wide range of adverse effects (Jackson and Blythe, 2013). In this study, it was determined that 5FU has caused the pathological change in the kidney, it was observed that degeneration in the distal tubules increased more and reached necrotic sizes in the kidney histology. Glomerular atrophy was observed from place to place in addition to the glomerular degeneration occurring in the form of changes in the glomerulus morphology. Upon the increase in the capsular area, the bowman's capsule disappeared as a result of the adhesion of glomerulus to the pari*et al* leaves of the bowman's capsule. Thus, it was found that glomerulus almost completely filled the bowman's capsule, and an appearance with no bowman's capsule was observed. Figures 2, 3 show the adapted tissue change frequency ratings of histopathological lesions in the kidney.

Also, administration of 5-FU was joined by a significant reduction in BCL-2 positive cells. Reactive oxygen species is generated by chemotherapy which is harmful to the DNA of epithelial cells and reduce the metabolism in progenitor cells and give raise of apoptosis (Vermes et al., 2000) these results were clarified and supported the findings of our investigators.

It was reported that the formation of reactive oxygen species (ROS) during inflammation induced liver injury (Assimakopoulos, 2006). However, since the drug is often administered in combination therapy, it is sometimes difficult to determine with certainty the contribution of 5-FU to the adverse drug reaction. Depending on the regimen, administration of 5-fluorouracil usually leads to severe toxicities in one third of the patients, and up to 3% of deaths have been reported in frail patients.

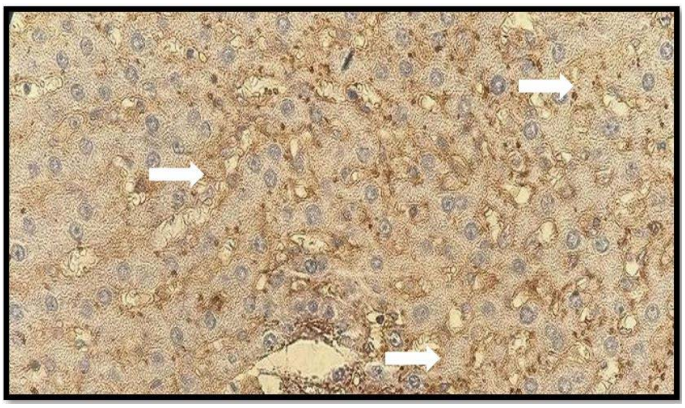


Figure 8: Immunohistochemical staining of liver in control group with Bcl2. A control group showing strong positive reaction.

Histopathological and immunohistological findings in the current study affirmed the study of (Bessa et al., 2012) showing that 5FU treatment leads to apoptosis and inflammation. Bcl2 is one of the most sensitive biomarkers. In accord with the report of (Assimakopoulos et al., 2006), our investigation showed that treatment with 5FU causes a distinct decrease bcl2 as compared to control group. The current investigation recorded important microscopic histological alterations in liver

tissue which indicate necrosis may follow virtually any lesion whose changes are significant, taking a toll on hepatocytes. Immunohistochemical staining of Bcl-2 was localized in the nuclei and cytoplasm of hepatocytes, it was demonstrated brown cytoplasmic staining. The liver tissues of the control group showed moderate expression of Bcl-2 proteins (Figures 8 and 9).

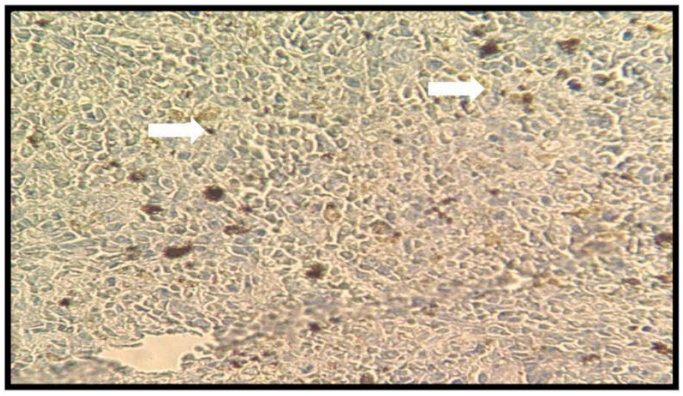


Figure 9: Immunohistochemical staining of liver in 5 FU treated group with Bcl2. A5 FU group showing weak reaction.

CONCLUSIONS AND RECOMMENDATIONS

The obtained results proposed immunomodulatory strategy of 5 FU in kidney and liver of rats. To investigate whether bcl2 expression play a role in the histologic lesions and atrophy. 5 FU demonstrated profound decreased the levels of Bcl-2 protein leading to increased apoptosis.

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NOVELTY STATEMENT

This study used 5-fluorouracil to show the effect of this substance on haematological parameters and on bcl-2 immunoreactivity, which very important in the apoptotic mechanism in kidney and the liver. The result showed this substance significantly decreased RBCs, WBCs and HB concentration in all treated animal as will as the 5-FU lead to pathological changes in the kidney tissue.

AUTHOR'S CONTRIBUTION

This work was contributed by all the authors. Each author has contributed to the study conception as well as the design, data of the study collection, analysis and writing as the report.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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