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Research Article

Evaluation of the Anticancer Effect of *Saussurea costus* Root Extract Against Induced Hepatic and Renal Cancer in White Mice: A Histopathological Study

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Abstract | The *Saussurea lappa* (Sc), traditionally known as Costus, is a perennial effective root extensively used to treat various ailments. This study intends to evaluate the anticancer effect of *Sc*- Root Extract (Sc-RE) against cancer induced by intraperitoneal injection of Polyvinyl pyrrolidone K-30 (PVP) in mice. 72 mice divided randomly into eight groups (G1, control group; G2, Sc-RE group; G3, chemotherapy alone; G4 cancer- group without treatment, G5, cancer-induced + chemotherapy; G6, cancer induced+ Sc-RE/ low dose; G7, cancer induced+ Sc-RE/ moderate dose; G8, cancer induced+ Sc-RE/ high dose). Liver and kidney samples were collected from all mice during different experiment periods, kept in 10% neutral buffered formalin, and sent for histological analysis. No abnormal histological features were seen in liver and kidney sections from the control and other mice groups before various treatments. The various treatment groups revealed diverse histopathological changes compared to the control groups. Various histological features were also seen in low, moderate, and high-dose Sc-RE treatment groups, accompanied by regeneration related to the treatment dose. Severe damage comprising necrosis of the hepatic cells, renal tubules, congestion, and infiltration of inflammatory cells was seen in the chemotherapy-treated group and mice suffering from cancer without treatment. This study also revealed variations in serum liver enzyme values between treatment groups. The values of ALT, AST, and ALP (U/L) evaluation in control groups were > 29.01±1.8, 87.55±2.9 and > 98.12±8.8, respectively. In conclusion, this study investigated that the ethanolic extract of Sc-RE has an anticancer effect that reduces the proliferation of cancer cells caused by the carcinogenic substance.

Keywords | Anticancer, Biochemical indices, Histopathology, Herbal medicine, *Saussurea costus* root extract

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INTRODUCTION

Cancer is a general name for many diseases that can affect any body part (Ferlay et al., 2020). Four characteristics distinguish cancer cells from normal cells: uncontrolled proliferation, a lack of capacity to differentiate, which leads to loss of function, invasiveness, and the ability to metastasis (Hanahan and Weinberg, 2011). Cancer is a worldwide public health problem that is likely to affect almost every person on the planet, with the estimation that one in three people will develop cancer at some point during their lifetime (Siegel et al., 2023). It is second only to heart disease as a leading cause of death in developed nations and is among the three leading causes of death for adults in developing countries (Nguyen et al., 2017).

Medicinal plants have contributed richly to the health of human beings. The vast potential of plant-based medicines

in treating numerous diseases has always contributed to the value of the plant community as a major area of research and development. Medicinal plants are a storehouse of numerous bioactive compounds with various therapeutic properties. The therapeutic potential of plants has been well explored over a very long period (Qassadi et al., 2023). The vast array of therapeutic effects associated with medicinal plants includes anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic (Raina et al., 2014).

The *Saussurea costus* (Family Asteraceae) is a perennial or biennial herb naturally found in sub-Himalayan regions (Amina et al., 2020). Sc is well-known in Islamic medicine and the Indian systems of medicine, and it is used either as a single drug or in combination with other drugs (Deabes et al., 2021). Sc roots are used mainly as an antispasmodic in asthma and cough and also in the treatment of cholera, chronic skin diseases, rheumatism (Choudhary et al., 2015), cold, quartan malaria, leprosy, persistent hiccups, hair wash, stomachache, toothache, and typhoid fever (Kulsoom et al., 2014). It is an important medicine for gout, erysipelas and promotes spermatogenesis, chronic inflammation of the lungs, and chest congestion (Pandey et al., 2007). Several previous studies have suggested that Sc has anticancer effects in neuroblastoma (Tabata et al., 2015), lung cancer (Hung et al., 2010), hepatocellular carcinoma (Hsu et al., 2009), gastric cancer (Ko et al., 2004), and prostate cancer (Tian et al., 2017). The hexane extract of Sc induced apoptosis in androgen-insensitive human prostate cells (Kim et al., 2009), and MgO nanoparticles (MgONPs) were synthesized using biomasses of Sc roots against breast cancer cells (Amina et al., 2020). A literature review revealed scarce publications on the activities of Sc-RE for treating epithelial cell cancer. Consequently, this study intends to detect the anticancer activities of Sc-RE against experimentally induced epithelial cell cancer in white mice *in vivo*.

MATERIALS AND METHODS

The Sc-Roots were purchased from an Iraqi herbal market and were free from defects and contaminants. The roots were dried at 40°C. The dried pieces were then ground into powder, aseptically dispensed into sterile plastic bags, and stored at −40°C. Extraction was performed using 80% ethanol at 25°C for 72 hours, as optimized in the previous study (Ahmed et al., 2016). The solvent was evaporated using a rotary vacuum evaporator (EYELA A-1000S, USA) at 40°C, and the subsequent extract was freeze-dried and stored in dark bottles at 4°C.

Seventy-two Albino Swiss mice (aged 8-10 weeks, with an average body weight of 28±1.5 g) obtained from (the Iraqi Center for Cancer and Medical Genetics Research/ Baghdad) were used in the present study. This study was approved by the Ethical and research committee/College of Science/ Al-Muthanna University (No. 404 Date 10-12-2021). The mice were allowed to acclimate to the standard laboratory conditions for 2 weeks and had free access to tap water and a chow diet for one week. Later on, the mice were randomly divided into eight groups (nine each) as follows: (G1, control group (Received distilled water only); G2, Sc-RE group (Received Sc-80% ethyl extract/ 600 mg/Kg) as a single oral dose only.; G3, chemotherapy alone/Mice received Ebetrex (2.5mg/Kg) chemotherapy alone.; G4, cancer- group without treatment; G5, cancer-induced + chemotherapy; G6, cancer induced+ Sc-RE / low dose; G7, cancer induced+ Sc-RE/moderate dose; G8, cancer induced+ Sc-RE / high dose (Table 1).

Cancer was induced by intraperitoneal injection of PVP-K30 at a 15 mg/ kg body mice dose. After 2 weeks, all mice were fed their diets, and mild, moderate, and high doses of Sc-RE were administered for G6, G7, and G8 through gastric intubation for four weeks. After the end of each treatment period, mice were euthanized.

Table 1: Shows the eight experimental groups including 5 control groups and 3 Sc-RE treated groups.

