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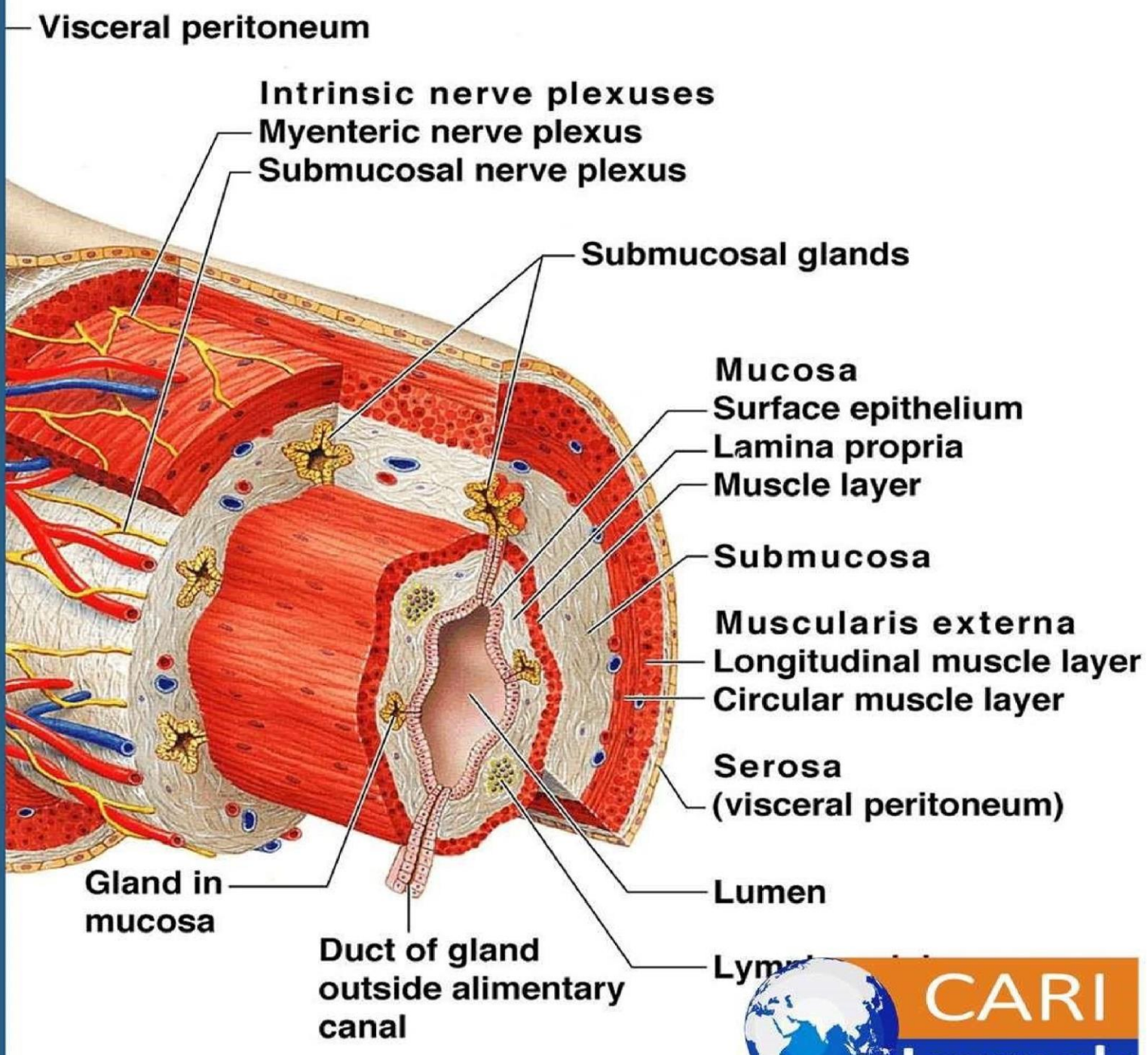

