# Evaluation of Nephroprotective Activity of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi Fruits against Prednisolone Induced ADPKD in Experimental Rats 

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#### Abstract

The study evaluated the nephroprotective activity of hydroalcoholic extracts of Trachyspermum ammi leaves and Citrus paradisi fruits against prednisolone-induced ADPKD in experimental rats. The hydroalcoholic extracts were given orally at 100 and $200 \mathrm{mg} / \mathrm{kg}$ for a period of 28 days. Kidney function was evaluated by measuring serum and urine creatinine, urea, uric acid, and proteinuria. Histopathological studies were also conducted. Materials and methods: Animal model was used to evaluate the nephroprotective effect of hydroalcoholic extract from $T$. ammi leaves \& C. paradisi fruits against prednisolone induced ADPKD in Wistar rats. Keywords: Trachyspermum ammi leaves, Citrus pardisi fruit, Nephroprotective activity, ADPKD, Prednisolone. International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.2.25 How to cite this article: Varalakshmi TN, Chitra V. Evaluation of Nephroprotective Activity of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi Fruits against Prednisolone Induced ADPKD in Experimental Rats. International Journal of Drug Delivery Technology. 2023;13(2):627-643. Source of support: Nil. Conflict of interest: None


## INTRODUCTION

Autosomal dominant polycystic kidney disease, also known as ADPKD, is a genetic condition that manifests itself as the development of many cysts inside the kidneys it is the most prevalent form of hereditary renal cystic illness is ADPKD, which belongs to group disorders with a shared but unique pathophysiology and is defined by the formation of renal cysts and a wide range of extra renal symptoms. ${ }^{1}$ Other organs can also be affected by autosomal dominant polycystic kidney disease. This includes cysts in arachnoid membrane, liver, vas deferens, pancreas, and abdominal wall hernias, along with intracranial aneurysms, dolichoectasias, aortic valve dilatation, aneurysm, mitral valve prolapse, and aortic root dilatation. This, in turn, causes gradual kidney damage, which may ultimately result in end-stage renal disease (ESRD). ${ }^{2}$

## Epidemiology

It is a common hereditary condition that affects somewhere between 1 in 500 and 1 in 1000 people in the general population. ${ }^{3}$ Dalgaard1 found $1 / 1000$ Copenhagen instances. According to a study in Olmsted County, Minnesota, 1 in 400 to 1 in 1000 people are clinically diagnosed (including seen and estimated post-mortem cases). France, Wales, and Japan were lowest. ${ }^{2,3} 1 / 4033$. 6. Black Seychellois were seldom infected,
whereas 1 in 544 whites were 72,144 Americans undergo dialysis or kidney transplantation yearly. 8 ADPKD-related end stage renal disorder (ESRD) is rare among African Americans since they have a higher risk of ESRD overall. 8.7 and 6.9 US, European, and Japanese men and women had ESRD due to autosomal dominant polycystic kidney disease in 1998-2001. 7.8 and 6.0 in 1999 and 4.0 and 4.0 in 2000. Advanced illness is more common in males. High blood pressure, recurrent infections of the urinary system, kidney stones, and stomach discomfort are some of the symptoms that may or may not be present. Symptoms may range from moderate to severe. ${ }^{4}$ It is essential to make an early diagnosis of ADPKD and begin treatment immediately to stop the disease and avoid further consequences to one's health. Depending on the severity of the condition, the patient may be offered a variety of treatment options, including dietary and lifestyle changes, medication, and even surgery.

This illness may be treated with prednisolone-induced medication, resulting in faster recovery than prior techniques.

## Overview on Trachyspermum ammi and Citrus paradisi

However, a rise in recent years in the exploration of natural herbs has provided nephroprotection which also includes $T$. ammi leaves and C. parasdisi fruits, which are considered

[^0]hydroalcoholic extracts, also have nephroprotective effect in natural forms, which are tested in experimental forms are discussed below.

Several studies have evaluated the nephroprotective activity of the hydroalcoholic extract of $T$. ammi leaves. ${ }^{5}$ This extract is believed to possess various bioactive components like flavonoids, alkaloids, saponins, and phenolic compounds which are thought to contribute to its nephroprotective effect. ${ }^{6,7}$ C. paradisi, commonly known as grapefruit, is a citrus fruit belonging to the family Rutaceae Practitioners of conventional medicine often use it to address including kidney diseases. Several studies have reported anti-inflammation, antioxidant, and antimicrobial actions of the hydroalcoholic extract of C. paradisi. However, nephroprotective activity of the hydroalcoholic extract of C. paradisi has not yet been studied.

## MATERIALS AND METHODS

Prednisolone - Hydroalcoholic extract of T. ammi leaves and C. paradisi fruits - Rats (Sprague-Dawley strain)-Glucose and lipid profile kits - Kidney function tests (serum creatinine and urea) - Histopathological staining kits. ${ }^{8}$

## Preparation of Hydroalcoholic Extract

- The leaves of T. ammi and fruits of C. paradisi were shade dried separately.
- Then they were powdered coarsely and exposed to subcritical hydroalcoholic extraction.
- This powder was mixed with 70:30 water: ethanol.
- This mixture was exposed to temperature of 110 to $200^{\circ} \mathrm{C}$ for 5 to 20 min under high pressure ( $100 \pm 10 \mathrm{~atm}$ ).


## Nephroprotective Effects of Hydroalcoholic Extract on Prednisolone-induced ADPKD

1. The only chemically induced model of PKD that has been thoroughly studied is the corticosteroid-induced model in mice. ${ }^{8}$
2. Steroids create an irreversible type of cystic illness if given outside a very specific window during the first neonatal week.
3. Cysts were seen mostly in the collecting ducts, with limited extension to the proximal tubules and glomeruli.
4. The response should be re-assessed once the dosage is adjusted after a few weeks. If the response is still inadequate, the dosage can be increased further. If the response is excessive, the dosage can be decreased.
5. The apical location of the $\mathrm{Na}+/ \mathrm{P}+$ ATPase was shown to be similar to heritable PKD shown affect.
6. The severity of the sickness was determined by the background strain chosen and was impacted by environmental variables.
7. Inbred strains of one week old rats will be weighed and administered intramuscularly in the hindquarter using prednisolone ( $250 \mathrm{mg} / \mathrm{kg}$ body weight) using a 27-gauge needle.
8. Rats were maintained under the room temperature $24^{\circ} \mathrm{C}$ using a cycle of 12 hours of sunlight and 12 hours of dark.
9. Blood in the urine and inflammation near kidney are the symptoms of ADPKD.
10. The exact method through which steroids may cause cystic illness is uncertain. ${ }^{9}$

## Mechanism

Steroid metabolic defect using strategies that suppress the Ke 6 and lip steroid dehydrogenase gene expression. ${ }^{10}$ The expression of these genes has been shown to be controlled by the transcriptional regulators HNF-4 and HNF-1, both of which have been shown to be down-regulated in PKD.