Groups			
Control groups	**Type of treatment**	**Treated group**	**Type of treatment**
G1	Received distilled water only.	G6	Received PVP (IPI) as a carcinogenic agent before two weeks from treatment with low dose (400 mg/Kg) of Sc 80% ethyl extract
G2	Received Sc-RE 80% ethyl extract (600 mg/Kg) as a single oral dose only.		
G3	Mice received Ebetrex (2.5mg/Kg) chemotherapy alone.	G7	Received PVP (IPI) as a carcinogenic agent before two weeks from treatment with moderate dose (600 mg/Kg) of Sc 80% ethyl extract
G4	Received PVP as a carcinogenic agent / intraperitoneal injection	G8	Received PVP (IPI) as a carcinogenic agent before two weeks from treatment with high dose (800 mg/Kg) of Sc 80% ethyl extract.
G5	Received PVP (IPI) as a carcinogenic agent before two weeks from treatment with Ebetrex (2.5mg/Kg) as chemotherapy		

Blood samples were collected by cardiac puncture, kept in test tubes without anticoagulant, and centrifuged at 3000 rpm at room temperature for 10 minutes. Later on, serum was collected and stored at -40 °C until further analysis. Liver and kidney specimens were collected in 10% neutral buffered formalin.

After fixation of the specimens for 48 hours, the specimens were dehydrated using ascending grades of ethyl alcohol (50-100%), then cleared using xylene (2 changes), then embedded in melted paraffin wax (59c), then blocked and sectioned with an ordinary microtome (3-6 micrometer thickness), stained by Hematoxylin and Eosin stain and examined under a light microscope. All sections were examined in magnification (X4, X10, X20, X40). Furthermore, images were acquired by a Leica image analyzer (Bancroft and Gamble, 2008). Serum was used to analyze the activities of liver aminotransferases (AST and ALT) and alkaline phosphatase (ALP) using Cobas C111 automated Chemistry analyzer Roche Company, Germany.

Statistical analysis

SPSS was used to examine the collected data. One-way analysis of variance (ANOVA) was used to compare the samples and controls statistically, and Tukey's post-test analysis was used to obtain statistical differences between the samples and the controls. Results were expressed as mean ± standard deviation (SD). A difference in the mean values with $p < 0.05$ was considered statistically significant (Mishra et al., 2019).

RESULTS AND DISCUSSION

The study's results revealed no significant differences in food and water intake among the mice (Figure 1A, B and C). Moreover, the cumulative tumor incidence was, on average and revealed 42% lower in the calorie-restricted groups. Multivariate regression analyses revealed that, regardless of the level of dietary fat, tumor incidence increased with increased caloric intake and body weight over a wide range of intakes, including moderate caloric restriction (i.e., 7-20%).

The results of the liver function tests revealed variations in serum liver enzyme values between treatment groups. The values of ALT (U/L) evaluation were > 29.01±1.8, > 37±1.1, > 360±8.7, > 175±7.5, > 313±5.4, > 53±1.1, > 46±1.5 and > 52±0.87 for G1, G2, G3, G4, G5, G6, G7 and G8, respectively. The results of AST (U/L) measurements were > 87.55±2.9, > 98.98±3.7, > 255±8.8, > 167±5.2, > 474±7.9, > 106±8.4, > 97±8.9, and > 99.4±7.6 for G1, G2, G3, G4, G5, G6, G7 and G8, respectively. While the results of ALP U/L determination were > 98.12±8.8, > 99±6.5, > 278±5.87, > 260±8.9, > 440±8.8, > 135.3±7.4, > 110.5±6.7 and > 125.2±7.6 for G1, G2, G3, G4, G5, G6, G7 and G8, respectively Table 2.

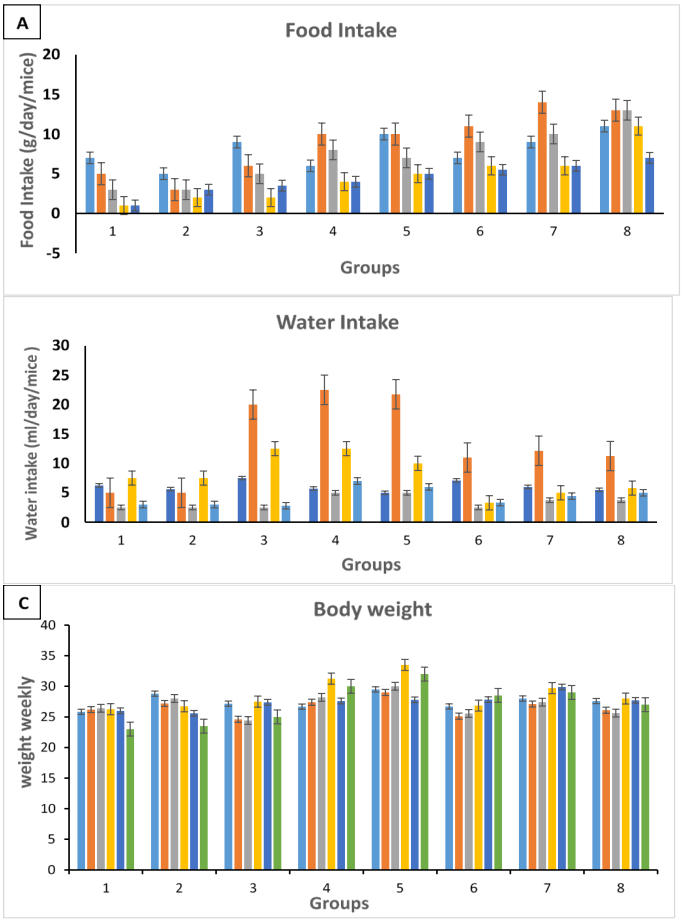

Figure 1: Shows the effects of date extract on (A) weekly food intake, (B) weekly water intake, and (C) body weights of the tested mice groups.

Table 2: Serum level of AST, ALT, and ALP in mice.

Groups	ALT UI/L	AST UI/L	ALP UI/L
1/ control group	29.01±1.8^{a}	87.55±2.9^{a}	98.12±8.8^{d}
2/ Sc-RE group	37±1.1^{b}	98.98±3.7^{b}	99±6.5^{e}
3/chemotherapy alone	360±8.7^{e}	255±8.8^{f}	278±5.87^{c}
4/ cancer- group without treatment	175±7.5^{b}	167±5.2^{c}	260±8.9^{b}
5/cancer-induced + chemotherapy	313±5.4^{f}	474±7.9^{f}	440±8.8^{c}
6/cancer induced+ Sc-RE/ low dose	53±1.1^{d}	106±8.4^{e}	135.3±7.4^{a}
7/ cancer induced+ Sc-RE/moderate dose	46±1.5^{c}	97±8.9^{c}	110.5±6.7^{b}
8/cancer induced+ Sc-RE/ high dose	52±0.87^{c}	99.4±7.6^{d}	125.2±7.6^{c}

Different letters indicate a significance difference ($p < 0.05$) between groups. Values are mean ± SD.

No abnormal histopathological changes were seen in the liver and kidney sections of mice in G1/ the control group

mice without treatment along the experimental period (Figures 2A, B, C, D and 3A, B, C, D).

