Isolation and Study of The Effect of Natural Products Isolated from Cordia Myxa Plant on The Level of Lysyl Oxidase Like -2 and Some Biochemical Variables in Mice with Bladder Cancer

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KEYWORDS

bladder cancer, lysyl oxidase like -2, mice, natural products, Cordia myxa

ABSTRACT

Using HPLC technology, I separated and diagnosed various natural products from the Cordia myxa plant, identifying the oily substance and its fatty acids. Some flavonoids and alkaloids were also separated. We identified the flavonoids using high-performance liquid chromatography (HPLC) technology. The effect of these products on lysyl oxidase like -2 (LOXL-2) was studied, and it was found that they had a positive effect on reducing the level of LOXL-2 and some other measured variables. In addition, the study examined the effect of these natural products on some biochemical parameters related to bladder cancer in the blood serum of mice induced with the disease. Biochemical variables included LOXL-2, glutathione peroxidase, glutathione, malondialdehyde (MDA), and tumor necrosis alpha (TNF-alpha). The results showed that the concentrations of LOXL-2, MDA, and TNF-α increased significantly in infected mice compared to healthy mice, and a significant decrease in the concentration of GPX and GSH was observed. Natural products demonstrated a positive therapeutic effect in mice, and tissue sections of experimental animals established the disease.

1. Introduction

Cancer is an inheritable condition that occurs during normal cell division processes, including several molecular changes that cause normal cells to differentiate into cancer cells. Cancer is defined by the unregulated development and spread of cells that are abnormal, which occur when some cells lose control of division and begin to divide uncontrollably to form a large number of cells. This new growth of abnormal cells is known as a tumor or neoplasm, which can be either benign or malignant (Zaahkouk *et al*., 2015). Bladder cancer is the ninth among all cancer globally (Antoni *et al*., 2017). It ranks seventeenth in female cancer cases and sixth in male carcinoma cases (Omorphose *et al*., 2022). Bladder cancer is the 2nd most prevalent urogenital cancer, accounting for approximately 90% of cases in developed countries and originating in the urothelium (a transition cell tumor) (Omorphose et al., 2022). twenty-five percent of carcinomas of the bladder are muscle-invasive, while 75 percent are not (Siegel *et al*., 2018). The majority of BCs are caused by outside exposure to poisons through the skin, gastrointestinal tract, or the lungs. The main risk factors for BC are tobacco smoke and carcinogens that exist in the workplace and environment. Tobacco smoke causes 50% of BCs, but the risk varies according to genders, smoked history, and what kind of smoke used (black and blonde cigarettes are treated by airflow and flue, respectively). Black tobacco is more carcinogenic for a greater quantity of nitrosamines, biphenyls, and arylamines. (Cumberbatch and Noon, 2019). Smoking cigarettes is the major cause for bladder carcinoma, causing between fifty percent and sixty of new cases each year. Tobacco raises the risk three to four times because to the carcinogenic substances found in tobacco smoke. Industrial exposure to carcinogenic substances, such as that found in manufacturing processes, is other major preventive risk factor (Saginala *et al*., 2020). For a long time, natural products were considered an important part of the treatment of bladder cancer. Numerous studies investigating natural compounds effective against bladder cancer have been carried out in recent decades, coinciding with an increase in interest in natural products, including medicinal herbs (Nagata *et al*., 2007). *Cordia myxa* is a flowering plant with spherical fruits and broad, crusty leaves. Flavonoids and other compounds are present in the fruits, as are alkaloids, phenolic acids, terpenoids, saponins, coumarins, and sterols. Urinary tract infections are treated with *C. myxa* fruits (Shahariari and Moghadamina, 2019). *C. myxa* has been shown to have antimicrobial, anti-inflammatory, antiparasitic, immunomodulator, and cardiovascular and breast cancer cell properties in pharmacological studies. Pharmacological studies of *C. myxa* have shown that fruits are astringent, anthelminthic, demulcent, and used for cough, chest complaints, urinary

tract, sore throat, lung, and spleen diseases. (Abidin, 2023).

2. Materials and Methods

A machine ground the *Cordia myxa* plant from one of Mosul's city locations into powder. The process of extracting oil from the *Cordia myxa* plant involves weighing 400 g of plant powder and then soaking it in petroleum ether solvent at a temperature of 60–80 C for a duration of one day. This process allows for the extraction of fatty acids and volatile oils. After that, a rotary evaporator was used to extract the solvent, and the resultant oil product was refrigerated and kept in a sealed tube for future tests. [The HPLC system used in this study included two SYKAM-GGerman solvent delivery system pumps as well as a spectrofluorometric detector with excitation and emission wavelengths of 265 and 315 nm, respectively. Shim-pack C18-ODS (250 mm * 4.6 mm) was the analytical column used. Acetonitrile-water gradient conditions were as follows: 85–15% from 0 to 4 minutes, 87–13% from 5 to 8 minutes, and 97–3% from 9 to 14 minutes. The column oven temperature was set at 50 °C, and the mobile phase was filtered, degassed, and pumped at 1.5 ml/min. The test took place in the Ministry of Science and Technology's laboratories in Baghdad, Department of Environment and Water. Derivatization: Add 250 µL of 9-fluorenylmethyl chloroformate solution to 1 mL of oil. After adding 25 µL of sodium phosphate buffer (0.05 M; pH 9.3) and briefly mixing, the samples were held at 40 °C for 10 minutes. Next, 100 µL of the reaction mixture was injected into the HPLC systems. The extraction of flavonoids from the plant involved utilizing the leftover material from *Cordia myxa* powder after oil extraction. We then dried this material to remove the petroleum ether. Subsequently, an extraction procedure was carried out for a duration of three days using 99% ethanol. The extracted product was stored in a sealed tube in a refrigerated environment for future investigations (Kato and Koike, 2010). Flavonoids were diagnosed using high-performance liquid chromatography (HPLC). After isolating both the oil and flavonoids, the residue was separated to obtain the alkaloids. We then used distilled water in the extraction apparatus to extract the residue for three days, ensuring the elimination of all ethanol. After soaking it in distilled water for a day, we placed the solution in a freeze-drying condition to remove the water, resulting in a dry extract that we later stored in a sealed tube for subsequent tests.