## Experimental Design

For all groups Inbred strains of one week old rats will be weighed, then given an intramuscular injection of Prednisolone ( $250 \mathrm{mg} / \mathrm{kg}$ body weight). Body weight average in grams is shown in Table 1 later after ADPKD induction treatment was done

- "Group I (ordinary): Oral administration of normal saline for 14 days
- Group II (Prednisolone): single dose of Prednisolone 250 $\mathrm{mg} / \mathrm{kg}$ body weight
- Group III: single dose of Prednisolone $250 \mathrm{mg} / \mathrm{kg}$ body weight and HTAL $200 \mathrm{mg} / \mathrm{kg}$ orally for 14 days
- Group IV: single dose of Prednisolone $250 \mathrm{mg} / \mathrm{kg}$ body weight and HTAL $400 \mathrm{mg} / \mathrm{kg}$ orally for 14 days
- Group V single dose of Prednisolone $250 \mathrm{mg} / \mathrm{kg}$ body weight and HCPF $200 \mathrm{mg} / \mathrm{kg}$ orally for 14 days
- Group VI single dose of Prednisolone $250 \mathrm{mg} / \mathrm{kg}$ body weight and HCPF $400 \mathrm{mg} / \mathrm{kg}$ orally for 14 days
- Group VII single dose of Prednisolone $250 \mathrm{mg} / \mathrm{kg}$ body weight and Tolvaptan $3 \mathrm{mg} / \mathrm{kg}$ orally for 14 days".
All the data results collected were analysed using ANOVA with post-hoc testing performed using Dunnet's test. Blood was drawn from the hearts of ether-anesthetized rats on day 15 to analyse serum biochemical markers. After that, both kidneys were surgically removed and cleaned in regular saline solution. We homogenised the second kidney in PBS ( $10 \mathrm{mmol} / \mathrm{L}, \mathrm{pH}$ 7.4) while preserving the first kidney in $10 \%$ formalin for histological analysis. For this experiment, kidney homogenate was kept at $20^{\circ} \mathrm{C}$ in the fridge biological parameters (Tables 2 and 3).


## Histopathology

Selection of dose of the extract: $\mathrm{LD}_{50}$ was chosen in accordance with OECD recommendations for determining the dosage for biological assessment. According to OECD criteria, the $\mathrm{LD}_{50}$ of leaf \& fruit extract is $2,000 \mathrm{mg} / \mathrm{kg}$, with no evidence of

Table 1: Body weight average in grams

| Groups | Body weight average in grams |
| :--- | :--- |
| Group I | $68.4 \pm 1.25$ |
| Group II | $43.5 \pm 1.56$ |
| Group III | $61.2 \pm 1.98$ |
| Group IV | $67.3 \pm 1.34$ |
| Group V | $59.7 \pm 1.23$ |
| Group VI | $66.8 \pm 1.36$ |
| Group VII | $69.1 \pm 1.62$ |

Table 2: Effect of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi Fruits against BUN, Serum Creatinine, Blood urea, Serum Sodium, Serum Potassium, Total proteins levels

| Groups | BUN $(\mathrm{mg} / \mathrm{dL})$ | Serum Creatinine $(\mathrm{mg} / \mathrm{dL})$ | Blood Urea $(\mathrm{mg} / \mathrm{dL})$ | Serum sodium <br> $\mathrm{mmol} / \mathrm{L}$ | Serum potassium <br> $\mathrm{mmol} / \mathrm{L}$ | Total Proteins <br> $\mathrm{g} / \mathrm{dL}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Group I | $15.4 \pm 0.95$ | $0.49 \pm 0.45$ | $16.7 \pm 0.36$ | $147.1 \pm 0.57$ | $4.98 \pm 0.27$ | $7.1 \pm 0.67$ |
| Group II | $29.6 \pm 0.69$ | $1.29 \pm 0.76$ | $27.5 \pm 0.47$ | $162.4 \pm 0.78$ | $7.12 \pm 0.21$ | $4.7 \pm 0.75$ |
| Group III | $21.4 \pm 0.75$ | $0.64 \pm 0.54$ | $22.4 \pm 0.39$ | $151.5 \pm 0.65$ | $6.46 \pm 0.19$ | $5.9 \pm 0.69$ |
| Group IV | $15.6 \pm 0.84$ | $0.61 \pm 0.67$ | $17.6 \pm 0.52$ | $149.9 \pm 0.48$ | $5.17 \pm 0.21$ | $6.7 \pm 0.74$ |
| Group V | $19.7 \pm 0.92$ | $0.73 \pm 0.43$ | $19.8 \pm 0.52$ | $155.1 \pm 0.79$ | $5.75 \pm 0.29$ | $5.7 \pm 0.73$ |
| Group VI | $16.1 \pm 0.75$ | $0.51 \pm 0.67$ | $16.9 \pm 0.58$ | $146.9 \pm 0.64$ | $5.01 \pm 0.31$ | $6.8 \pm 0.64$ |
| Group VII | $15.3 \pm 0.82$ | $0.45 \pm 0.48$ | $17.1 \pm 0.61$ | $144.2 \pm 0.51$ | $4.81 \pm 0.26$ | $7.01 \pm 0.67$ |

Table 3: Effect of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi Fruits on SOD, Glutathione and Malondialdehyde levels

| Groups | SOD Unit/gram of tissue | Glutathione unit/gram of tissue | Malondialdehyde unit/gram of tissue |
| :--- | :--- | :--- | :--- |
| Group I | $9.32 \pm 1.27$ | $23.87 \pm 1.34$ | $5.98 \pm 1.43$ |
| Group II | $3.75 \pm 1.38$ | $10.54 \pm 1.49$ | $2.19 \pm 1.39$ |
| Group III | $6.23 \pm 1.14$ | $17.65 \pm 1.32$ | $3.96 \pm 1.36$ |
| Group IV | $8.47 \pm 1.02$ | $23.01 \pm 1.27$ | $6.01 \pm 1.42$ |
| Group V | $5.98 \pm 1.24$ | $15.92 \pm 1.41$ | $3.65 \pm 1.48$ |
| Group VI | $9.27 \pm 1.17$ | $21.32 \pm 1.34$ | $5.49 \pm 1.37$ |
| Group VII | $9.49 \pm 1.09$ | $24.01 \pm 1.25$ | $6.23 \pm 1.32$ |



Figure 1: histopathology of order: 1, 2, 3, 4, 5, 6, 7 under microscope 11
acute toxicity. The biological testing was done at dosages of 200 and $400 \mathrm{mg} / \mathrm{kg}$ body weight.

## Statistical Analysis

"The data was analysed by one-way analysis of variance (ANOVA) and Tukey's post-test for multiple comparisons was used for the post-study evaluation. A p-value $<0.05$ was considered to be statistically significant".

## Biochemical Parameters

The tests were conducted under the biochemical parameters mentioned below. ${ }^{12}$

- Serum parameters - Creatinine, Uric acid, Urea, Total protein levels
- Antioxidant parameters-SOD, LPO, Glutathione, Catalase etc.
- Liver function tests (LFT's) and BP assessment
- Kidney histopathology studies.
- Cell proliferation and viability Test
- Urine Analysis - blood, pus cells, pH etc.


## RESULTS

The findings of the analysis are given in the tabulated in Table 4 to 19 .

## Body weight

From the ANOVA Table 4 of the case blood weight we can say that there is a significant difference among the groups as the $p$-value is lesser than 0.05 .

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean differences and their significance values are tabulated in Table 5.
"From Table 2, it is justifiable that there is a significant difference of means of group-1 with respect to the 2,3 , and

| Table 4: Body weight |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| ANOVA |  |  |  |  |  |
| Values | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
|  | 6 | 373.655 | 135.004 | .000 |  |
| Between <br> groups | 2241.928 |  |  |  |  |
| Within <br> groups | 58.123 | 21 | 2.768 |  |  |
| Total | 2300.051 | 27 |  |  |  |

group-5, respectively. On the other hand, 4, 6, and group-7 don't have any significant differences.

From Table 2, it is justifiable that there is a significant difference of means of group-2 with respect to $1,3,4,5,6$, group-7.

From Table 2, it is justifiable that there is a significant difference of the means of group- 3 with respect to $1,2,4,6$, group- 7 , respectively. On the other hand, group-5 doesn't have any significant difference.

From Table 2 it is justifiable that there is a significant difference of means of group-4 with respect to 2,3 , group5, respectively. On the other hand, 1, 6, 7 don't have any significant difference.