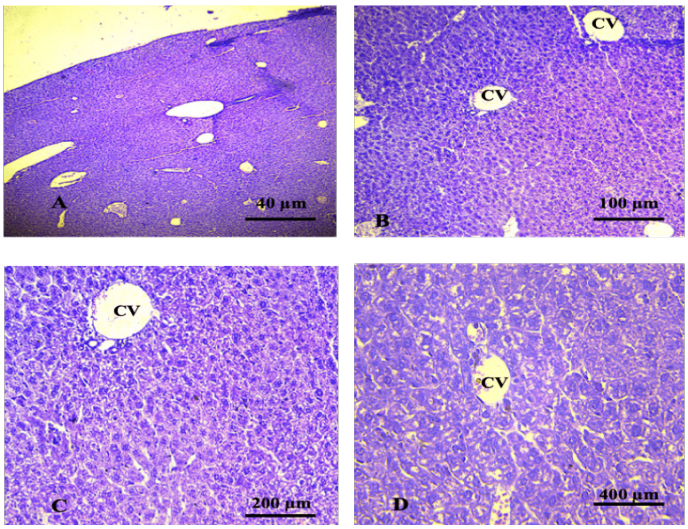

Figure 2: Shows liver of the control group showing normal histological features. The sections revealed normal organisation of the hepatic cells surrounded the central vein. (CV): central vein. (A. X 4, B. X10, C.X20, D. X40) (H and E).

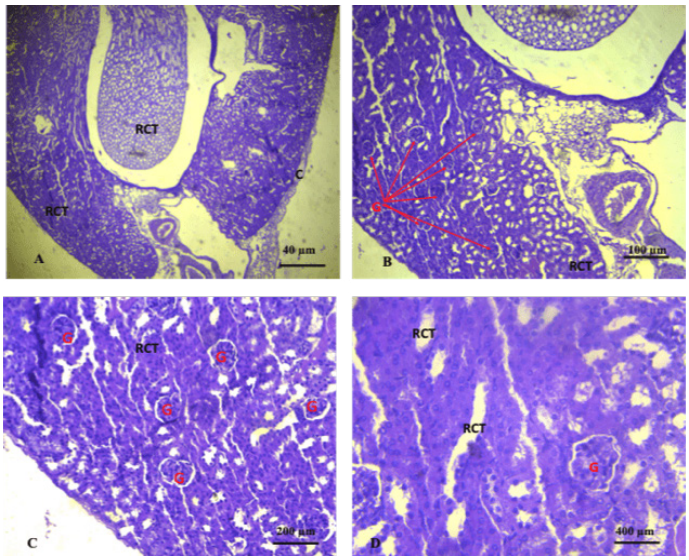

Figure 3: Shows section of the kidney of the control group. The sections revealed normal glomeruli (G) with normal urinary space (US) and renal convoluted tubules (RCT) (A. X 4, B. X10, C.X20, D. X40) (H and E).

The histological features of the kidney and liver from G2 (mice received Sc-RE only) revealed normal histological features similar to those seen in control group 1 (Figures 4A, B, C, D and 5A, B, C, D).

Liver and kidney sections of mice in control group 3/ chemotherapy alone (2.5mg/Kg Ebetrex) revealed various severe histopathological changes at different treatment periods. The liver sections revealed severe necrotic areas and heavy infiltration of inflammatory cells around the central vein. Moreover, various degenerative changes were seen in the hepatic cells comprising cytoplasmic condensation. Congestion and free RBCs were also seen in central veins and the hepatic parenchyma (Figure 6A, B, C, D). The kidney sections in the 5th week revealed severe degenerative and necrosis of glomeruli and renal tubules with obvious congestion and free red blood cells. Infiltration of inflammatory cells was also observed (Figure 7A, B, C, D).

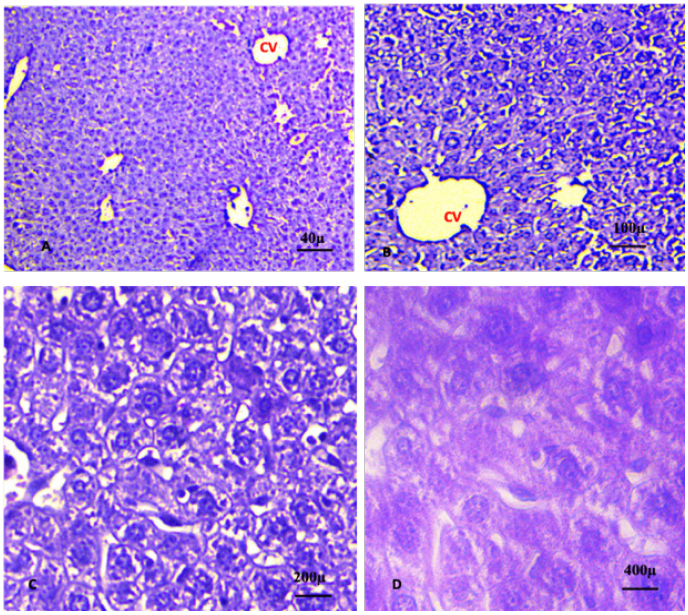

Figure 4: A, B, C, D shows liver of the control group 3 (plant extract alone). The sections revealed normal organisation of the hepatic cells surrounded the central vein. (CV): central vein. (A. X 4, B. X10, C.X20, D. X40). (H and E).

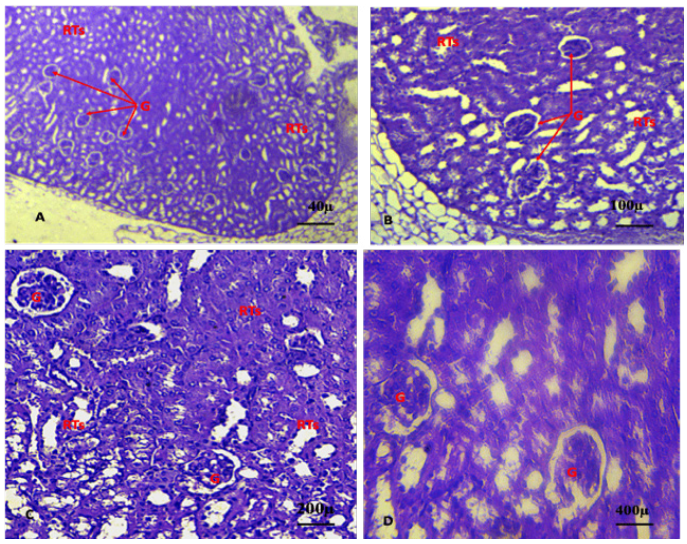

Figure 5: A, B, C, D shows the kidneys of the control group 2 (plant extract alone). Sections showing normal histological features of the kidney. The sections revealed normal glomeruli (G) with normal urinary space (US) and renal convoluted tubules (RCT) (A. X 4, B. X10, C.X20, D. X40). (H and E).