Experimental Animals

The study used 25 adult Swiss albino mice as its experimental animals. The study included participants aged three months and weighing between 25 and 30 g. These were stored in a specialized chamber at the University of Mosul's veterinary medicine college, which provided optimal lighting, temperature, ventilation, and feeding conditions. Athical approval number: UM.VET.2023.7780, dated December 21, 2023.

Induction of bladder cancer Disease

We created bladder cancer by injecting N-methyl-N-nitrosurea into the peritoneal cavity for a month and a half at a dose of 4 mg/kg (Faustino-Rocha *et al.*, 2015). A later histological investigation's results led to the diagnosis of the new disease. Following the induction of the disease, the collection of blood and tissue samples began.

Injecting animals with bladder cancer

We divided the experimental animals into five groups, each containing five rats. Group 1 consisted of the negative control group, which received water exclusively, and four other groups that received the carcinogenic chemical N-methyl-N-nitrosurea. Following this, three groups received the products extracted from the plant's seeds (*Cordia myxa*) using a needle. Group No. 2 received an injection of 0.1 ml of physiological saline into the peritoneal cavity, representing an untreated infected group (the infected control group). Group 3 received an injection of the oil extract into the peritoneal cavity at a dose of 1.24 mg/kg of body weight, while Group 4 also received the same injection. Group 5 received an injection of the alkaloid extract at a dose of 7.0 mg/kg, while the third group (3) received an injection of flavonoids at a dose of 60 mg/kg (Salah Najim *et al*., 2022).

Sample collection (blood and tissue)

We used ether to anesthetize the animals after the experiment. Because of its quick action and large safety, ether is a powerful anesthetic for use in research. We took blood samples by puncturing the orbital sinus using capillary tubes. We then collected the blood into simple tubes that were devoid of anticoagulants. After allowing the drawn blood to coagulate, we separated the serum using centrifugation for fifteen minutes at a velocity of three thousand xg. Following this, we carefully transferred the serum to specialized tubes and stored it at -10 °C until we conducted the next round of experimental tests. We extracted and preserved the bladder tissue in tubes containing a 10% formalin solution for histological examination.

This ELISA kit uses Sandwich-ELISA, with a pre-coated Micro Elisa strip plate containing an antibody specific to LOXL2. We add the standards or samples, incubate them, add the TMB substrate solution, and measure the optical density (OD) at 450 nm. We compare the OD of the samples to the standard curve to ascertain their LOX2 concentration.We measured TNF-a levels in blood serum using a sandwich ELISA assay to determine the TNF-a concentration.We measured the effective concentration of glutathione peroxidase according to the researchers' method (Sunderman and Nomoto, 1970).Serum glutathione was measured using the researchers' modified method (Sedlak & Lindsay, 1968).The amount of malondialdehyde in the blood serum was determined using the technique employed by the researcher (Buege and Aust, 1973).The amount of malondialdehyde in the blood serum was determined using the technique employed by the researcher (Buege and Aust, 1973).

Statistical Analysis

We used SPSS-19 statistical software to analyze the clinical examination data. We used a one-way analysis of variance to calculate the average and the standard deviation of the mean (SD). Duncan's test evaluates differences between groups using the p-value. A p-value of less than 0.01 indicates a significant difference (Kirkpatrick & Feeney,2012).

3. Results

We identified oils using the HPLC technique. We used the HPLC technique to analyze fatty acids in isolated oils from the *Cordia Myxa* plant, resulting in multiple peaks (Figure 1). This agrees with the researcher (Al-Snafi, 2020). *Cordia myxa* seeds contain 2.2% oil, which includes palmitic, stearic, oleic, and linolenic acids. We tested the biological efficacy of treating mice with newly induced bladder cancer. We analyzed the fatty acid composition of the plant's oil extract using the HPLC technique. The results indicate that palmatic acid, oleic acid, stearic acid, linolic acid, and linolenic acid are present. Figure 1 displays the chromatogram of oil extracted from the *Cordia myxa* plant. Table 1 displays the fatty acid peaks and retention times.