From Table it is justifiable that there is a significant difference of means of group- 5 with respect to $1,2,4,6$, group7 , respectively. On the other hand, group- 3 doesn't have any significant difference.

From Table it is justifiable that there is a significant difference of means of group- 6 with respect to the 2,3 , group-

Table 5: Mean differences and their significance values

| Multiple comparisons |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dependent variable: values |  |  |  |  |  |  |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. Error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | 27.61413* | 1.17638 | . 000 | 23.7900 | 31.4383 |
|  | 3 | $7.94960^{*}$ | 1.17638 | . 000 | 4.1254 | 11.7738 |
|  | 4 | 1.68695 | 1.17638 | . 778 | -2.1372 | 5.5111 |
|  | 5 | 10.65884* | 1.17638 | . 000 | 6.8347 | 14.4830 |
|  | 6 | 3.25878 | 1.17638 | . 129 | -. 5654 | 7.0829 |
|  | 7 | 1.36046 | 1.17638 | . 902 | -2.4637 | 5.1846 |
| 2 | 1 | -27.61413** | 1.17638 | . 000 | -31.4383 | -23.7900 |
|  | 3 | -19.66453** | 1.17638 | . 000 | -23.4887 | -15.8404 |
|  | 4 | -25.92719** | 1.17638 | . 000 | -29.7513 | -22.1030 |
|  | 5 | -16.95530** | 1.17638 | . 000 | -20.7795 | -13.1311 |
|  | 6 | -24.35536** | 1.17638 | . 000 | -28.1795 | -20.5312 |
|  | 7 | -26.25367* | 1.17638 | . 000 | -30.0778 | -22.4295 |
| 3 | 1 | $-7.94960^{*}$ | 1.17638 | . 000 | -11.7738 | -4.1254 |
|  | 2 | 19.66453* | 1.17638 | . 000 | 15.8404 | 23.4887 |
|  | 4 | -6.26266* | 1.17638 | . 000 | -10.0868 | -2.4385 |
|  | 5 | 2.70923 | 1.17638 | . 288 | -1.1149 | 6.5334 |
|  | 6 | -4.69083* | 1.17638 | . 010 | -8.5150 | -. 8667 |
|  | 7 | -6.58914* | 1.17638 | . 000 | -10.4133 | -2.7650 |
| 4 | 1 | -1.68695 | 1.17638 | . 778 | -5.5111 | 2.1372 |
|  | 2 | 25.92719* | 1.17638 | . 000 | 22.1030 | 29.7513 |
|  | 3 | $6.26266{ }^{*}$ | 1.17638 | . 000 | 2.4385 | 10.0868 |
|  | 5 | $8.97189^{*}$ | 1.17638 | . 000 | 5.1477 | 12.7960 |
|  | 6 | 1.57183 | 1.17638 | . 828 | -2.2523 | 5.3960 |
|  | 7 | -. 32648 | 1.17638 | 1.000 | -4.1506 | 3.4977 |
| 5 | 1 | $-10.65884^{*}$ | 1.17638 | . 000 | -14.4830 | -6.8347 |
|  | 2 | $16.95530^{*}$ | 1.17638 | . 000 | 13.1311 | 20.7795 |
|  | 3 | -2.70923 | 1.17638 | . 288 | -6.5334 | 1.1149 |
|  | 4 | -8.97189** | 1.17638 | . 000 | -12.7960 | -5.1477 |
|  | 6 | $-7.40006^{*}$ | 1.17638 | . 000 | -11.2242 | -3.5759 |
|  | 7 | -9.29837* | 1.17638 | . 000 | -13.1225 | -5.4742 |
|  |  |  |  |  |  | Table contin... |


| 6 | 1 | -3.25878 |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
|  | 2 | $24.35536^{*}$ | 1.17638 | .129 | -7.0829 | .5654 |
|  | 3 | $4.69083^{*}$ | 1.17638 | .000 | 20.5312 | 28.1795 |
|  | 4 | -1.57183 | 1.17638 | .010 | .8667 | 8.5150 |
|  | 5 | $7.40006^{*}$ | 1.17638 | .828 | -5.3960 | 2.2523 |
|  | 7 | -1.89831 | 1.17638 | .000 | 3.5759 | 11.2242 |
|  | 1 | -1.36046 | 1.17638 | .676 | -5.7225 | 1.9258 |
|  | 2 | $26.25367^{*}$ | 1.17638 | .902 | -5.1846 | 2.4637 |
|  | 3 | $6.58914^{*}$ | 1.17638 | .000 | 22.4295 | 30.0778 |
|  | 4 | .32648 | 1.17638 | .000 | 2.7650 | 10.4133 |
|  | 5 | $9.29837^{*}$ | 1.17638 | 1.000 | -3.4977 | 4.1506 |
|  | 6 | 1.89831 | 1.17638 | .000 | 5.4742 | 13.1225 |

*.The mean difference is significant at the 0.05 level.

Table 6: Effect of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi fruits on body weight

| ANOVA |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :---: |
|  |  |  |  |  | Sig. |
| Values of squares | $d f$ | Mean square | $F$ | 218.970 | .000 |
| Between <br> groups | 646.797 | 6 | 107.800 |  |  |
| Within <br> groups <br> Total | 10.338 | 21 | .492 |  |  |

5, respectively. On the other hand, $1,4,7$ doesn't have any significant difference.
From Table it is justifiable that there is a significant difference of means of group-7 with respect to the $2,3,5$, respectively. On the other hand, 1,4 , group- 6 doesn't have any significant difference".

## Blood Urea Nitrogen

"From the above ANOVAa table of the case Blood urea nitrogen, we can say that there is a significant difference among the groups as the $p$-value is lesser than 0.05 ".

Table 7: Multiple Comparisons

| Dependent variable: values |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. Error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | $-14.17825^{*}$ | . 49614 | . 000 | -15.7911 | -12.5654 |
|  | 3 | $-5.50922^{*}$ | . 49614 | . 000 | -7.1221 | -3.8964 |
|  | 4 | -. 35541 | . 49614 | . 990 | -1.9682 | 1.2574 |
|  | 5 | -4.88227* | . 49614 | . 000 | -6.4951 | -3.2694 |
|  | 6 | -. 84341 | . 49614 | . 623 | -2.4562 | . 7694 |
|  | 7 | . 06879 | . 49614 | 1.000 | -1.5440 | 1.6816 |
| 2 | 1 | 14.17825* | . 49614 | . 000 | 12.5654 | 15.7911 |
|  | 3 | 8.66903* | . 49614 | . 000 | 7.0562 | 10.2819 |
|  | 4 | 13.82284* | . 49614 | . 000 | 12.2100 | 15.4357 |
|  | 5 | 9.29598* | . 49614 | . 000 | 7.6831 | 10.9088 |
|  | 6 | $13.33484^{*}$ | . 49614 | . 000 | 11.7220 | 14.9477 |
|  | 7 | 14.24704* | . 49614 | . 000 | 12.6342 | 15.8599 |
| 3 | 1 | 5.50922* | . 49614 | . 000 | 3.8964 | 7.1221 |
|  | 2 | -8.66903* | . 49614 | . 000 | -10.2819 | -7.0562 |
|  | 4 | 5.15382* | . 49614 | . 000 | 3.5410 | 6.7666 |
|  | 5 | . 62695 | . 49614 | . 860 | -. 9859 | 2.2398 |
|  | 6 | 4.66581* | . 49614 | . 000 | 3.0530 | 6.2786 |
|  | 7 | 5.57802* | . 49614 | . 000 | 3.9652 | 7.1908 |