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Methanolic Extract of *Cannabis sativa* Ameliorates Rifampin-Induced Nephrotoxicity and Tubular Necrosis in Rats

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Abstract

Purpose: The study was conducted for the renal protective activity of the methanolic extract of *Cannabis sativa* against renal toxicity induced by rifampin injection in rats. This study selected *Cannabis sativa*, a member of the Cannabinaceae family, for its widespread use in treating gout, constipation, pain, insomnia, kidney inflammation, and other diseases. The results showed decreased levels of serum urea, creatinine, sodium (Na), and potassium (K) inhibited the induced toxicity. Histopathological examination revealed that the kidney was protected from the marked necrosis of renal tubules caused by rifampin.

Methodology: Forty Wister white (albino) rats weighing 100-150 gm were used. We produced renal toxicity by injecting rifampin i/p- at a dose rate of 0.8 mL/kg BW for 28 days, which led to nephrotoxicity. We administered the plant extract by simultaneously administering a methanolic extract of Cannabis sativa orally at dose rates of 300 and 600 mg/kg for 28 days.

Findings: Rifampin-induced nephrotoxicity increased potassium, urea, and creatinine levels while decreasing sodium levels. When Cannabis sativa is administered with rifampin, sodium concentrations increase. A decrease in potassium, urea, and creatinine accompanies the increase in sodium levels.

Unique Contribution to Theory, Practice, and Policy: Flavonoids may be responsible for the nephroprotective effect of Cannabis sativa extract; therefore, more research to identify the active component is necessary.

Keywords: *Cannabis Sativa, Rifampin Nephrotoxicity, Sodium, Potassium, Urea, Creatinine*

Introduction:

The kidneys are located in the back of the abdomen. Their functions include filtration of the blood as well as controlling the body's fluid balance and regulating the balance of electrolytes (Atef et al. 2015). One of the most common kidney disorders, nephrotoxicity, arises when the body cannot excrete an accumulation of drugs or toxins (Adebisi and KESINA, 2000). Several therapeutic drugs, such as antibiotics and chemotherapeutic agents, can harm the kidney, leading to acute renal failure or chronic nephritis. Aminoglycosides, Gentamycin, and non-steroid anti-inflammatory drugs (NSAIDs) can cause renal toxicity (Martinez et al., 2014). Chronic kidney disease is a progressive loss of function over months or years, unlike acute kidney failure, where the reduction in kidney function is present early. Drugs used in chemotherapy, such as cisplatin and carboplatin, will lead to renal toxicity as well as biological therapy, such as interleukin 2 or interferon α (Kurschel et al., 1991).

Mycobacterium tuberculosis causes tuberculosis, a serious disease. It is a growing international health concern (Abdelaol et al., 2009). Rifampicin is one of the drugs that treat tuberculosis. It is effective against M. tuberculosis, both intracellular and extracellular. Upon injection, the body widely distributes rifampicin, also known as rifampin. It is present in effective concentration in many organs and fluids, including cerebrospinal fluid (Pal et al., 2006). Oxidative stress represents the central key in the pathogenesis of drug-induced renal damage (Lopez et al., 2011). Therefore, the use of antioxidants could offer protection against drug-induced renal damage. People use herbal medicines and botanical supplements for both prevention and treatment. It's a common practice all over the world. Sudan is rich in plants used for herbal medicine (Elhardallan, 2011).

This study selects *Cannabis sativa* due to its widespread use in many countries for treating gout and constipation (Mariuana, 1975 and Brioofeman , Albazia, 2017) also use it to treat pain and insomnia. *Cannabis sativa* is an annual herbaceous flowering plant found in Eastern Asia as well as Africa. It is the most common type of cannabis plant. It originated in central Asia and later spread to Europe. It is known among many cultures for its medicinal potential. Hussain et al. (2015) use it to treat epilepsy. Samia (2011) conducted another study using Cannabis sativa to treat trypanosomosis, demonstrating strong trypanocidal activity.