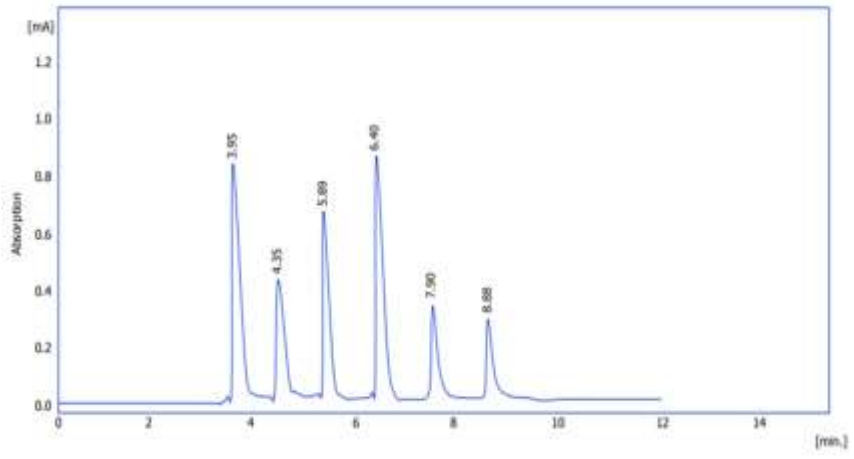
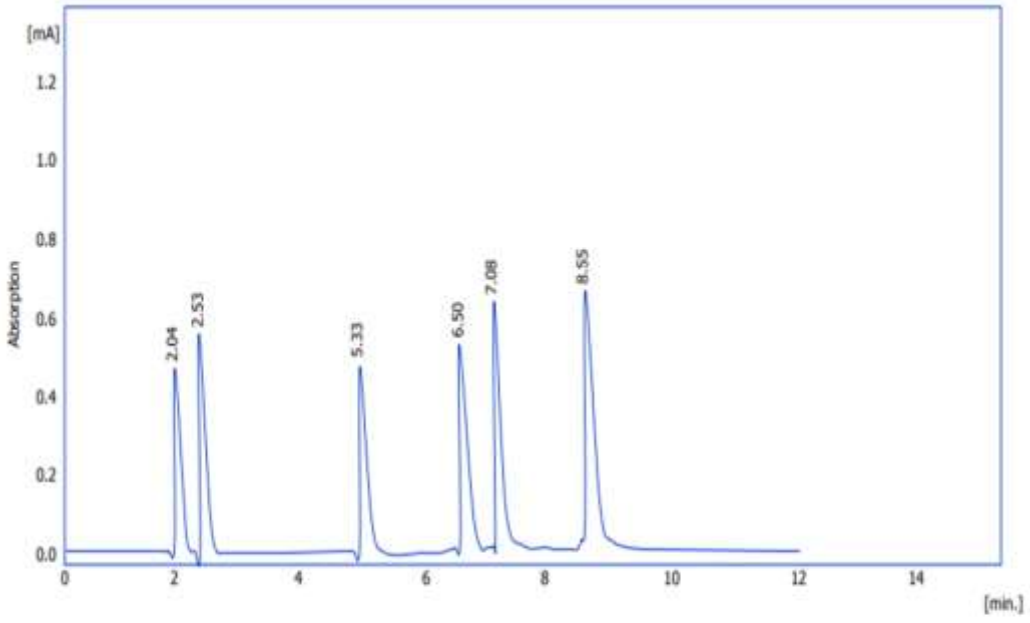


Figure (1): HPLC of oils isolated from *Cordia Myxa*

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.95	250412.65	800.65	24.00	24.00	0.15	
2	4.35	120447.90	240.58	11.00	11.00	0.08	
3	5.89	168985.77	684.74	17.00	17.00	0.10	
4	6.40	310250.88	804.88	24.00	24.00	0.15	
5	7.90	95654.32	350.21	12.00	12.00	0.05	
6	8.88	74512.71	310.44	12.00	12.00	0.05	
	Total	1020264.23	3191.50	100.00	100.00		

Table (1) Analysis of the standard sample to diagnose the oil yield of *Cordia Myxa*

We used HPLC technology to identify compounds in the flavonoid extract of the *Cordia myxa* plant (see figure 2). We identified phenols in the flavonoids of the *Cordia myxa* plant. We purified the results of the standard sample analysis using the high-performance liquid chromatography technique (Figure 2), identifying the sex peaks of the phenolic compounds. We identified these compounds by comparing their detention time (Rt) with the retention time (Rt) of the standard chromatogram, as illustrated in Figure 2. The *Cordia myxa* plant contains ferulic acid, rutin, kaempferol, caffeic acid, isorhamnetin, and gallic acid.



Result chromatography Table (Uncal - F:\ sample)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.04	20325.65	450.12	14.00	14.00	0.10	
2	2.53	25647.90	520.66	16.00	16.00	0.15	
3	5.33	22478.98	465.99	14.00	14.00	0.10	
4	6.50	24516.99	500.32	17.00	17.00	0.10	
5	7.08	30221.47	630.65	19.00	19.00	0.15	
6	8.55	31336.62	640.12	20.00	20.00	0.15	
	Total	152574.49	3207.49	100.00	100.00		

Figure (2) Analysis of the standard sample to determine the flavonoid production of the *Cordia Myxa* plant by (HPLC) Chromatogram.

Table (2): Estimation of biochemical variables in experimental animals with induced bladder cancer

treated with *Cordia myxa* plant extracts.

Biochemical variables	Group 1 control		Group 2 Patients(N-methyl-N-nitrosourea) 4 mg/kg		Group3 Oil (1.24 mg/kg)		Group 4 Flavonoid (61 mg/kg)		Group 5 Alkaloid (7.0mg/kg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LOXL-2	136.96e	1.98	74981a	3.56	350.08c	1.95	141.62d	1.37	424.33b	1.26
GPX	120 a	1.64	54.03 d	3.71	84.42 c	3.51	116.06 a	3.63	94.15 b	2.96
GSH	10.71 a	0.354	3.63 d	0.353	6.57 c	0.43	10.39 a	0.18	8.50 b	0.32
MDA	2.00 d	0.028	7.56 a	0.28	4.47 b	0.16	1.77 d	0.31	3.76 c	0.20
TNF-a	24.98 e	1.09	890.9 a	6.10	215.89 c	4.01	53.37 d	2.29	489.08 b	2.96

** Different letters indicate a significant difference at the probability level ($P \leq 0.01$).

Histological study of induced bladder cancer and the role of *Cordia Myxa* plant extracts in treating it