| 4 | 1 | . 35541 | . 49614 | . 990 | -1.2574 | 1.9682 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 2 | $-13.82284^{*}$ | . 49614 | . 000 | -15.4357 | -12.2100 |
|  | 3 | $-5.15382^{*}$ | . 49614 | . 000 | -6.7666 | -3.5410 |
|  | 5 | $-4.52687^{*}$ | . 49614 | . 000 | -6.1397 | -2.9140 |
|  | 6 | -. 48800 | . 49614 | . 952 | -2.1008 | 1.1248 |
|  | 7 | . 42420 | . 49614 | . 975 | -1.1886 | 2.0370 |
| 5 | 1 | $4.88227^{*}$ | . 49614 | . 000 | 3.2694 | 6.4951 |
|  | 2 | $-9.29598^{*}$ | . 49614 | . 000 | -10.9088 | -7.6831 |
|  | 3 | -. 62695 | . 49614 | . 860 | -2.2398 | . 9859 |
|  | 4 | $4.52687^{*}$ | . 49614 | . 000 | 2.9140 | 6.1397 |
|  | 6 | $4.03886^{*}$ | . 49614 | . 000 | 2.4260 | 5.6517 |
|  | 7 | $4.95106^{*}$ | . 49614 | . 000 | 3.3382 | 6.5639 |
| 6 | 1 | . 84341 | . 49614 | . 623 | -. 7694 | 2.4562 |
|  | 2 | $-13.33484^{*}$ | . 49614 | . 000 | -14.9477 | -11.7220 |
|  | 3 | $-4.66581^{*}$ | . 49614 | . 000 | -6.2786 | -3.0530 |
|  | 4 | . 48800 | . 49614 | . 952 | -1.1248 | 2.1008 |
|  | 5 | $-4.03886^{*}$ | . 49614 | . 000 | -5.6517 | -2.4260 |
|  | 7 | . 91220 | . 49614 | . 539 | -. 7006 | 2.5250 |
| 7 | 1 | $\text { -. } 06879$ | . 49614 | 1.000 | -1.6816 | 1.5440 |
|  | 2 | $-14.24704^{*}$ | . 49614 | . 000 | -15.8599 | -12.6342 |
|  | 3 | -5.57802* | . 49614 | . 000 | -7.1908 | -3.9652 |
|  | 4 | -. 42420 | . 49614 | . 975 | -2.0370 | 1.1886 |
|  | 5 | $-4.95106^{*}$ | . 49614 | . 000 | -6.5639 | -3.3382 |
|  | 6 | -. 91220 | . 49614 | . 539 | -2.5250 | . 7006 |

*. The mean difference is significant at the 0.05 level.

Table 8: Blood Urea

| ANOVA |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
| Values | 342.370 | 6 | 57.062 | 190.858 | .000 |
| Between <br> groups |  |  |  |  |  |
| Within <br> groups <br> Total | 6.278 | 21 | .299 |  |  |

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean differences and their significance values are tabulated in Table 3.
"From Table 3, it is justifiable that there is a significant difference of means of group-1 with respect to 2,3 and group5 , respectively. On the other hand, 4,6 , group- 7 doesn't have any significant difference.

From Table 3, it is justifiable that there is a significant difference of the means of group-2 with respect to group-1, $3,4,5,6$, group-7.

Table 9: Effect of Hydroalcoholic Extract of Trachyspermum ammi leaves and Citrus paradisi fruits on body weight

| Multiple comparisons |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dependent variable: values |  |  |  |  |  |  |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. Error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | -10.14176* | . 38664 | . 000 | -11.3986 | -8.8849 |
|  | 3 | -4.69100* | . 38664 | . 000 | -5.9479 | -3.4341 |
|  | 4 | -. 38619 | . 38664 | . 949 | -1.6431 | . 8707 |
|  | 5 | -2.96650* | . 38664 | . 000 | -4.2234 | -1.7096 |
|  | 6 | -. 10790 | . 38664 | 1.000 | -1.3648 | 1.1490 |
|  | 7 | -. 08194 | . 38664 | 1.000 | -1.3388 | 1.1749 |

Table contin...

| 2 | 1 | $10.1417{ }^{*}$ | . 38664 | . 000 | 8.8849 | 11.3986 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 3 | $5.45076{ }^{*}$ | . 38664 | . 000 | 4.1939 | 6.7076 |
|  | 4 | $9.75556^{*}$ | . 38664 | . 000 | 8.4987 | 11.0124 |
|  | 5 | $7.17526^{*}$ | . 38664 | . 000 | 5.9184 | 8.4321 |
|  | 6 | $10.03385^{*}$ | . 38664 | . 000 | 8.7770 | 11.2907 |
|  | 7 | $10.05982^{*}$ | . 38664 | . 000 | 8.8030 | 11.3167 |
| 3 | 1 | $4.69100^{*}$ | . 38664 | . 000 | 3.4341 | 5.9479 |
|  | 2 | -5.45076* | . 38664 | . 000 | -6.7076 | -4.1939 |
|  | 4 | $4.30480^{*}$ | . 38664 | . 000 | 3.0479 | 5.5617 |
|  | 5 | $1.72449^{*}$ | . 38664 | . 003 | . 4676 | 2.9814 |
|  | 6 | $4.58309^{*}$ | . 38664 | . 000 | 3.3262 | 5.8400 |
|  | 7 | $4.60906^{*}$ | . 38664 | . 000 | 3.3522 | 5.8659 |
| 4 | 1 | . 38619 | . 38664 | . 949 | -. 8707 | 1.6431 |
|  | 2 | $-9.75556^{*}$ | . 38664 | . 000 | -11.0124 | -8.4987 |
|  | 3 | $-4.30480^{*}$ | . 38664 | . 000 | -5.5617 | -3.0479 |
|  | 5 | $-2.58031^{*}$ | . 38664 | . 000 | -3.8372 | -1.3234 |
|  | 6 | . 27829 | . 38664 | . 990 | -. 9786 | 1.5352 |
|  | 7 | . 30426 | . 38664 | . 984 | -. 9526 | 1.5611 |
| 5 | 1 | $2.96650^{*}$ | . 38664 | . 000 | 1.7096 | 4.2234 |
|  | 2 | $-7.17526^{*}$ | . 38664 | . 000 | -8.4321 | -5.9184 |
|  | 3 | -1.72449** | . 38664 | . 003 | -2.9814 | -. 4676 |
|  | 4 | $2.58031^{*}$ | . 38664 | . 000 | 1.3234 | 3.8372 |
|  | 6 | $2.85860^{*}$ | . 38664 | . 000 | 1.6017 | 4.1155 |
|  | 7 | $2.88457{ }^{*}$ | . 38664 | . 000 | 1.6277 | 4.1414 |
| 6 | 1 | . 10790 | . 38664 | 1.000 | -1.1490 | 1.3648 |
|  | 2 | $-10.03385^{*}$ | . 38664 | . 000 | -11.2907 | -8.7770 |
|  | 3 | -4.58309* | . 38664 | . 000 | -5.8400 | -3.3262 |
|  | 4 | -. 27829 | . 38664 | . 990 | -1.5352 | . 9786 |
|  | 5 | $-2.85860^{*}$ | . 38664 | . 000 | -4.1155 | -1.6017 |
|  | 7 | . 02597 | . 38664 | 1.000 | -1.2309 | 1.2828 |
| 7 | 1 | . 08194 | . 38664 | 1.000 | -1.1749 | 1.3388 |
|  | 2 | $-10.05982^{*}$ | . 38664 | . 000 | -11.3167 | -8.8030 |
|  | 3 | $-4.60906{ }^{*}$ | . 38664 | . 000 | -5.8659 | -3.3522 |
|  | 4 | -. 30426 | . 38664 | . 984 | -1.5611 | . 9526 |
|  | 5 | -2.88457* | . 38664 | . 000 | -4.1414 | -1.6277 |
|  | 6 | -. 02597 | . 38664 | 1.000 | -1.2828 | 1.2309 |

*. The mean difference is significant at the 0.05 level.