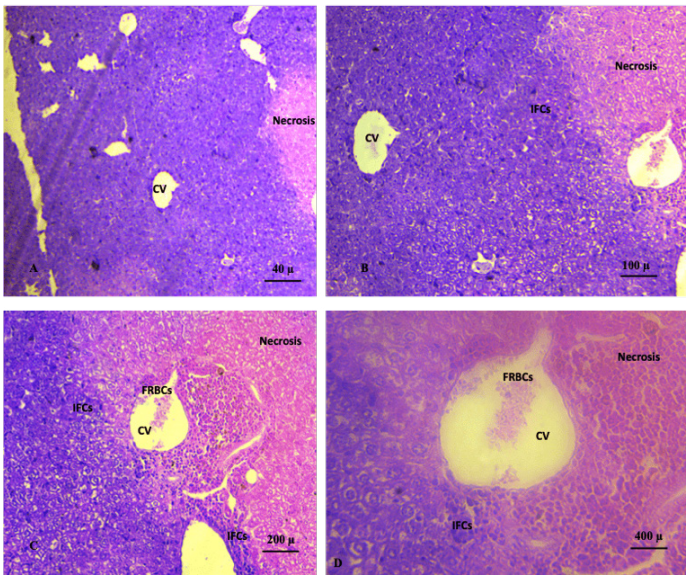

Figure 6: 5th weeks: Shows liver section for Group 3/ chemotherapy alone (2.5mg/Kg Ebetrex) revealing large necrosis hepatic area and heavy infiltration of inflammatory cells around central vein. A congestion and free RBCs were also seen in the section. (A. X 4, B. X10, C.X20, D. X40). (H and E).

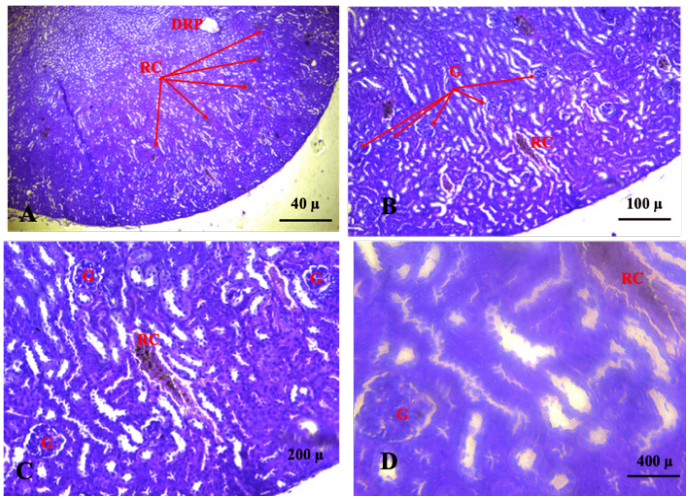

Figure 7: 3rd and 4th and 5th weeks: Shows kidney sections with various degenerative changes of the glomeruli and renal tubules. Congestion was also seen. (G: Glomeruli, RC: Renal Congestion, DRP: Damage renal parenchyma). (A. X 4, B. X10, C.X20, D. X40). (H and E).

Various histopathological features were seen in mice's liver and kidney sections in group 4/ mice treated with carcinogenic PVP-K30 only. Mice in this group showed advanced stages of cancer in kidney and liver sections in the 5th week. The liver sections revealed hepatic cell degeneration associated with nuclear pyknosis (orange arrow) and multiple aggregations of cancer cells around the portal area, accompanied by the infiltration of inflammatory cells and congestion. There was severe damage of renal parenchyma, including degeneration of convoluted renal tubules and invading cancer cells with Intra-tumoral fibrosis (ITF) related to poor prognosis of this cancer (Figures 8A, B, C, D and 9A, B, C, D).

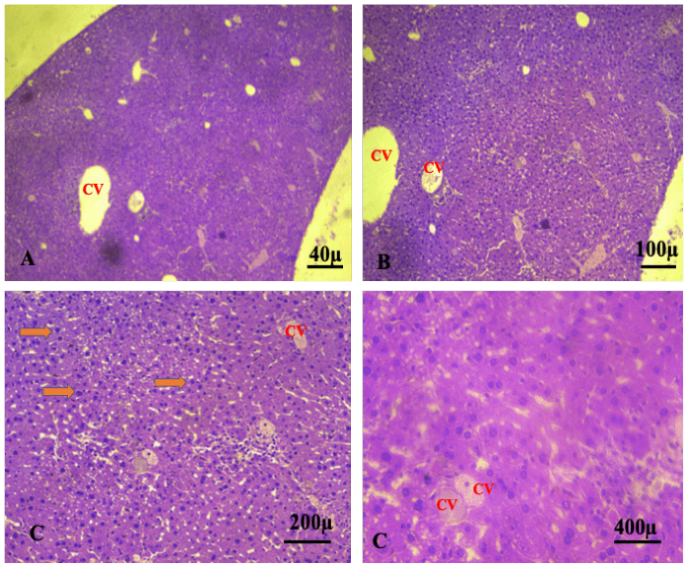

Figure 8: A, B, C, D shows the liver control group 3 (Cancer alone without treatment) at 5th week of intraperitoneal injection of Polyvinyl pyrrolidone K-30. Hepatic cell degeneration associated with nuclear pyknosis (orange arrow) and cancer cell aggregation (ACs) around the portal area, accompanied by the infiltration of inflammatory cell and congestion (CV: central vein). (A. X 4, B. X10, C.X20, D. X40) (H and E).

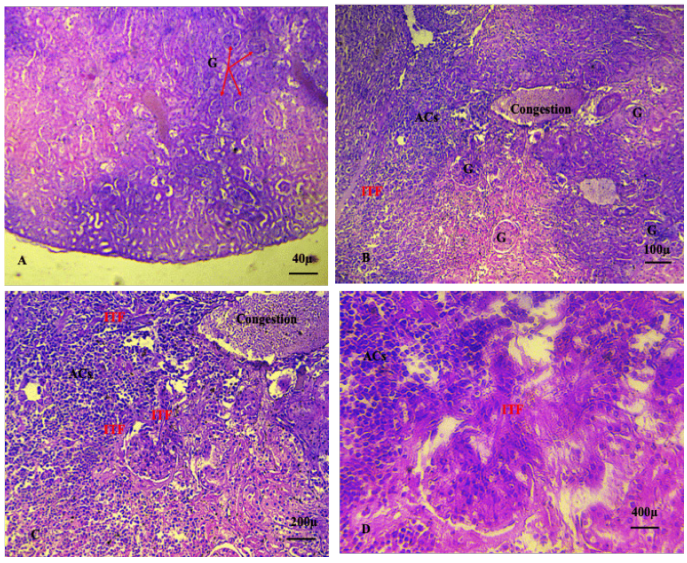

Figure 9: A, B, C, D shows advanced stages of renal cancer characterised by Intra-tumoral fibrosis (ITF), severe damage of renal parenchyma, including degeneration of renal convoluted tubules and congestion. (ACs: Abnormal cell aggregation, G: glomeruli). (A. X 4, B. X10, C.X20, D. X40) (H and E).