In mice, bladder cancer was induced, according to the study. The study showed this by looking at how transitional epithelial cells die and are destroyed, how epithelial cells in the cavity shed and slough off their skin, how inflammatory cells move and swell, and how mucous layer A thins out. Figure 3 shows that in mice with bladder cancer, the transitional epithelial cells lacked a normal arrangement. This was different from healthy animal tissues, which had a normal transitional epithelium covering the mucous layer and normal, symmetrical fibers in the muscular layer. Furthermore, Table 2 links the current bladder cancer model to an abnormally high concentration of LOXL-2 and tumor necrosis factor. Figure 7 depicts a histological section of a mouse's bladder taken from a group of mice infected with *Cordia myxa* plant oil extract. The therapeutic role We note the therapeutic role of the oil extract, which led to a slight degeneration of transitional epithelial cells, a slight invasion of inflammatory cells, edema, and thickening of the muscle layer. The flavone extract mitigated the induced cancer's impact, as evidenced by the death of some transitional epithelial cells and the invasion of others. ed. The alkaloid extract of the *Cordia myxa* plant (Figure 9) demonstrated less efficiency than the oily extract. There are a few pleomorphic tumor cells, some irregularities in the transitional epithelial cells, and some swelling in the mucous layer, but not as much as in the untreated group. Using *Cordia myxa* oil, flavonoid, and alkaloid extracts to treat the condition not only stops inflammation but also lowers hyperplasia. The biochemical results in Table 2 were also consistent with the anatomical results. (Goradel *et al*., 2019) support these findings. In 2006, Kim et al. found that the bioactive polyphenolic and flavonoid phytochemicals in *Cordia myxa* fruit (CMF) have many biological activities, such as the ability to fight cancer and free radicals. Olyphenols are naturally occurring compounds found primarily in fruits and vegetables that are known for their strong antioxidant properties (Wahle *et al*., 2010). They are more potent antioxidants than vitamins C, E, and carotenoids. These compounds protect biological systems by scavenging free radicals, which lowers the risk of cancer and prevents oxidative damage to macromolecules such as carbohydrates, proteins, fats, and DNA. Polyphenols serve a variety of biological functions, including modulating carcinogenesis. Studies have demonstrated their ability to reduce the number and expansion of tumors in human cancer cell lines. Their anti-cancer mechanisms include antioxidant activity, reactive oxygen species neutralization, and carcinogenesis inhibition. Zyme inhibition is the process of deactivating carcinogen-metabolizing enzymes, inhibiting their activity and synthesis. Flavonoids suppress NF-κB activation and gene expression, leading to increased apoptosis. This establishes the molecular basis for polyphenols' anticancer properties. Their effects on angiogenesis—the growth of new blood vessels—are critical for tumor growth and metastasis. Key activators include fibroblast growth factor (FGF), interleukin 8 (IL-8), transforming growth factor α (TGFα), and vascular endothelial growth factor (VEGF). Polyphenols can inhibit angiogenesis and tumor growth by suppressing these factors (Basli *et al*., 2017). Alants primarily contain alpha-linolenic acid (ALA), an essential omega-3 fatty acid that may reduce markers of inflammation such as C-reactive protein and TNF-alpha. Additionally, inhibiting nuclear factor kappa B (NF-kB) may reduce inflammation by reducing the production of inflammatory mediators. theory (Stark *et al*.,

2008).

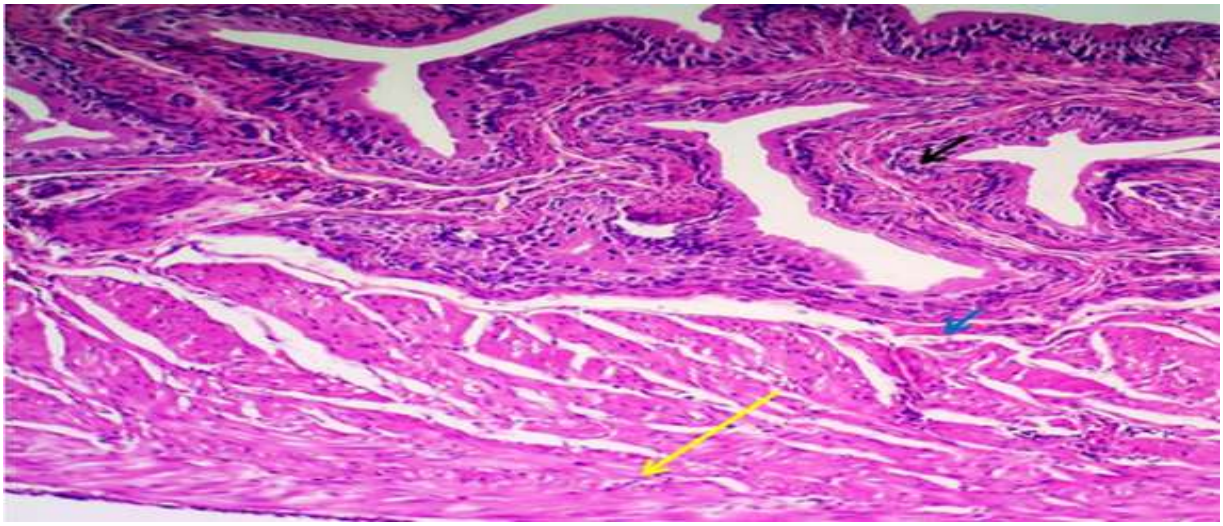


Figure 3 depicts a tissue section of a healthy mouse bladder. A histological section of the urinary bladder of mice from the control group demonstrates the normal histological features of the bladder layers, including the mucous layer, which contains the transitional epithelial cells (black arrow), the submucosal layer (blue arrow), and the muscle layer (yellow arrow). Hematoxylin and eosin stain, 100.X