Material and Methods:

Animals:

Forty Wister white (albino) rats weighing 100–150 gm were obtained from the Atomic Energy Research Institute. The animals were housed in the rats laboratory cages and maintained in a room with standard environmental conditions, including a controlled temperature of 27^{o}C, relative humidity of 60%, free access to water, and a rat feed formula consisting of corn powder and protein. We identified the animals by their color tail marks, and they were in good health. The animals were allowed 10 days as a preliminary adaptive period.

Plant material:

Cannabis sativa, belonging to the family Cannabinaceae, is a shrub with a strong fragrance that grows in different parts of the world. A forensic evidence institute obtained the whole plant of C. sativa from Niala, South Darfur (Sudan). The plant was cleaned and dried.

Preparation of plant extract:

The entire (C. sativa) 500 mg of the cleaned and dried plant was used. The the plant powder was successively extracted by soaking it in 80% methanol for 4 hours and then filtered it. We used a rotary evaporator to evaporate the filtrate. The residue was kept as stock for future use. The extraction was carried out according to Sukhdev et al.'s (2008) method.

Phytochemical screening

We used the Martinez & Valencia (1999) methods for phytochemical screening to find the active constituents of C. sativa.

Experimental design:

Group A: Serving as the control for 28 days.

Group B: Injected with rifampin i/p- at a dose rate of 0.8 mL/kg BW for 28 days to produce nephrotoxicity.

Group C: Injected with rifampin I/P- at a dose rate of 0.8 mL/kg BW in combination with 300 mL/kg of the plant methanolic extract for 28 days.

Group D: Injected with rifampin i/p- at a dose rate of 0.8 mL/kg BW, together with 600 mg/kg of the plant methanolic extract, for 28 days. The rats received the plant extract orally through nasogastric tubes.

Biochemical analysis:

We collected blood for serum analysis every week for 28 days. We determined the analysis of urea, creatinine, sodium, and potassium using the colorimetric method and commercial kits from Randox Laboratories LTD (U.K.).

Statistical analysis:

The data were analyzed using ANOVA. A probability of P0.05 and P<0.01 was considered statistically significant. Results were expressed as mean ± standard error.

Results:

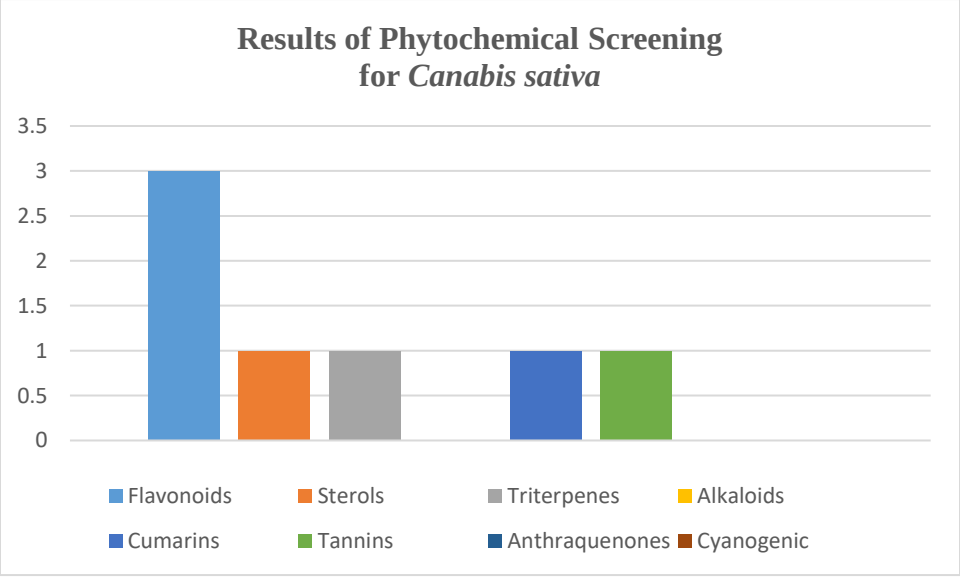
The phytochemical screening resulted in the presence of flavonoids with high concentration (+++) while sterols , Triterpenes, Cumarins and Tannins with adequate amounts.