Table 10: Effect of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi fruits on multiple comparisons

| ANOVA |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
| Values | 6 | 138.127 | 337.271 | .000 |  |
| Between <br> groups | 828.759 | 21 | .410 |  |  |
| Within <br> groups <br> Total | 8.600 | 837.360 | 27 |  |  |

From Table 3, it is justifiable that there is a significant difference of the means of group-3 with respect to $1,2,4,6$, group- 7 , respectively. On the other hand, group- 5 doesn't have any significant difference.

From Table 3, it is justifiable that there is a significant difference of means of group- 4 with respect to the 2,3 , group- 5 , respectively. On the other hand, 1,6 , group- 7 don't have any significant difference.

From Table 3, it is justifiable that there is a significant difference of means of group-5 with respect to $1,2,4,6$, group7, respectively. On the other hand, group-3 doesn't have any significant difference.

Evaluation of Nephroprotective Activity of Trachyspermum ammi Leaves and Citrus paradisi Fruits

| Table 11: Effect of Hydroalcoholic Extract of Trachyspermum ammi leaves and Citrus paradisi fruits on body weight |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Multiple comparisons |  |  |  |  |  |  |
| Dependent variable: values |  |  |  |  |  |  |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | -14.35335** | . 45252 | . 000 | -15.8244 | -12.8823 |
|  | 3 | -4.16935** | . 45252 | . 000 | -5.6404 | -2.6983 |
|  | 4 | $-2.98060^{*}$ | . 45252 | . 000 | -4.4516 | -1.5096 |
|  | 5 | -7.65560* | . 45252 | . 000 | -9.1266 | -6.1846 |
|  | 6 | . 19926 | . 45252 | . 999 | -1.2718 | 1.6703 |
|  | 7 | 3.25865* | . 45252 | . 000 | 1.7876 | 4.7297 |
| 2 | 1 | $14.35335^{*}$ | . 45252 | . 000 | 12.8823 | 15.8244 |
|  | 3 | 10.18399** | . 45252 | . 000 | 8.7130 | 11.6550 |
|  | 4 | $11.37275^{*}$ | . 45252 | . 000 | 9.9017 | 12.8438 |
|  | 5 | 6.69775* | . 45252 | . 000 | 5.2267 | 8.1688 |
|  | 6 | $14.55261^{*}$ | . 45252 | . 000 | 13.0816 | 16.0236 |
|  | 7 | $17.61200^{*}$ | . 45252 | . 000 | 16.1410 | 19.0830 |
| 3 | 1 | 4.16935* | . 45252 | . 000 | 2.6983 | 5.6404 |
|  | 2 | -10.18399* | . 45252 | . 000 | -11.6550 | -8.7130 |
|  | 4 | 1.18875 | . 45252 | . 168 | -. 2823 | 2.6598 |
|  | 5 | -3.48624* | . 45252 | . 000 | -4.9573 | -2.0152 |
|  | 6 | $4.36862^{*}$ | . 45252 | . 000 | 2.8976 | 5.8396 |
|  | 7 | 7.42801* | . 45252 | . 000 | 5.9570 | 8.8990 |
| 4 | 1 | $2.98060^{*}$ | . 45252 | . 000 | 1.5096 | 4.4516 |
|  | 2 | -11.37275* | . 45252 | . 000 | -12.8438 | -9.9017 |
|  | 3 | -1.18875 | . 45252 | . 168 | -2.6598 | . 2823 |
|  | 5 | -4.67500* | . 45252 | . 000 | -6.1460 | -3.2040 |
|  | 6 | $3.17986^{*}$ | . 45252 | . 000 | 1.7088 | 4.6509 |
|  | 7 | 6.23925* | . 45252 | . 000 | 4.7682 | 7.7103 |
| 5 | 1 | 7.65560* | . 45252 | . 000 | 6.1846 | 9.1266 |
|  | 2 | -6.69775* | . 45252 | . 000 | -8.1688 | -5.2267 |
|  | 3 | $3.48624^{*}$ | . 45252 | . 000 | 2.0152 | 4.9573 |
|  | 4 | $4.67500^{*}$ | . 45252 | . 000 | 3.2040 | 6.1460 |
|  | 6 | $7.85486^{*}$ | . 45252 | . 000 | 6.3838 | 9.3259 |
|  | 7 | 10.91425* | . 45252 | . 000 | 9.4432 | 12.3853 |
| 6 | 1 | -. 19926 | . 45252 | . 999 | -1.6703 | 1.2718 |
|  | 2 | -14.55261* | . 45252 | . 000 | -16.0236 | -13.0816 |
|  | 3 | $-4.36862^{*}$ | . 45252 | . 000 | -5.8396 | -2.8976 |
|  | 4 | -3.17986* | . 45252 | . 000 | -4.6509 | -1.7088 |
|  | 5 | -7.85486* | . 45252 | . 000 | -9.3259 | -6.3838 |
|  | 7 | 3.05939** | . 45252 | . 000 | 1.5884 | 4.5304 |
| 7 | 1 | $-3.25865^{*}$ | . 45252 | . 000 | -4.7297 | -1.7876 |
|  | 2 | -17.61200** | . 45252 | . 000 | -19.0830 | -16.1410 |
|  | 3 | -7.42801* | . 45252 | . 000 | -8.8990 | -5.9570 |
|  | 4 | -6.23925* | . 45252 | . 000 | -7.7103 | -4.7682 |
|  | 5 | -10.91425* | . 45252 | . 000 | -12.3853 | -9.4432 |
|  | 6 | $-3.05939^{*}$ | . 45252 | . 000 | -4.5304 | -1.5884 |

*. The mean difference is significant at the 0.05 level.

Table 12: Effect of Hydroalcoholic Extract of Trachyspermum ammi leaves and Citrus paradisi fruits on body weight

| ANOVA |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Values |  |  |  |  |  |
|  | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
| Between <br> groups | 18.897 | 6 | 3.150 | 48.587 | .000 |
| Within <br> groups | 1.361 | 21 | .065 |  |  |
| Total | 20.259 | 27 |  |  |  |

From Table 3, it is justifiable that there is a significant difference of means of group-6 with respect to 2,3 and group-5,
respectively. On the other hand, 1,4 , group- 7 don't have any significant difference.
From Table 3, it is justifiable that there is a significant difference of means of group- 7 with respect to 2,3 and group- 5 , respectively. On the other hand, 1,4 , group- 6 don't have any significant difference".

## Blood Urea

"From the above anova table of the case Blood urea we can say that there is a significant difference among the groups as the p- value is lesser than $0.05^{\prime \prime}$.