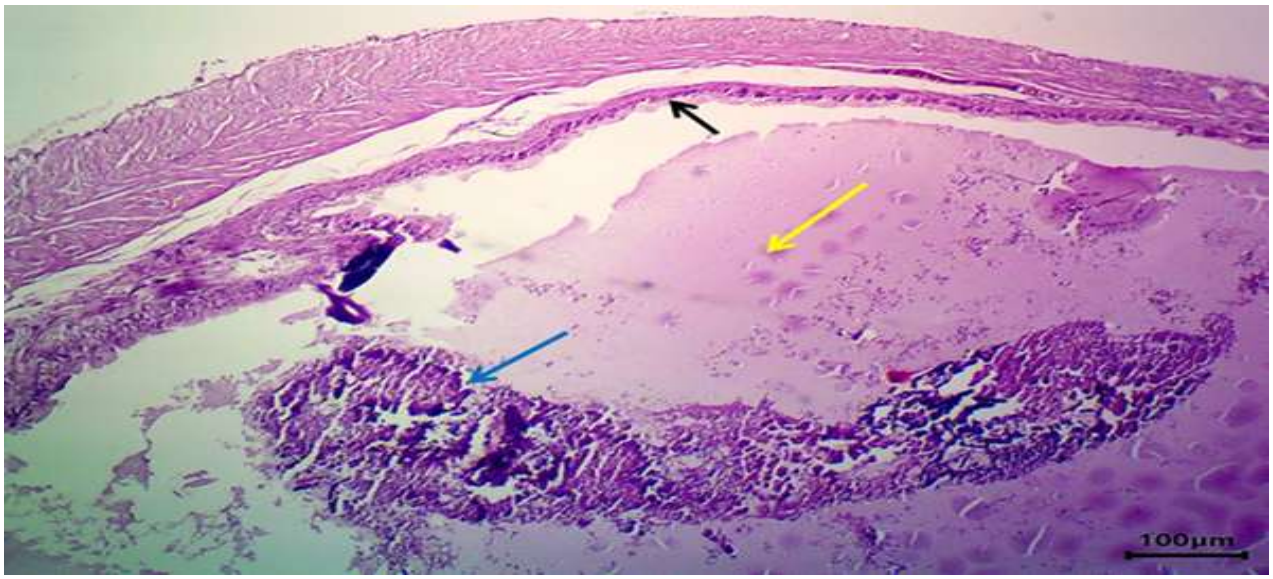


Figure 4 (histological section of a mouse's bladder with induced bladder cancer) Mice from the infected group display a histological section of their bladders, revealing the existence of epithelial cancer cells in the mucous layer, their thinning, the necrosis of transitional epithelial cells (black arrow), the desquamation and shedding of epithelial cells in the lumen (blue arrow), and the presence of edema (yellow arrow). Hematoxylin and eosin stain, 40x.

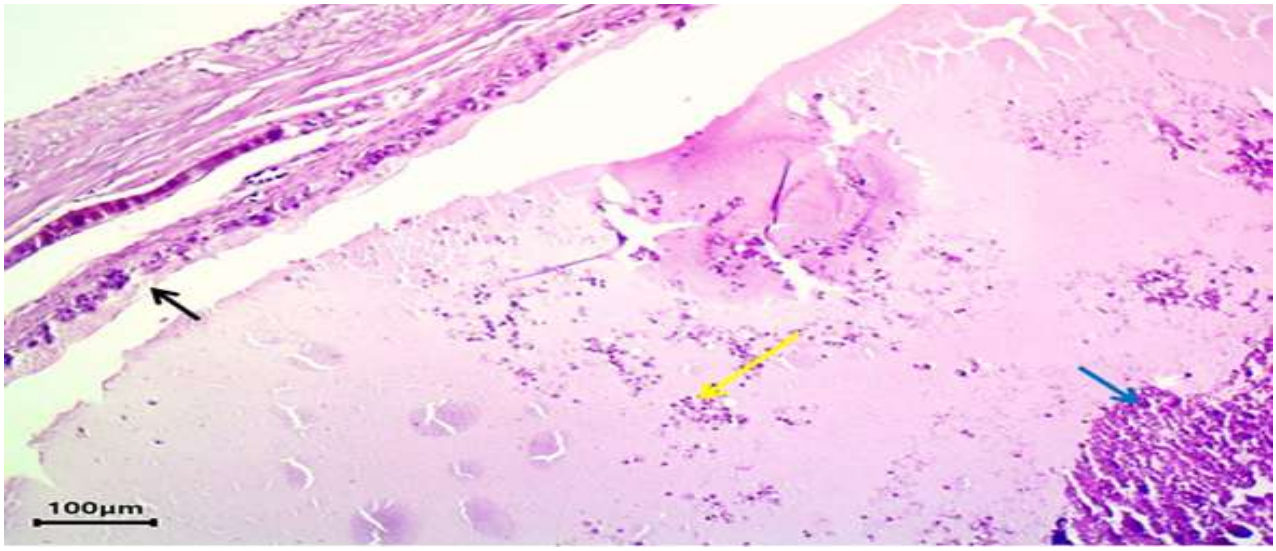


Figure 5: (Histological section of a mouse's bladder with induced bladder cancer) Mice from the infected group display a histological section of their bladders, revealing the presence of epithelial cancer cells in the mucous layer, thinning of the mucous layer, necrosis of transitional epithelial cells (black arrow), desquamation and shedding of epithelial cells in the lumen (blue arrow), and infiltration of inflammatory cells and edema. Hematoxylin and eosin stain 100X.