Table (1): Result of phytochemical screening *Cannabis sativa*.

Test	Sonchus oleraceus
Flavonoids	+++
Sterols	+
Triterpenes	+
Alkaloids	-
Cumarins	+
Tannins	+
Anthraquenones	-
Cyanogenic	-

Plants have no anthraquinone, Cyanogenic, and Alkaloids

Key: + Trace ++ Moderate +++ High - Negative



The group that received rifampin alone showed a noticeable increase in urea and creatinine. The group that received both rifampin and 300 mg/kg of C. sativa methanolic extract showed a decrease in urea and creatinine levels (tables 1 and 2). At 600 mg/kg of the extract, the levels of urea and

creatinine gradually decreased until the end of the experiment, when they returned to their normal levels.

Table 3 presents the sodium results. Rifampin caused a significant decrease in the level of sodium ($P< 0.05$). Administration of C. sativa increased the concentration of sodium, and the increase was better with 600 mg/kg of the plant extract. We found that rifampin alone decreased the potassium concentration. When administered in conjunction with rifampin, the potassium concentration gradually increased until the end of the experimental period. We found that the high dose of the plant extract (600 mg/kg) marked the increase.

Table (2): Concentration of urea in rats injected with rifampin and treated with methanolic extract of *Cannabis sativa*

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
A	28.00 ±0.48	29.01± 4.12	29.33± 3.36	28.67± 2.36	29.00± 2.46
B	27.02 ±1.02	38.12±3.18	43.21±2.34	49.32±3.18°	52.21±1.53°°
C	20.26±1.20	36.64±2.30	38.67±2.33	46.02±2.48°	50.45±3.24
D	21.34±2.08	42.02±1.46	32.12±2.16	32.12± 2.16°	41.48±3.21°°

Group A: Serves as control.

Group B: Injected rifampin I/P 0.8 ml/kg BW

Group C: Injected rifampin together with 300 mg/kg BW of the plant extract.

Group D: Injected rifampin together with 600 mg/kg BW of the plant extract.

The difference was found to be significant (°$P<0.05$, (°°$P<0.01$) when compared with group B (control).

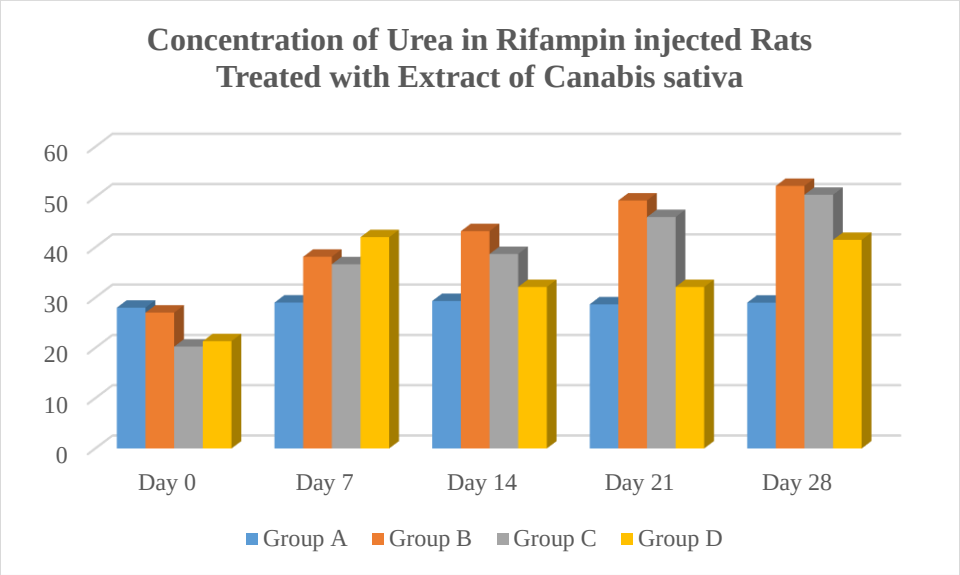


Table (3): Concentration of Creatinine in rats injected with rifampin and treated with methanolic extract of *Cannabis sativa*