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean

Table 13: Effect of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi fruits on multiple comparisons of Serum potassium

| Serum potassium |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Multiple comparisons |  |  |  |  |  |  |
| Dependent variable: values |  |  |  |  |  |  |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | -2.06578* | . 18003 | . 000 | -2.6510 | -1.4805 |
|  | 3 | -1.41852* | . 18003 | . 000 | -2.0038 | -. 8333 |
|  | 4 | -. 02378 | . 18003 | 1.000 | -. 6090 | . 5615 |
|  | 5 | -. $70569^{*}$ | . 18003 | . 012 | -1.2909 | -. 1204 |
|  | 6 | . 06145 | . 18003 | 1.000 | -. 5238 | . 6467 |
|  | 7 | . 28364 | . 18003 | . 698 | -. 3016 | . 8689 |
| 2 | 1 | $2.06578^{*}$ | . 18003 | . 000 | 1.4805 | 2.6510 |
|  | 3 | . $64726^{*}$ | . 18003 | . 024 | . 0620 | 1.2325 |
|  | 4 | $2.04200^{*}$ | . 18003 | . 000 | 1.4568 | 2.6272 |
|  | 5 | $1.36009^{*}$ | . 18003 | . 000 | . 7749 | 1.9453 |
|  | 6 | $2.12723^{*}$ | . 18003 | . 000 | 1.5420 | 2.7125 |
|  | 7 | $2.34942 *$ | . 18003 | . 000 | 1.7642 | 2.9347 |
| 3 | 1 | $1.41852^{*}$ | . 18003 | . 000 | . 8333 | 2.0038 |
|  | 2 | -.64726* | . 18003 | . 024 | -1.2325 | -. 0620 |
|  | 4 | $1.39474^{*}$ | . 18003 | . 000 | . 8095 | 1.9800 |
|  | 5 | . $71284^{*}$ | . 18003 | . 011 | . 1276 | 1.2981 |
|  | 6 | $1.47997^{*}$ | . 18003 | . 000 | . 8947 | 2.0652 |
|  | 7 | $1.70217^{*}$ | . 18003 | . 000 | 1.1169 | 2.2874 |
| 4 | 1 | . 02378 | . 18003 | 1.000 | -. 5615 | . 6090 |
|  | 2 | -2.04200* | . 18003 | . 000 | -2.6272 | -1.4568 |
|  | 3 | -1.39474* | . 18003 | . 000 | -1.9800 | -. 8095 |
|  | 5 | -.68191* | . 18003 | . 016 | -1.2672 | -. 0967 |
|  | 6 | . 08523 | . 18003 | . 999 | -. 5000 | . 6705 |
|  | 7 | . 30742 | . 18003 | . 619 | -. 2778 | . 8927 |
| 5 | 1 | . $70569^{*}$ | . 18003 | . 012 | . 1204 | 1.2909 |
|  | 2 | -1.36009* | . 18003 | . 000 | -1.9453 | -. 7749 |
|  | 3 | -. $71284^{*}$ | . 18003 | . 011 | -1.2981 | -. 1276 |
|  | 4 | .68191* | . 18003 | . 016 | . 0967 | 1.2672 |
|  | 6 | . $76714{ }^{*}$ | . 18003 | . 005 | . 1819 | 1.3524 |
|  | 7 | . $98933{ }^{*}$ | . 18003 | . 000 | . 4041 | 1.5746 |

Table contin...

| 6 | 1 | -. 06145 | . 18003 | 1.000 | -. 6467 | . 5238 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 2 | -2.12723* | . 18003 | . 000 | -2.7125 | -1.5420 |
|  | 3 | -1.47997* | . 18003 | . 000 | -2.0652 | -. 8947 |
|  | 4 | -. 08523 | . 18003 | . 999 | -. 6705 | . 5000 |
|  | 5 | $-.76714^{*}$ | . 18003 | . 005 | -1.3524 | -. 1819 |
|  | 7 | . 22219 | . 18003 | . 873 | -. 3630 | . 8074 |
| 7 | 1 | -. 28364 | . 18003 | . 698 | -. 8689 | . 3016 |
|  | 2 | $-2.34942^{*}$ | . 18003 | . 000 | -2.9347 | -1.7642 |
|  | 3 | $-1.70217^{*}$ | . 18003 | . 000 | -2.2874 | -1.1169 |
|  | 4 | -. 30742 | . 18003 | . 619 | -. 8927 | . 2778 |
|  | 5 | $-.98933^{*}$ | . 18003 | . 000 | -1.5746 | -. 4041 |
|  | 6 | -. 22219 | . 18003 | . 873 | -. 8074 | . 3630 |

*. The mean difference is significant at the 0.05 level.

Table 14: Total proteins

| ANOVA |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Values |  |  |  |  |  |
|  | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
| Between <br> groups | 27.467 | 6 | 4.578 | 6.174 | .001 |
| Within <br> groups <br> Total | 15.570 | 21 | .741 |  |  |

differences and their significance values are tabulated in Table 5.
"From the above table it is justifiable that there is a significant difference of means of group-1 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-4, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-2 with respect to the group-1, group-3, group-4, group-5, group-6, group-7.

From the above table it is justifiable that there is a significant difference of means of group-3 with respect to the

Table 15: Effect of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi fruits on multiple comparisons

| Multiple comparisons |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dependent variable: values |  |  |  |  |  |  |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | $2.87660^{*}$ | . 60887 | . 002 | . 8973 | 4.8559 |
|  | 3 | 1.53399 | . 60887 | . 202 | -. 4453 | 3.5133 |
|  | 4 | . 63954 | . 60887 | . 936 | -1.3398 | 2.6188 |
|  | 5 | $2.25314^{*}$ | . 60887 | . 019 | . 2738 | 4.2324 |
|  | 6 | . 80933 | . 60887 | . 831 | -1.1700 | 2.7886 |
|  | 7 | . 23628 | . 60887 | 1.000 | -1.7430 | 2.2156 |
| 2 | 1 | $-2.87660^{*}$ | . 60887 | . 002 | -4.8559 | -. 8973 |
|  | 3 | -1.34261 | . 60887 | . 334 | -3.3219 | . 6367 |
|  | 4 | -2.23705* | . 60887 | . 020 | -4.2164 | -. 2578 |
|  | 5 | -. 62345 | . 60887 | . 943 | -2.6027 | 1.3558 |
|  | 6 | $-2.06727^{*}$ | . 60887 | . 037 | -4.0466 | -. 0880 |
|  | 7 | -2.64031* | . 60887 | . 005 | -4.6196 | -. 6610 |
| 3 | 1 | -1.53399 | . 60887 | . 202 | -3.5133 | . 4453 |
|  | 2 | 1.34261 | . 60887 | . 334 | -. 6367 | 3.3219 |
|  | 4 | -. 89445 | . 60887 | . 759 | -2.8737 | 1.0848 |
|  | 5 | . 71915 | . 60887 | . 894 | -1.2601 | 2.6984 |
|  | 6 | -. 72466 | . 60887 | . 890 | -2.7040 | 1.2546 |
|  | 7 | -1.29771 | . 60887 | . 371 | -3.2770 | . 6816 |

Table contin...

| 4 | 1 | -. 63954 | . 60887 | . 936 | -2.6188 | 1.3398 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 2 | 2.23705* | . 60887 | . 020 | . 2578 | 4.2164 |
|  | 3 | . 89445 | . 60887 | . 759 | -1.0848 | 2.8737 |
|  | 5 | 1.61360 | . 60887 | . 161 | -. 3657 | 3.5929 |
|  | 6 | $\text { . } 16979 .$ | $\text { . } 60887$ | $1.000$ | -1.8095 | $2.1491$ |
|  | 7 | -. 40326 | . 60887 | . 993 | -2.3826 | 1.5760 |
| 5 | 1 | $-2.25314^{*}$ | . 60887 | . 019 | -4.2324 | -. 2738 |
|  | 2 | $62345 .$ | . 60887 | . 943 | -1.3558 | 2.6027 |
|  | 3 | -. 71915 | . 60887 | . 894 | -2.6984 | 1.2601 |
|  | 4 | -1.61360 | . 60887 | . 161 | -3.5929 | . 3657 |
|  | 6 | -1.44381 | . 60887 | . 259 | -3.4231 | . 5355 |
|  | 7 | $-2.01686^{*}$ | . 60887 | . 044 | -3.9962 | -. 0376 |
| 6 | 1 | -. 80933 | . 60887 | . 831 | -2.7886 | 1.1700 |
|  | 2 | $2.06727^{*}$ | . 60887 | . 037 | . 0880 | 4.0466 |
|  | 3 | . 72466 | . 60887 | . 890 | -1.2546 | 2.7040 |
|  | 4 | -. 16979 | . 60887 | 1.000 | -2.1491 | 1.8095 |
|  | 5 | 1.44381 | . 60887 | . 259 | -. 5355 | 3.4231 |
|  | 7 | -. 57305 | . 60887 | . 961 | -2.5523 | 1.4062 |
| 7 | 1 | -. 23628 | . 60887 | 1.000 | -2.2156 | 1.7430 |
|  | 2 | $2.64031^{*}$ | . 60887 | . 005 | . 6610 | 4.6196 |
|  | 3 | 1.29771 | . 60887 | . 371 | -. 6816 | 3.2770 |
|  | 4 | . 40326 | . 60887 | . 993 | -1.5760 | 2.3826 |
|  | 5 | $2.01686^{*}$ | . 60887 | . 044 | . 0376 | 3.9962 |
|  | 6 | . 57305 | . 60887 | . 961 | -1.4062 | 2.5523 |