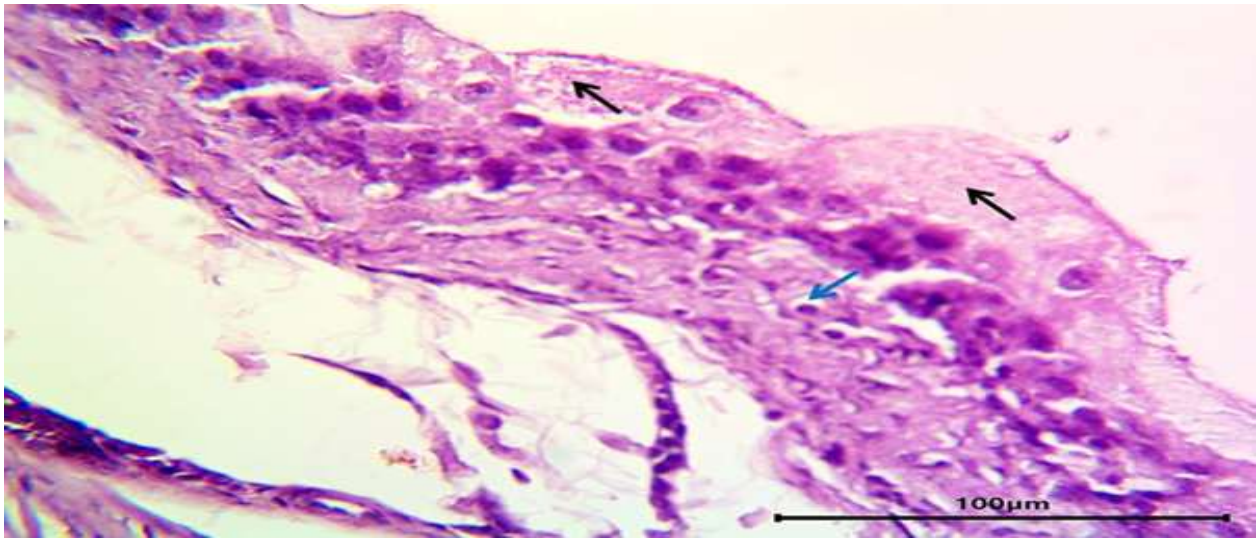


Figure 6 (histological section of a mouse's bladder with induced bladder cancer) A histological section of the urinary bladder of mice from the infected group shows the presence of epithelial cancer cells in the mucous layer, necrosis of transitional epithelial cells (black arrow), and degeneration of others (blue arrow). Hematoxylin and eosin stain 400X.

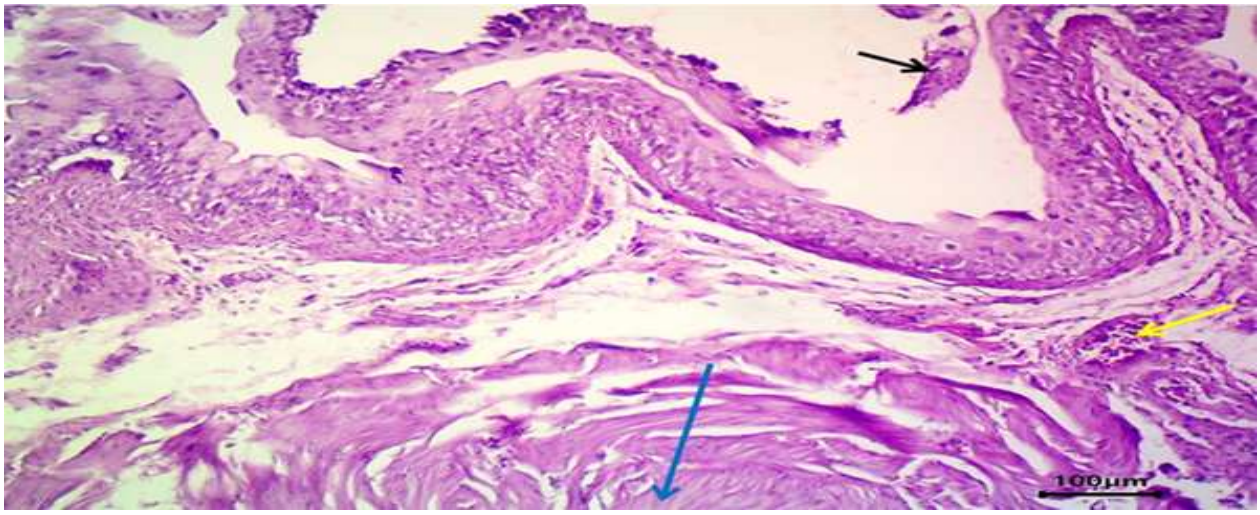


Figure 7A shows a section of the bladder of a mouse with bladder cancer that was treated with an oil extract from *Cordia myxa* seeds. It also shows a section of the bladder of mice from the AH group that has edema (blue arrow) and some degeneration of the transitional epithelial cells (black arrow). Hematoxylin and eosin stain, 400X, shows a slight infiltration of inflammatory cells (yellow arrow).