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
A	0.33 ±0.33	0.63± 0.42	0.67± 0.07	0.83± 0.17	0.86± 0.32
B	0.47 ±0.07	2.53±0.42	3.57±0.38°	4.73±0.17°	5.40±0.32°°
C	0.53±0.03	0.57±0.03	1.83±0.15	1.82±0.06°	1.86±0.07°
D	0.48±0.02	0.47±0.07	1.72±0.25	1.68± 0.12	1.43±0.03°°

Group A: Serves as control.

Group B: Injected rifampin I/P 0.8 ml/kg BW

Group C: Injected rifampin together with 300 mg/kg BW of the plant extract.

Group D: Injected rifampin together with 600 mg/kg BW of the plant extract

The difference was found to be significant (°P<0.05, (°°P<0.01) when compared with group B (control).

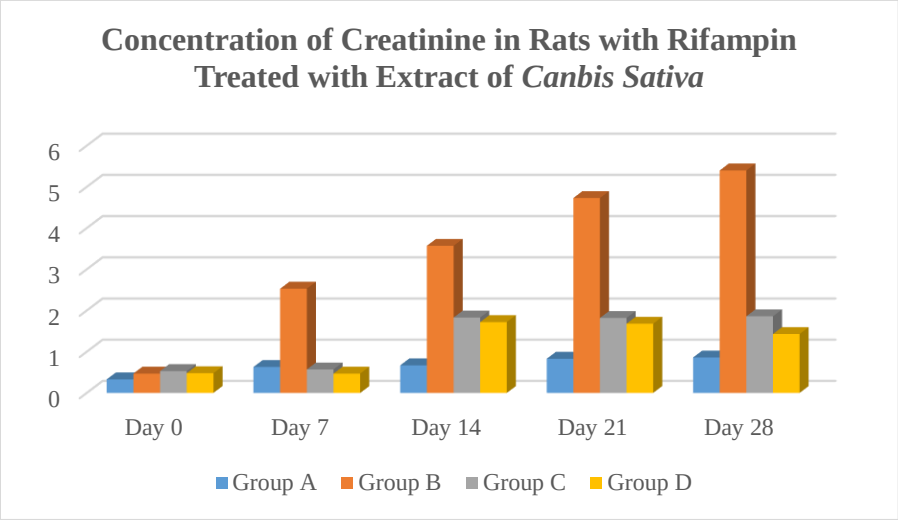


Table (4): Concentration of Sodium in rats injected with rifampin and treated with methanolic extract of *Cannabis sativa*

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
A	88.10 ±1.10	105.1± 1.70	86.51± 3.29	94.42± 0.06	128.00± 0.05
B	123.33 ±0.33	138.67±1.20	96.48±0.26	128.24±1.24°	150.67±0.88°°
C	136.00±0.88	143.67±0.33	124.24±0.84	136.67±0.86°	148.00±1.53°
D	126.24±1.21	138.67±1.20	98.62±0.64	132.24± 1.45	145.00±0.02°°

Group A: Serves as control.

Group B: Injected rifampin I/P 0.8 ml/kg BW

Group C: Injected rifampin together with 300 mg/kg BW of the plant extract.

Group D: Injected rifampin together with 600 mg/kg BW of the plant extract.

The difference was found to be significant (°P<0.05, (°°P<0.01) when compared with group B (control).