*. The mean difference is significant at the 0.05 level.

Table 16: SOD

| Table 16: SOD |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| ANOVA |  |  |  |  |  |
| Values | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
|  | 6 | 22.063 | 14.642 | .000 |  |
| Between <br> groups | 132.376 |  |  |  |  |
| Within <br> groups | 31.642 | 21 | 1.507 |  |  |
| Total | 164.019 | 27 |  |  |  |

group-1, group-2, group-4, group-5, group-6, and group-7 respectively.
From the above table it is justifiable that there is a significant difference of means of group-4 with respect to the group-2, group-3 and group-5 respectively. On the other hand, group1 , group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-5 with respect to the group-1, group-2, group-3, group-4, group-6, group-7, respectively.

From the above table it is justifiable that there is a significant difference of means of group-6 with respect to the group-2,

Table 17: Multiple comparisons

| Multiple comparisons |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dependent variable: values |  |  |  |  |  |  |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | 4.94723* | . 86798 | . 000 | 2.1256 | 7.7688 |
|  | 3 | $3.13914^{*}$ | . 86798 | . 023 | . 3175 | 5.9608 |
|  | 4 | -1.13582 | . 86798 | . 841 | -3.9574 | 1.6858 |
|  | 5 | 2.36198 | . 86798 | . 141 | -. 4596 | 5.1836 |
|  | 6 | -. 62151 | . 86798 | . 990 | -3.4431 | 2.2001 |
|  | 7 | -. 73815 | . 86798 | . 976 | -3.5598 | 2.0835 |


| 2 | 1 | -4.94723* | . 86798 | . 000 | -7.7688 | -2.1256 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 3 | -1.80809 | . 86798 | . 397 | -4.6297 | 1.0135 |
|  | 4 | -6.08305* | . 86798 | . 000 | -8.9047 | -3.2614 |
|  | 5 | -2.58525 | . 86798 | . 087 | -5.4069 | . 2364 |
|  | 6 | $-5.56875^{*}$ | . 86798 | . 000 | -8.3904 | -2.7471 |
|  | 7 | $-5.68538^{*}$ | . 86798 | . 000 | -8.5070 | -2.8638 |
| 3 | 1 | -3.13914* | . 86798 | . 023 | -5.9608 | -. 3175 |
|  | 2 | 1.80809 | . 86798 | . 397 | -1.0135 | 4.6297 |
|  | 4 | -4.27496* | . 86798 | . 001 | -7.0966 | -1.4533 |
|  | 5 | -. 77716 | . 86798 | . 969 | -3.5988 | 2.0445 |
|  | 6 | $-3.76066^{*}$ | . 86798 | . 005 | -6.5823 | -. 9390 |
|  | 7 | $-3.87729^{*}$ | . 86798 | . 003 | -6.6989 | -1.0557 |
| 4 | 1 | 1.13582 | . 86798 | . 841 | -1.6858 | 3.9574 |
|  | 2 | $6.08305^{*}$ | . 86798 | . 000 | 3.2614 | 8.9047 |
|  | 3 | $4.27496^{*}$ | . 86798 | . 001 | 1.4533 | 7.0966 |
|  | 5 | $3.49780^{*}$ | . 86798 | . 009 | . 6762 | 6.3194 |
|  | 6 | . 51430 | . 86798 | . 996 | -2.3073 | 3.3359 |
|  | 7 | . 39766 | . 86798 | . 999 | -2.4239 | 3.2193 |
| 5 | 1 | -2.36198 | . 86798 | . 141 | -5.1836 | . 4596 |
|  | 2 | 2.58525 | . 86798 | . 087 | -. 2364 | 5.4069 |
|  | 3 | . 77716 | . 86798 | . 969 | -2.0445 | 3.5988 |
|  | 4 | $-3.49780^{*}$ | . 86798 | . 009 | -6.3194 | -. 6762 |
|  | 6 | $-2.98350{ }^{*}$ | . 86798 | . 034 | -5.8051 | -. 1619 |
|  | 7 | $-3.10014^{*}$ | . 86798 | . 025 | -5.9217 | -. 2785 |
| 6 | 1 | . 62151 | . 86798 | . 990 | -2.2001 | 3.4431 |
|  | 2 | $5.56875^{*}$ | . 86798 | . 000 | 2.7471 | 8.3904 |
|  | 3 | $3.76066^{*}$ | . 86798 | . 005 | . 9390 | 6.5823 |
|  | 4 | -. 51430 | . 86798 | . 996 | -3.3359 | 2.3073 |
|  | 5 | $2.98350^{*}$ | . 86798 | . 034 | . 1619 | 5.8051 |
|  | 7 | -. 11664 | . 86798 | 1.000 | -2.9382 | 2.7050 |
| 7 | 1 | . 73815 | . 86798 | . 976 | -2.0835 | 3.5598 |
|  | 2 | $5.68538^{*}$ | . 86798 | . 000 | 2.8638 | 8.5070 |
|  | 3 | $3.87729^{*}$ | . 86798 | . 003 | 1.0557 | 6.6989 |
|  | 4 | -. 39766 | . 86798 | . 999 | -3.2193 | 2.4239 |
|  | 5 | $3.10014^{*}$ | . 86798 | . 025 | . 2785 | 5.9217 |
|  | 6 | . 11664 | . 86798 | 1.000 | -2.7050 | 2.9382 |

*. The mean difference is significant at the 0.05 level.

Table 18: Glutathione

| ANOVA |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Values |  |  |  |  |  |
|  | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
| Between <br> groups | 612.445 | 6 | 102.074 | 64.528 | .000 |
| Within <br> groups | 33.219 | 21 | 1.582 |  |  |
| Total | 645.664 | 27 |  |  |  |

group-3 and group-5 respectively. On the other hand, group-1, group-4, group-7 doesn't have any significant difference.
From the above table it is justifiable that there is a significant difference of means of group-7 with respect to the group-2, group-3 and group-5 respectively. On the other hand, group1 , group-4, group-6 doesn't have any significant difference".