Sections of the liver and kidney of mice in group 5/ cancer treated with chemotherapy (2.5mg/Kg Ebetrex) revealed various severe histopathological changes at different treatment periods. The liver sections revealed advanced

stages of cancer, severe degeneration, and necrosis of the hepatic cells accompanied by congestion and infiltration of inflammatory cells. Besides, the liver sections in the 5th week revealed loss of the central vein and portal area's structural organization and extensive hepatic parenchyma necrosis (Figure 10A, B, C, D). Moreover, kidney sections showed severe degeneration and necrosis of glomeruli accompanied by a widening of the glomerular wall and damaged renal tubules. Aggregations of immature cancer cells heavily invaded the renal parenchyma, leading to the loss of normal histological features. Intra-tumoral fibrous tissue was also seen that indicating the advanced stages of renal cancer and an unfavorable prognosis. The kidney sections also showed severe infiltration of the inflammatory cells and congestion (Figure 11A, B, C, D).

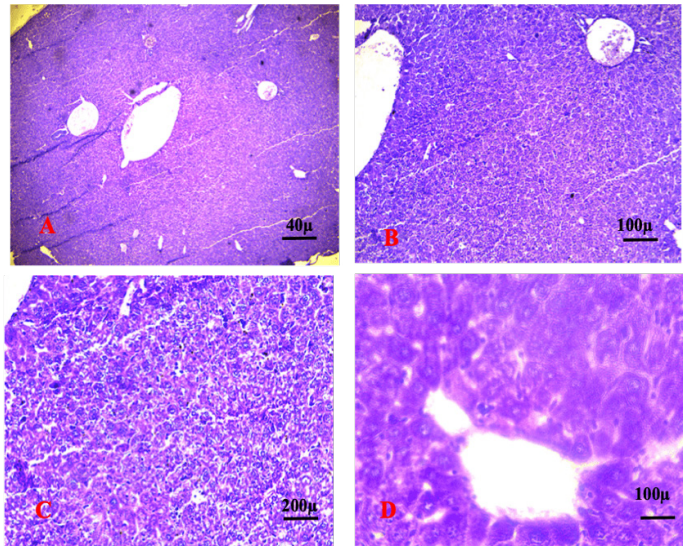

Figure 10: A, B, C, D shows liver sections with sever degeneration and necrosis of the hepatic cells accompanied by congestion and infiltration of inflammatory cells. (A. X 4, B. X10, C.X20, D. X40). (H and E).

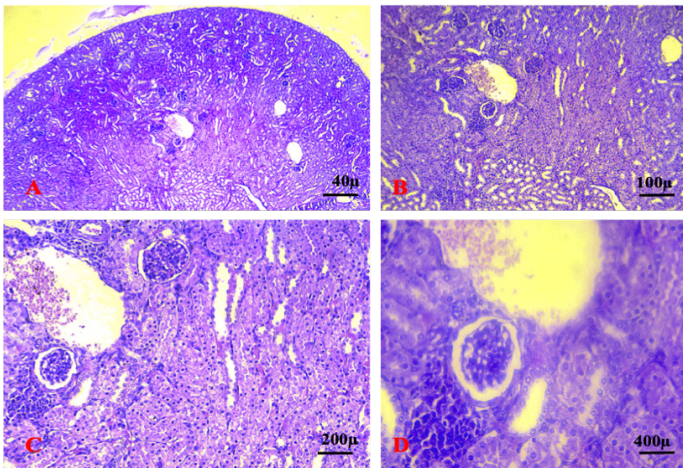

Figure 11: A, B, C and D shows the liver section at 5th week revealing the loss of the structural organization of the central vein and portal area accompanied with extensive necrosis of hepatic parenchyma. (A. X 4, B. X10, C.X20, D. X40). (H and E).

The liver and kidney sections of mice in treatment group 6 (mice received PVP as a carcinogenic agent two weeks after treatment with a low dose (400 mg/Kg) of Sc-RE) revealed various histopathological changes at different periods of treatment. Liver sections in 5th week revealed aggregation of immature cancer cells surrounding the central veins and portal areas.

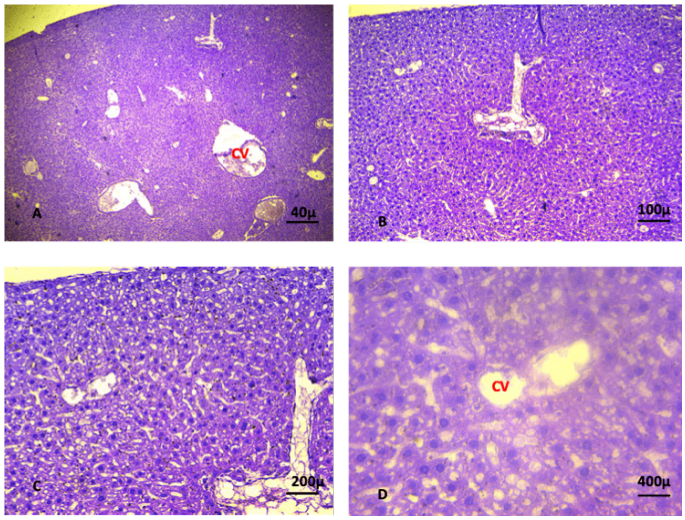

Figure 12: A, B, C, D shows sections of the liver at the 5th week of the experiment revealed slight degeneration of the hepatic cells, congestion, fatty degeneration, necrosis of hepatic cells around the central vein and absence of immature cancer cells (A. X 4, B. X10, C.X20, D. X40). (H and E).

Degeneration and necrosis of the hepatic cells and necrosis of hepatic parenchyma were also noticeable. Liver sections revealed structural organization loss accompanied by congestion and infiltration of inflammatory cells (Figure 12A, B, C, D). At the same time, kidney sections in the 5th week revealed severe degeneration and necrosis of the renal cortex involving the renal tubules. Degeneration of glomeruli and widening of its wall was also observed. Small aggregations of immature cancer cells were seen in the renal parenchyma. The kidney sections also showed infiltration of the inflammatory cells and congestion (Figure 13A, B, C, D).

The liver and kidney sections of mice in treatment group 7 (mice received PVP as a carcinogenic agent two weeks from treatment with a moderate dose of 600 mg/Kg of Sc-RE) revealed diverse histopathological changes at different periods of treatment. Liver sections revealed slight degeneration and a few infiltrations of inflammatory cells. Additionally, normal structural organization of the central vein and portal area was seen, and no apparent immature cancer cell was seen (Figure 14A, B, C, D). Section of the kidney at 5th week revealed degeneration and necrosis of the renal cortex involving the renal tubules. There was an obvious degeneration of the glomeruli and extension of

its wall. The kidney sections also showed infiltration of the inflammatory cells and congestion, but no apparent aggregations of immature cancer cells were seen in the renal parenchyma (Figure 15A, B, C, D).