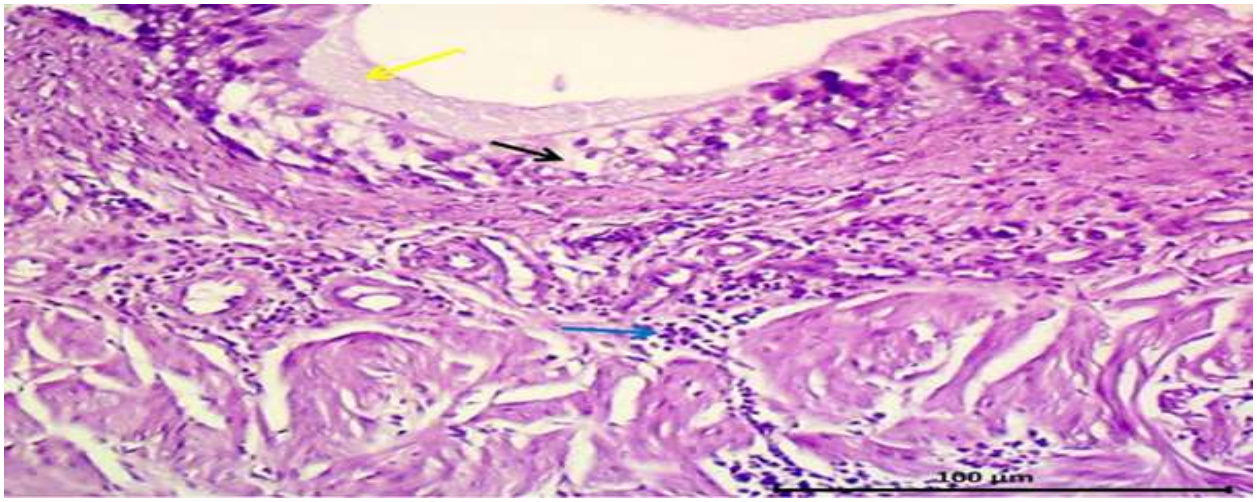


Figure 8: B, a histological section of the bladder of a mouse with induced bladder cancer treated with the flavonoid extract of *Cordia myxa* seeds, illustrates the slight necrosis of transitional epithelial cells (black arrow), slight infiltration of inflammatory cells (blue arrow), and edema (yellow arrow). Hematoxylin and eosin stain,400 X.

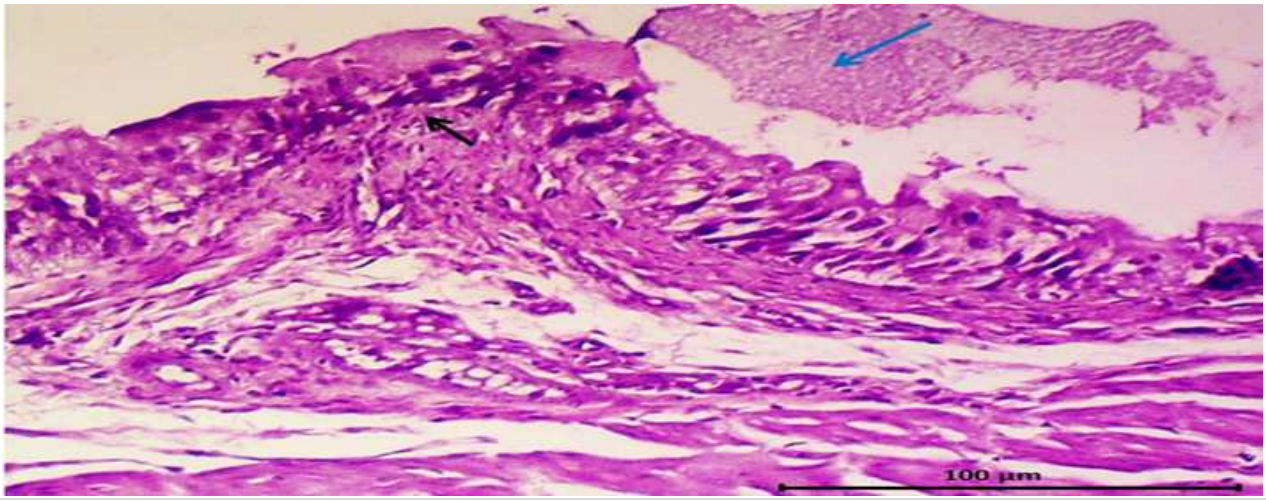


Figure 9 shows the histological section of the bladder of a mouse with induced bladder cancer, treated with the alkaloid extract of *Cordia myxa* seeds. The mucous layer exhibits a small number of pleomorphic tumor cells, irregular transitional epithelial cells (black arrow), and a slight edema (blue arrow). Hematoxylin and eosin stain, 400X.

Discussion

The study on using plant oils and flavonoids from the *Cordia myxa* plant for bladder cancer treatment shows promise due to their anti-inflammatory, immune-boosting, and antioxidant properties. Flavonoids may inhibit cancer cell growth and induce apoptosis. The *Cordia myxa* plant, known for its traditional medicinal uses, contains beneficial compounds like flavonoids and oil. However, extensive clinical trials and standardized dosages are needed to confirm the safety and efficacy of these treatments. These studies focus on targeting and inhibiting the growth of cancer cells and preventing cancer development by protecting against cell damage.

The effect of natural products isolated from *Cordia myxa* plants on certain biochemical parameters:

Previous research has shown that pro-inflammatory cytokines, such as tumor necrosis factor-a, play an important role in modulating the mucosal immune system in bladder cancer. LOXL-2 promotes angiogenesis and metastasis in cancer. As a result, we measured the enzymes LOXL-2 with Tumor Necrosis Factor levels and discovered that the current model of bladder cancer is associated with an increase in these pro-inflammatory and carcinogenic markers (Zang *et al*.,2018). Results obtained from biochemical examinations estimated in blood serum of laboratory animals are shown in Table 3-18, which included the estimation of indicators associated with the progression of induced bladder cancer as well as their response to treatment with extracts prepared from the seeds of the *Cordia myxa* plant, as the results showed a significant effect of treatment with *Cordia myxa* plant extracts on the variables studied in biochemistry.The quantity of lysyl oxidase-like-2 in mice: LOXL-2 levels were significantly higher ($P \leq 0.01$) in the blood's serum of mice that were given N-methyl-N-nitrosourea compared to mice that were not given the chemical. A rise in LOXL-2 Numerous factors, such as oxidative stress and mitochondrial dysfunction, contribute to this increase. Elevated LOXL-2 levels in blood serum can serve as a biomarker to assess the severity of bladder cancer. The study found that natural products from the *Cordia myxa* plant, including oil, flavonoids, and alkaloids, decreased LOXL-2 levels in mice with N-methyl-N-nitrosourea-induced bladder cancer. Visit the control group. Treatment daily with natural products within 21 days at doses of 1.24, 61, and 7.0 mg/kg body weight resulted in a significant decrease ($P \leq 0.01$) in LOXL-2 concentrations compared to the control group, as shown in Table 3-15. The decrease in LOXL-2 levels caused by the natural metabolites derived from the *Cordia myxa* plant may be attributed to their ability to regenerate and repair cells in the bladder, thereby decreasing LOXL-2 levels. The LOXL-2 plant contains antioxidants such as fatty acids, phenolic compounds, vitamins, and minerals.Level of Glutathione (GSH) in mice: the study found a significant reduction ($P \leq 0.01$) in serum GSH levels in mice induced with N-methyl-N-nitrosourea compared to the control group. This decrease in GSH levels may be attributed to increased levels of lipid peroxides observed in mice exposed to N-methyl-N-nitrosourea. GSH, a non-enzymatic antioxidant, helps eliminate free radicals in the body. In its inactive form, GSSG interacts with proteins to form a compound called protein-glutathione disulfide. The formation of this compound stimulates the thiol transferase enzyme, reducing the amount of active GSH available (Pradhan *et al*., 2020). Natural products from the *Cordia Myxa* plant significantly increased. ($P \leq 0.01$) GSH concentration serum from the blood of mice with N-methyl-N-nitrosourea-induced bladder cancer disease, as presented in Table 3–18. GSH levels increased due to activation of the gamma-glutamyl transpeptidase enzyme or improved glutathione reductase activity. The enzyme converts oxidized glutathione to its reduced form, assisted by the NADPH enzyme (Santacroce *et al*., 2023). Mice's levels of malondialdehyde (MDA) The study found that N-methyl-N-nitrosourea-induced mice had significantly higher MDA levels were significantly higher (P

≤ 0.01) than in the control group is most likely due to higher ROS production. (Peña-Bautista *et al*., 2019) found compromised antioxidant defense mechanisms. Mice treated with natural products, including oil, flavonoids, and alkaloids, showed a significant drop (P ≤ 0.01) in comparison to the N-methyl-N-nitrosourea-induced group. Oils and flavonoids have the ability to eliminate free radicals and possess antioxidant mechanisms, which explains the significant decrease in MDA concentration. The activation of enzymatic antioxidants may lead to a reduction in free radicals and MDA levels in blood serum (Cosme *et al*., 2020). The level of tumor necrosis factor-alpha in mice was measured. The results of Table 3 showed the significant role of *Cordia Myxa* plant extracts in reducing the concentration of tumor necrosis factor in experimental animals infected with induced bladder cancer, as it is noted that the group of infected and untreated animals recorded the highest significant averages of 890.86 pg/ml, while *Cordia Myxa* plant extracts led to reducing the concentration. Tumor necrosis factor was 53.37 pg/mL, with a significant difference from the infected group. The flavonoid extract was more efficient by recording the lowest average concentration of tumor necrosis factor among the infected group, which was 53.37 pg/ml, while the healthy group reached the significantly lowest concentration, which was 24.99 pg/ml. Flavonoids are more effective because they contain kaempferol, which induces apoptosis (programmed cell death) in cancer cells by influencing mitochondrial pathways and increasing the activity of caspases, enzymes that play critical roles in apoptosis. This has been observed in various cancer types. (Amjad *et al*., 2022). Chronic inflammation has been linked to an increased risk of cancer. Kaempferol inhibits key inflammatory mediators and pathways, including NF-κB, that contribute to cancer initiation and progression. Molecules Kaempferol's Therapeutic Importance in Cancer Treatment via Cell Signaling Pathway Modulation (Qattan *et al*., 2022).

Level of glutathione peroxidase in mic The results of table (3) showed the significant role of *Cordia Myxa* plant extracts in increasing the concentration of glutathione peroxidase in experimental animals infected with induced bladder cancer, as it is noted that the group of infected and untreated animals recorded the lowest significant averages of 54.03 U/L, while *Cordia Myxa* plant extracts led to increasing the concentration of glutathione peroxidase to 116.06 U/L, with a significant difference from the infected group. The flavonoid extract was more efficient by recording the highest average concentration of glutathione peroxidase among the infected group, which was 116.06 U/L, while the healthy group reached the significantly highest concentration, which was 120.0 U/L. Flavonoids are more effective because they contain rutin, a flavonoid compound that belongs to the flavonoid class. Rutin is naturally occurring in many plants. Rutin is an antioxidant and anti-inflammatory with potential health benefits. It is regarded as an important compound for maintaining a healthy circulatory system. Rutin may help to relieve bloating and inflammation in the body. Rutin is considered a powerful antioxidant because it protects cells from free radical damage and contributes to the delay of the cellular aging process (Hosseinzadeh and Nassiri-Asl, 2014). A histological study was conducted on induced bladder cancer, examining the role of *Cordia myxa* plant extracts in its treatment.

Conclusions

The study found a link between increasing the concentration of lysyl oxidase 2 and bladder cancer. This study discovered that natural products isolated from the *Cordia myxa* plant have a therapeutic effect on bladder cancer, reducing inflammation and oxidative stress.

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