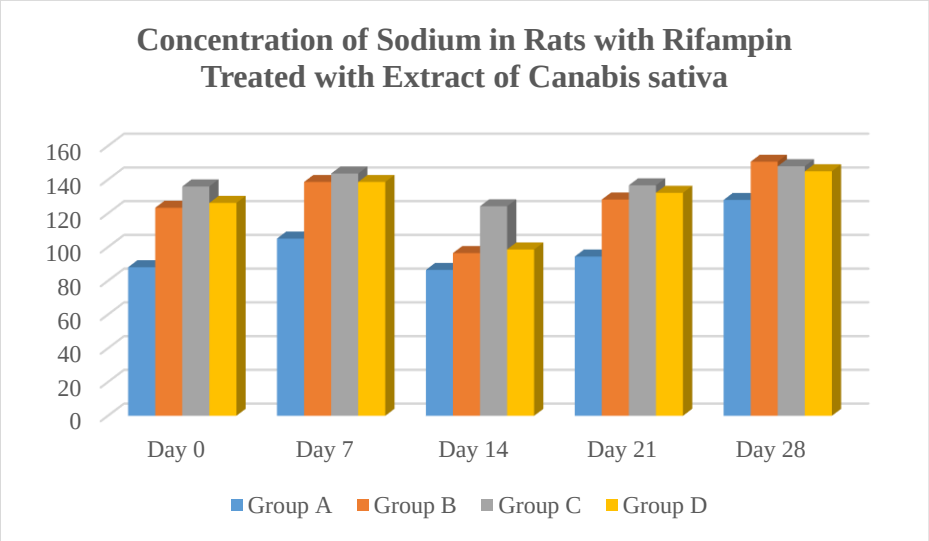


Table (5): Concentration of Potassium in rats injected with rifampin and treated with methanolic extract of *Cannabis sativa*

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
A	2.86 ±1.15	3.88± 0.3	4.73± 0.03	4.23± 0.09	4.03± 0.09
B	2.22 ±0.20	3.60±0.02	4.13±0.08	5.48±0.40°	4.03±0.04
C	3.14±0.16	3.53±0.09	4.26±0.03	5.23±0.13	5.83±0.21°
D	2.80±0.15	3.67±0.12	3.89±0.08	5.83± 0.21°	6.90±0.06°

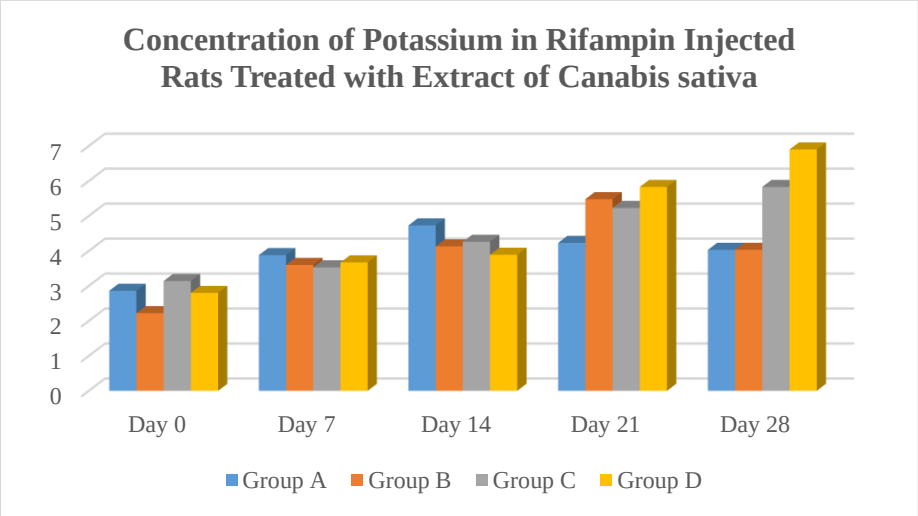
Group A: Serves as control.

Group B: Injected rifampin I/P 0.8 ml/kg BW

Group C: Injected rifampin together with 300 mg/kg BW of the plant extract.

Group D: Injected rifampin together with 600 mg/kg BW of the plant extract.

The difference was found to be significant (°P<0.05, (°°P<0.01) when compared with group B (control).