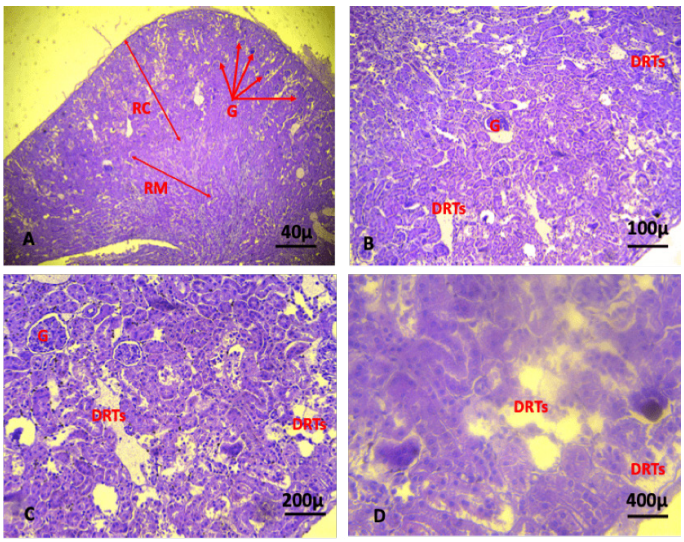

Figure 13: A, B, C, D shows section of kidney at the 5th week of the experiment. The kidney section revealed severe degeneration and necrosis of renal parenchyma, damage of renal tubules, infiltration of the inflammatory cells and severe congestion and absence of immature cancer cell aggregation (Damage of renal tubules: DRTs; renal cortex: RC; Renal medulla: RM; Glomeruli: G). (A. X 4, B. X10, C.X20, D. X40). (H and E).

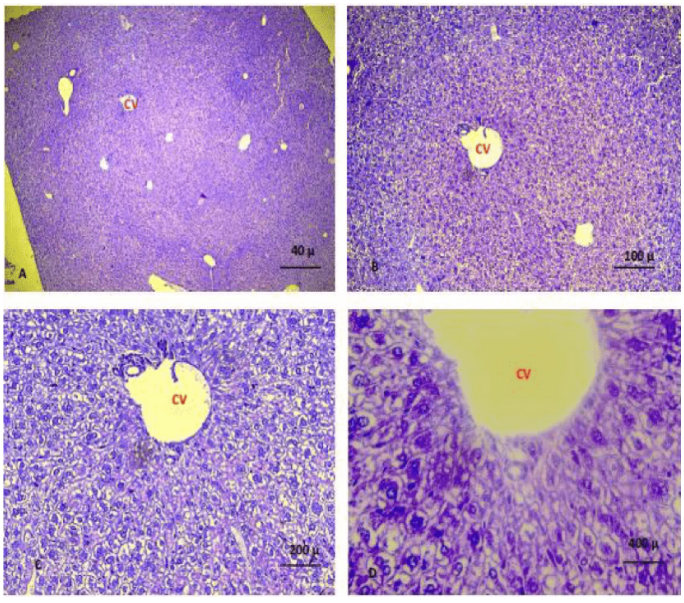

Figure 14: A, B, C, D shows liver sections of mice in group 7 at 4th week of experiment. Liver sections revealed slight degeneration and few infiltrations of inflammatory cells. Additionally, normal structural organization of the central vein and portal area was seen and no obvious immature cancer cell was seen (A. X 4, B. X10, C.X20, D. X40). (H and E).

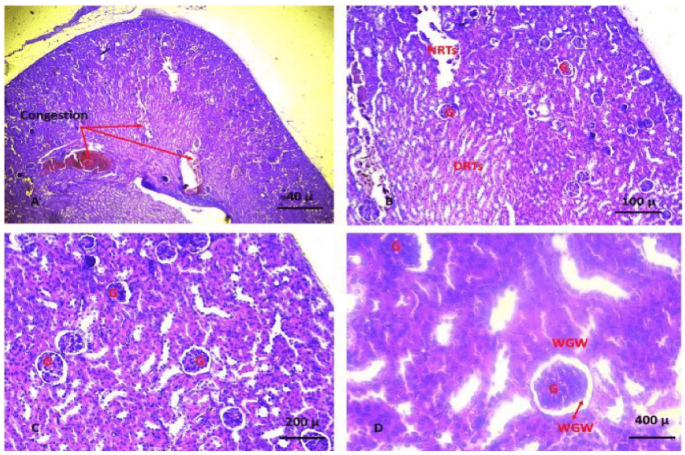

Figure 15: A, B, C, D shows section of kidney of group 7 at the 5th week of the experiment. The kidney sections revealed severe congestion, degeneration and necrosis of renal parenchyma and renal tubules, infiltration of the inflammatory cells, absence of immature cancer cell aggregation (Damaged renal tubules: DRTs; Necrosis of renal tubules: NRTs; Glomeruli: G; Widening of glomerular wall: WGW). (A. X 4, B. X10, C.X20, D. X40). (H and E).

The liver and kidney sections of mice in treatment group 8 (mice received PVP as a carcinogenic agent two weeks from treatment with a high dose of 800 mg/Kg of Sc-RE) revealed various histopathological changes at different periods of treatment.

The liver section revealed slight degeneration and a few infiltrations of inflammatory cells. Additionally, severe congestion of the central vein and portal area was seen. No prominent immature cancer cell was seen (Figure 16A, B, C, D).

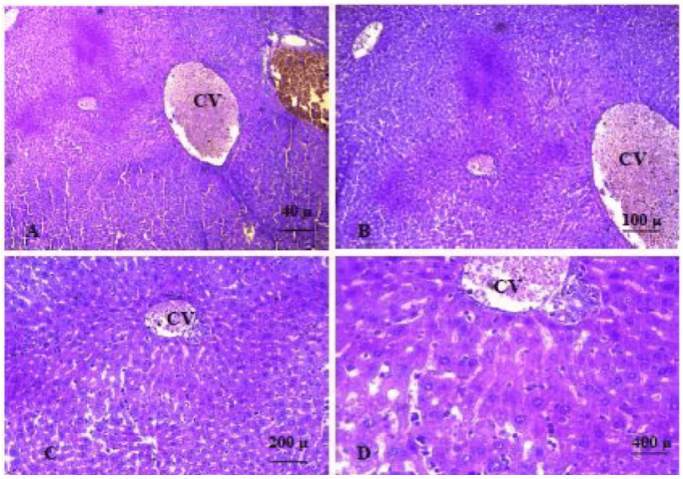

Figure 16: A, B, C, D shows liver of mice in group 8 at 5th week of the experiments, the liver section showed slight degeneration and few infiltrations of inflammatory cells. Additionally, severe congestion of the central vein and portal area was seen. No obvious immature cancer cell was seen. (A. X 4, B. X10, C.X20, D. X40). (H and E).

Section of the kidney in 5th week revealed slight degeneration of the renal cortex and medulla that involved the renal tubules. Additionally, there were infiltration of the inflammatory cells, an obvious degeneration of glomeruli, and extending of its wall. The kidney sections also showed congestion, but no apparent aggregations of immature cancer cells were seen in the renal parenchyma (Figure 17A, B, C, D).