Histopathological changes:

In group B (Fig 1) The injection of rifampin resulted in severe hyperemia in the area surrounding the central veins. The kidney exhibited extensive vacuolar degeneration, and massive necrosis of cortical tubules and glomeruli with haemorrhage. Group C(Fig 2) (300 mg/kg) showed mild to moderate necrosis of glomeruli and tubules, numerous vacuoles in the cytoplasm, and most cells showed small vaculation and lymphocyte infiltration. Group D(Fig 3)(600 mg/kg) experienced mild to moderate vacuolar degeneration of cells, slight congestion and fatty changes and infiltration of inflammatory cells.

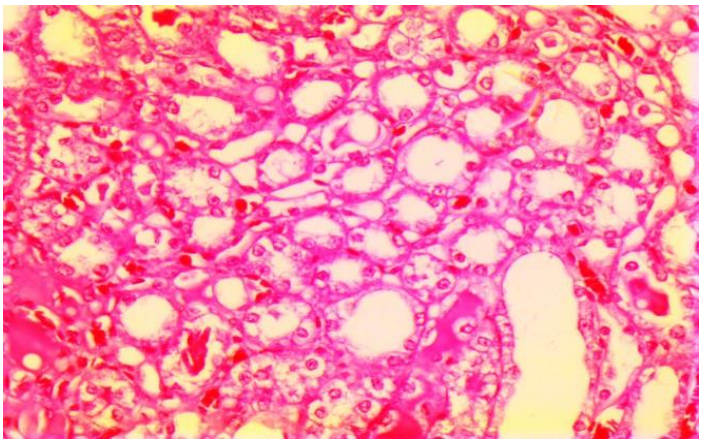


Figure 1. Histopathological changes in rat kidney tissue following rifampin administration.

The kidney section from a representative rat in Group B was treated with rifampin (0.8 ml/kg body weight) for 28 days. The image showed severe hyperemia, extensive vacuolar degeneration, massive necrosis, and hemorrhage, indicating significant damage to kidney structure and function induced by rifampin.

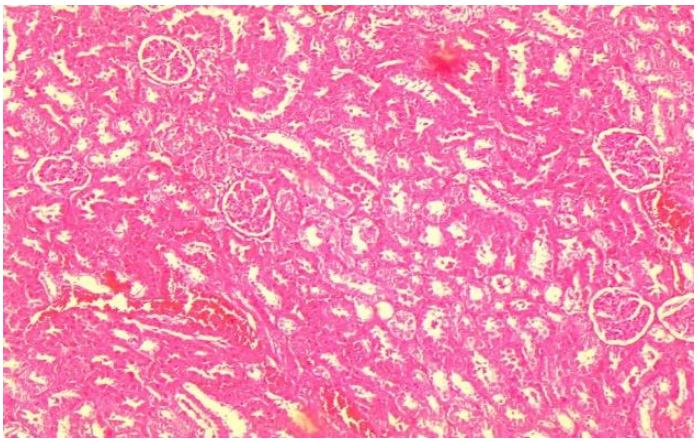


Figure 2: Histopathological changes in rat kidney tissue following rifampin administration and treatment with 300 mg/kg* Cannabis sativa *extract.

Kidney sections from a representative rat in Group C were treated with rifampin (0.8 ml/kg body weight) and Cannabis sativa extract (300 mg/kg body weight) for 28 days. The image shows reduced pathological changes compared to severe damage in Group B, with mild to moderate necrosis in glomeruli and tubules, numerous vacuoles, and lymphocyte infiltration, indicating an ongoing inflammatory response.