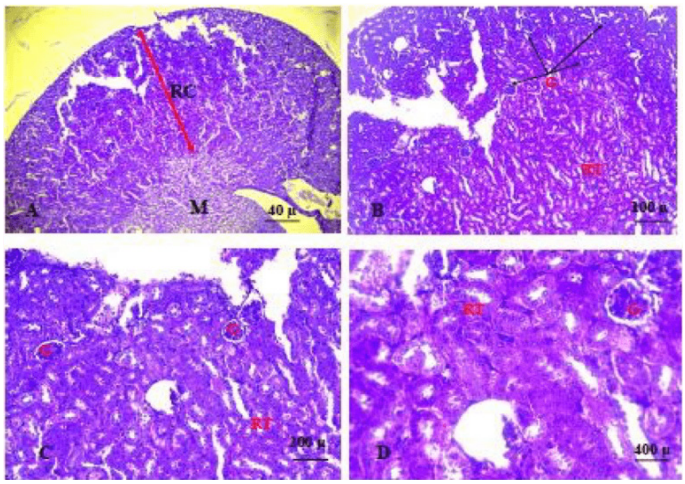

Figure 17: A, B, C, D shows liver of mice in group 8 at 5th week of the experiments, the liver section showed slight degeneration and few infiltrations of inflammatory cells. Additionally, severe congestion of the central vein and portal area was seen. No obvious immature cancer cell was seen. (A. X 4, B. X10, C.X20, D. X40). (H and E).

Polyvinyl pyrrolidone K-30 (PVP-K-30) is a macromolecule with 35.000-40.000 molecular weight used in the pharmaceutical industry (Leopold et al., 1954). Repeated subcutaneous Polyvinyl pyrrolidone injections was induced local sarcoma in vivo in mice, rats, and rabbits. Additionally, its single or several intraperitoneal implantations revealed tumor development at distant sites comprising the reticuloendothelial system (IARC, 1999). However, the inhalation of Polyvinyl pyrrolidone to Sprague-Dawley rats for 24 months revealed adenomas and adenocarcinoma of the nasal cavity accompanied by hepatocellular carcinomas significantly related to the dose. Larynx squamous carcinoma was also observed in the high-dose group (Klimisch et al., 1997). The results of the current study approved the development of liver and kidney cancer in experimental groups (G4, G5, G6, G7, G8) due to intraperitoneal injections of mice with a high dose of PVP-K-30.

Moreover, various advanced histopathological features comprising cancer cells were seen in the last weeks of the experiment in group 4 (mice treated with carcinogenic substance PVP-K30 only). These results are compatible with previously reported studies that approved the development of local and distant cancer in various experimental animals (mice, rats, rabbits) due to repeated administration of Polyvinyl pyrrolidone by several routes using materials of different molecular weights. The reported cancers were adenoma and adenocarcinoma of the nasal cavity, hepatocellular carcinoma, and larynx squamous carcinoma (Klimisch et al., 1997; IARC, 1999). These studies approved the development of tumors due to injection or implantation of PVP that was documented by various macroscopical and microscopical changes in the liver and kidney of experimental animals. Likewise, liver biopsy collected from patients injected intravenously with PVP revealed mild inflammation accompanied by basophilic globular deposits in Kupffer cells. Additionally, a foam-cell storage phenomenon was seen due to the accumulation of PVP in reticulated nuclei. Likewise, PVP can be excreted via the kidneys, and autopsy investigation approved its accumulation in the kidneys, liver, spleen, and lymph nodes (IARC, 1999).

The results of the current study revealed no significant differences in water and food intake between experimental groups, with 42% lower in the calorie-restricted groups and average cumulative tumor incidence. Nonetheless, dietary fat and tumor incidence increased with increased caloric intake and body weight over a wide range of intakes, including moderate caloric restriction (i.e., 7-20%). These data indicate that total caloric intake is an important determinant of tumorigenesis in mice. Body weight may be a more sensitive indicator for this effect than caloric intake alone (Albanes, 1987). Previous studies approved that the food intake increased significantly in cachectic tumor-bearing mice (TB), synchronously with losing body weight (Dwarkasing et al., 2014). However, another study revealed variation in fed mice housed at 22 °C consumed 30% more calories than ad libitum–fed mice at 27 °C, although there was no difference between groups in body composition or cancer progression (Huffman et al., 2007).

Liver enzyme tests consider obvious prognostic factors of survival time in advanced cancer patients and are useful for patients with hepatobiliary cancer or liver metastasis. The current study revealed variations in the liver enzyme evaluation between experimental groups compared to the values of the control group. The serum levels of liver enzymes ALT, AST, and ALP (U/L) in mice fed with the Sc-RE for 4 weeks were significant ($P < 0.05$) compared to that of mice in the control group. Nonetheless, significant increases were seen in G3, G4, and G5. Moreover, slight increases were observed in G6, G7, and G8, compared to the control group, but minor variations were seen within Sc-RE low, moderate, and high treatment doses. These results are compatible with previous studies that approved an increase in the values of liver enzymes in treatment groups indicating damage to the hepatocytes (Dar et al.,

2019). A previous study also revealed that two times higher AST and ALT levels, prolonged PT, and hypoalbuminemia are likely prognostic factors of poor survival in advanced cancer patients. These parameters provide new insights into prognostication in advanced cancer (Tsai et al., 2014).

The primary importance of measuring alkaline phosphatase (ALP) is to check the possibility of bone diseases or liver disease. The transaminase enzymes (ALT and AST) are important in producing various amino acids. Measuring the concentrations of various transaminases is important in diagnosing and tracking many diseases (Odiegwu et al., 2021). The liver is crucial in the metabolism (i.e., inactivation or activation) of many commonly used anticancer agents cytotoxic or new biological agents. Therefore, the assessment of liver function is a fundamental part of the initial work-up and management of patients with cancer (Lala et al., 2023).

Histopathological examination of liver and kidney sections of mice in different experimental groups is considered a diagnostic tool to evaluate cancer and malignancy's progress and treatment efficacy in different tissues. The results of the current study showed normal liver and kidney histological features of the control group 1, in accordance with reference histological features described in the textbook (Azevedo et al., 2022). Additionally, normal histological features similar to those in the control group were also seen in the kidney and liver sections of G2 (mice received Sc-RE only).

These results are compatible with the results of the previously reported researcher (Ghada et al., 2020), who approved the notable protective effect of Sc-ethanolic extract via its anti-apoptotic, anti-inflammatory, and antioxidant capability. Consequently, they considered Sc, the best nominee, a natural antioxidant that faces the harmful effects of glucocorticoids.