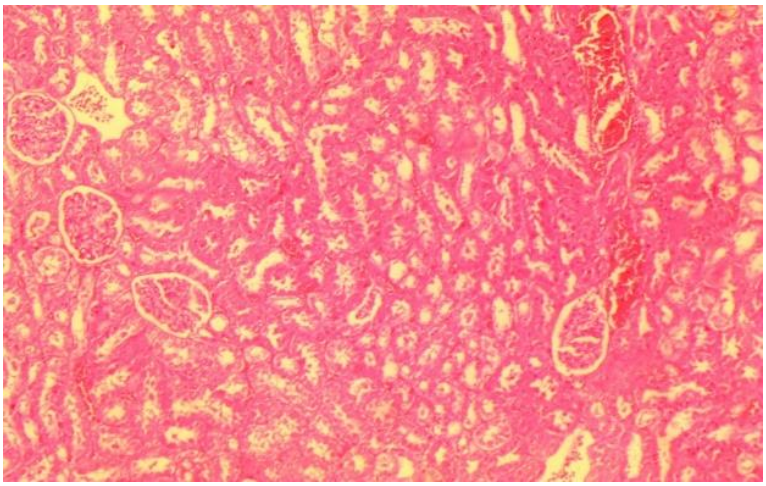


Figure 3. Histopathological changes in rat kidney tissue following rifampin administration and treatment with 600 mg/kg* Cannabis sativa *extract.

Kidney section from a representative rat in Group D, treated with rifampin (0.8 ml/kg body weight) and Cannabis sativa *extract (600 mg/kg body weight) for 28 days. The image shows improved histopathological features compared to Group C (300 mg/kg Cannabis sativa extract), with mild to moderate vacuolar degeneration, slight congestion, fatty changes, and less pronounced*

inflammation. The higher dose of Cannabis sativa extract provides greater protection against rifampin-induced nephrotoxicity.

Figure 4. **Cannabis sativa** ***plant.***

The image illustrates the distinctive morphology of the Cannabis sativa plant, characterized by its palmately compound leaves that feature serrated leaflets. This study uses the methanolic extract from this plant to investigate its nephroprotective properties against rifampin-induced renal toxicity.

Discussion:

Long-term use of rifampicin antibiotics has been known to induce nephrotoxicity. They can bind to 1-antigen on blood cells, leading to hemolysis and heme pigment release. Heme pigment is nephrotoxic to the renal tubules, which can lead to kidney injury. In this study, elevated serum urea and creatinine evidenced rifampin-induced renal injury and glomerular dysfunction. Researchers often regard these parameters as reliable markers of renal damage (Adebisi et al., 2000; Isleniet et al., 2011). The elevated serum markers of renal toxicity agree with Hashimi et al.'s (2017) study, which reported increased serum levels of urea and creatinine in albino rabbits following the administration of anti-tuberculosis drugs isoniazid and rifampin to induce nephrotoxicity. The administration of the methanolic extract of C. sativa resulted in a marked decrease in the concentrations of urea and creatinine, particularly with the high dose of 600 mg/kg of the plant extract. Renal toxicity can also lead to insufficient excretion of sodium and fluids from the body, which causes an increase in sodium levels in the blood. The injection of rifampin intraperitoneally into the rats demonstrates this fact.

Rifampin administration of the C. sativa extract increased the sodium concentration. The potassium level decreases as the sodium level rises. Potassium deficiency augmented the

detrimental impact of sodium excess. The injection of Rifampin into the rats led to a decrease in potassium levels, which subsequently increased upon administration of C. sativa extract. Yang et al. (2002), who reported a case of a man with nephrotoxicity from consuming A-braccolita for 5 months, agreed with the decrease in potassium concentration. The man showed hypokalemia and hypophosphatemia. These results were consistent with those of Hala et al. (2021), who used Fagonia cretica ethanolic extract as a nephroprotective against gentamycin injection in rats.

Based on the results obtained, it was clear that the methanolic extract of C. sativa has a protective effect on nephrotoxicity induced by rifampin. We observed the effect at a high dose of 600 mg/kg of the extract. The presence of flavonoids, which can act as antioxidants, may be responsible for the nephroprotective effect of C. sativa. Antioxidants are protective against oxidation and damage due to inflammation of the kidney (Koyner et al., 2008).

Conclusion:

We conclude that the methanolic extract of Cannabis sativa protects rats from rifampin-induced nephrotoxicity. The high dose of plant extract gave better results.

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