In the current study, liver and kidney sections of mice in Group 5/ cancer treated with chemotherapy (2.5mg/Kg Ebetrex) revealed diverse severe histopathological sections at different treatment periods, which became more severe at 5th week, indicating the advanced stages of cancer. These results agree with previously reported studies (Hersh et al., 1966; Tobias and Auerbach, 1973; Doha et al., 2018). These studies approved the hepatotoxicity, kidney damage, and administration disorders of Ebetrex as anticancer. Methotrexate is the active component of Ebetrex. It is a chemotherapy agent and immunosuppressive. Ebetrex treats breast, leukemia, lung, lymphoma, gestational trophoblastic diseases, and osteosarcoma. Moreover, it is also used as an antimetabolite and antirheumatic (https://web.archive.org/web/20161008130258/; https:/www.drugs.com/monograph/methotrexate.html). Though, Methotrexate has adverse effects on the treated patients. The previous studies approved the adverse effects, including hepatotoxicity, blood abnormalities (leukopenia, anemia, and thrombocytopenia), stomatitis, increased risk of infection, hair loss, nausea, fatigue, acute pneumonitis, renal impairment and mucositis (Oliff et al., 1979; Kamen et al., 1981; Rossi, 2013). Consequently, the severe histopathological features in this group might be related to the adverse effects of Methotrexate used as chemotherapy to treat the induced cancer in experimental mice. Nonetheless, these observations were evident in the histopathological examination of the liver and kidney sections of control group 3, where the animal received only chemotherapy (2.5mg/Kg Ebetrex). In 5th week, both liver and kidney sections revealed severe degenerative and necrosis changes accompanied by heavy infiltration of inflammatory cells. These results are compatible with the previous study that approved Methotrexate administration induced an acute reaction, tissue edema, marked vascular congestion, and leucocyte infiltration. Other studies found that Methotrexate induces oxidative and nitrative stresses accompanied by activation of nuclear factor-KB (NF- KB) and p38 pathways. Additionally, numerous degenerative changes, including karyorrhexis nuclei, cellular vacuolization that displays the smooth endoplasmic reticulum's dilatation, and tissue architecture disruption (Kim et al., 2009; Mukherjee et al., 2013).

In groups 6, 7, and 8, mice received PVP (IPI) as a carcinogenic agent; two weeks later, mice received Sc-RE treatment at 400 mg/kg, 600 mg/kg, and 800mg/kg for G6, G7, and G8, respectively. The sections of the liver and kidney from these mice revealed numerous diverse histopathological features related to various treatment groups. In 5th week, the liver sections of G6 revealed aggregation of immature cancer cells. The kidney sections revealed severe degeneration and necrosis of the renal cortex with small aggregations of immature cancer cells in the renal parenchyma. Additionally, the liver sections of G7 revealed slight degeneration, few infiltrations of inflammatory cells, nearly typical structural organization of the hepatic central vein and portal area, and no apparent immature cancer cells. At the same time, kidney sections showed degeneration and necrosis of the renal cortex. There was an obvious degeneration of glomeruli but no apparent aggregations of immature cancer cells in renal parenchyma. Nonetheless, the liver sections of G8 revealed slight degeneration and few infiltrations of inflammatory cells accompanied by severe congestion of the central vein and portal area with no apparent immature cancer cells. While kidney sections revealed slight degeneration of the renal cortex and medulla, accompanied by infiltration of the inflammatory cells. However, no apparent aggregations of immature cancer cells were seen in the renal parenchyma.

These results are compatible with previously reported studies (Sun et al., 2003; Robinson et al., 2008; Liu et al., 2011; Nageswara et al., 2013; Rasul et al., 2013; Hua et al., 2014; Alaa et al., 2020; Patel et al., 2020).

These *in vitro* studies approved the presence of the active anticancer substance in the roots of *Saussurea lappa*. They found a new sesquiterpene lactone, which induced apoptosis in cancer cells mediated via ROS generation and mitochondrial dysfunction. Korean, Japanese, and China traditional medicine uses SC-dried root for the treatment of different diseases and conditions, such as abdominal pain. A previous study investigated two major components of Sesquiterpene lactones in the root of Sc.

The costunolide and dehyrocostus lactones own numerous biological activities, comprising antitumor activity (Ko et al., 2005). At the same time, another researcher determined the bioactive compound a costunolide in the Sc (*S. lappa*) root. This substance has been explored for its cause of inhibition of human leukemia cells and its hypothetical action pathway. The costunolide is a potent apoptosis inducer enabling its action by forming reactive oxygen species, mediating mitochondrial permeability change, and liberating cytochrome C (Lee et al., 2001). The root extract of *S. lappa* is approved to treat gastric cancer cells via G2- growth arrest. It can produce growth inhibition of gastric cancer cells and is also effective on leukemia and intestinal carcinoma cells *in vitro*.

Nonetheless, (Ko et al., 2004) approved the cure with *S. lappa* that drastically diminished the viabilities of cells in a dose and time-dependent manner. *In vitro*, cytotoxicity was determined by *S. lappa* bioactive Costunolide, dehydrocostus lactone, and lappa dilactone. Likewise, *S. lappa* root cynaropicrin was approved to have potential against some leukocyte cancer cells like leukemia or lymphoma (Elsebai et al., 2016).

Consequently, the evaluation of the histopathological features for all treatment groups found that a low dose of 400 mg\ kg Sc-RE was less effective in inhibiting the cancer cells in the liver and kidney. In contrast, moderate 600 mg/ kg and high 800 mg/ kg treatment doses inhibited the growth of cancer cells in the liver and kidney. These results are compatible with previously reported studies (Alaa et al., 2020; Ghada et al., 2020; Alotaibi et al., 2021; Elgharabawy et al., 2021), they approved that *Saussurea lappa* ethanolic extract can reduce the pulmonary and splenic tissue damage caused by triamcinolone acetonide through modulation of the apoptosis, inflammation, and oxidative stress. However, severe damages were seen in the histological structure of the kidney in the last weeks of treatment due to the excretion and accumulation of the Sc-RE in the renal tubules. These observations must be addressed by adjusting the treatment dose to prevent these adverse effects on the kidney.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study approved the activities of Sc-RE to inhibit in vivo-induced hepatic and kidney cancer in mice. Therefore, *Saussurea costus* may be a promising agent for inhibiting induced cancer growth due to various carcinogenic substances. The authors suggest a future study investigating the pathway of *Saussurea costus*-Root extract effects as an anticancer and determining the anticancer bioactive components.

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NOVELTY STATEMENT

For the authors knowledge, this is the first study approved the activities of *Saussurea costus* to inhibit *in vivo* induced hepatic and kidney cancer in mice.

AUTHOR'S CONTRIBUTION

The study was carried out by ZAA, HKS, and KAA, and all authors contributed equally. In addition, KAA and ZAA made essential contributions to the experimental experiment. HKS organization and analysis of the data for this work. All authors above consented to be held responsible for all parts of the work and participated in its preparation, drafting, and revision. They also provided final permission for the version that was published.

Ethical consideration

The authors have no any ethical issues such as plagiarism, information fabrication, misconduct and/or falsification, permission to publish, duplicate publication and/or submission, and redundancy.

Conflict of interest

The authors have declared no conflict of interest.

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