## Serum Sodium

"From the above anova table of the case serum sodium we can say that there is a significant difference among the groups as the p-value is lesser than $0.05^{\prime \prime}$.
Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean

*. The mean difference is significant at the 0.05 level.

| Table 20: Malondialdehyde |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| ANOVA |  |  |  |  |  |
|  | Sum of squares | $d f$ | Mean square | $F$ | Sig. |
| Values | 6 | 16.376 | 9.710 | .000 |  |
| Between <br> groups | 98.257 | 21 | 1.687 |  |  |
| Within <br> groups | 35.418 | 27 |  |  |  |
| Total | 133.675 | 27 |  |  |  |

differences and their significance values are tabulated in Table 6.
"From the above table it is justifiable that there is a significant difference of means of group-1 with respect to the group-2, group-3, group-4, group-5, group-7 respectively. On the other hand, group-6, doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-2 with respect to the group-1, group-3, group-4, group-5, group-6, group-7.

From the above table it is justifiable that there is a significant difference of means of group-3 with respect to the group-1, group-2, group-5, group-6, and group-7 respectively. On the other hand, group-4 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group- 4 with respect to the

Table 21: Effect of Hydroalcoholic Extract of Trachyspermum ammi Leaves and Citrus paradisi fruits on multiple comparisons

| Multiple comparisons |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dependent variable: values |  |  |  |  |  |  |
| Tukey HSD |  |  |  |  |  |  |
| (I) Replications | (J) Replications | Mean difference (I-J) | Std. error | Sig. | 95\% Confidence interval |  |
|  |  |  |  |  | Lower bound | Upper bound |
| 1 | 2 | 4.56934* | . 91831 | . 001 | 1.5841 | 7.5546 |
|  | 3 | $3.28631{ }^{*}$ | . 91831 | . 025 | . 3011 | 6.2715 |
|  | 4 | . 41933 | . 91831 | . 999 | -2.5659 | 3.4046 |
|  | 5 | 1.72185 | . 91831 | . 517 | -1.2634 | 4.7071 |
|  | 6 | -.67926 | . 91831 | . 988 | -3.6645 | 2.3060 |
|  | 7 | -. 53959 | . 91831 | . 997 | -3.5248 | 2.4456 |
| 2 | 1 | -4.56934* | . 91831 | . 001 | -7.5546 | -1.5841 |
|  | 3 | -1.28303 | . 91831 | . 797 | -4.2683 | 1.7022 |
|  | 4 | -4.15001* | . 91831 | . 003 | -7.1352 | -1.1648 |
|  | 5 | -2.84749 | . 91831 | . 068 | -5.8327 | . 1377 |
|  | 6 | -5.24860* | . 91831 | . 000 | -8.2338 | -2.2634 |
|  | 7 | -5.10893* | . 91831 | . 000 | -8.0942 | -2.1237 |
| 3 | 1 | -3.28631* | . 91831 | . 025 | -6.2715 | -. 3011 |
|  | 2 | 1.28303 | . 91831 | . 797 | -1.7022 | 4.2683 |
|  | 4 | -2.86698 | . 91831 | . 065 | -5.8522 | . 1182 |
|  | 5 | -1.56446 | . 91831 | . 621 | -4.5497 | 1.4208 |
|  | 6 | $-3.96557^{*}$ | . 91831 | . 005 | -6.9508 | -. 9803 |
|  | 7 | -3.82590* | . 91831 | . 007 | -6.8111 | -. 8407 |
| 4 | 1 | -. 41933 | . 91831 | . 999 | -3.4046 | 2.5659 |
|  | 2 | 4.15001* | . 91831 | . 003 | 1.1648 | 7.1352 |
|  | 3 | 2.86698 | . 91831 | . 065 | -. 1182 | 5.8522 |
|  | 5 | 1.30252 | . 91831 | . 786 | -1.6827 | 4.2877 |
|  | 6 | -1.09859 | . 91831 | . 888 | -4.0838 | 1.8866 |
|  | 7 | -. 95892 | . 91831 | . 937 | -3.9441 | 2.0263 |
| 5 | 1 | -1.72185 | . 91831 | . 517 | -4.7071 | 1.2634 |
|  | 2 | 2.84749 | . 91831 | . 068 | -. 1377 | 5.8327 |
|  | 3 | 1.56446 | . 91831 | . 621 | -1.4208 | 4.5497 |
|  | 4 | -1.30252 | . 91831 | . 786 | -4.2877 | 1.6827 |
|  | 6 | -2.40111 | . 91831 | . 171 | -5.3863 | . 5841 |
|  | 7 | -2.26144 | . 91831 | . 223 | -5.2467 | . 7238 |


| 6 |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
|  | 1 | .67926 | .91831 | .988 | -2.3060 | 3.6645 |
|  | 2 | $5.24860^{*}$ | .91831 | .000 | 2.2634 | 8.2338 |
|  | 3 | $3.96557^{*}$ | .91831 | .005 | .9803 | 6.9508 |
|  | 4 | 1.09859 | .91831 | .888 | -1.8866 | 4.0838 |
|  | 5 | 2.40111 | .91831 | .171 | -.5841 | 5.3863 |
|  | 7 | .13967 | .91831 | 1.000 | -2.8456 | 3.1249 |
|  | 1 | .53959 | .91831 | .997 | -2.4456 | 3.5248 |
|  | 2 | $5.10893^{*}$ | .91831 | .000 | 2.1237 | 8.0942 |
|  | $3.82590^{*}$ | .91831 | .007 | .8407 | 6.8111 |  |
|  | 4 | .95892 | .91831 | .937 | -2.0263 | 3.9441 |
|  | 2.26144 | .91831 | .223 | -.7238 | 5.2467 |  |
|  | -.13967 | .91831 | 1.000 | -3.1249 | 2.8456 |  |

*. The mean difference is significant at the 0.05 level.
group-1, group-2, group-5, group-6, group-7, respectively. On the other hand, group-3 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-5 with respect to the group-1, group-2, group-3, group-4, group-6, group-7, respectively.

From the above table it is justifiable that there is a significant difference of means of group-6 with respect to the group-2, group-3, group-4, group-5, group-7 respectively. On the other hand, group-1, doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-7 with respect to the group-1, group-2, group-3, group-4, group-5, group-6, respectively".

## Serum Potassium

"From the above anova table of the case serum potassium can say that there is a significant difference among the groups as the $p$-value is lesser than $0.05^{\prime \prime}$.

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean differences and their significance values are tabulated in Table 8.
"From Table 8 it is justifiable that there is a significant difference of means of group-1 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-4, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-2 with respect to the group-1, group-3, group-4, group-5, group-6, group-7.

From the above table it is justifiable that there is a significant difference of means of group-3 with respect to the group-1, group-2, group-4, group-5, group-6, group-7 respectively.

From the above table it is justifiable that there is a significant difference of means of group-4 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-1, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group- 5 with respect to the group-1, group-2, group3, group-4, group-6, group-7, respectively.

From the above table it is justifiable that there is a significant difference of means of group-6 with respect to the group-2, group-5 respectively. On the other hand, group-1, group-3, group-4, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-7 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-1, group-4, group-6 doesn't have any significant difference".

## Total Proteins

"From the ANOVA Table 9 of the case Blood urea nitrogen we can say that there is a significant difference among the groups as the p -value is lesser than 0.05 ".

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean differences and their significance values are tabulated in Table 10.
"From Table 10it is justifiable that there is a significant difference of means of group-1 with respect to the group-2, group- 5 respectively. On the other hand, group-3, group-4, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-2 with respect to the group-1, group-4, group-6, group-7. On the other hand, group3 , group-5 doesn't have any significant difference.

From the above table it is justifiable that there is a no significant difference of means to group-3.

From the above table it is justifiable that there is a significant difference of means of group- 4 with respect to the group-2, respectively. On the other hand, group-1, group-3, group-5 group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group- 5 with respect to the group-1, group-7, respectively. On the other hand, group-2, group-3, group-4, group-6 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-6 with respect to the group-2, group-3 and group-5 respectively. On the other hand, group-1, group-4, group-3, group-5, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-7 with respect to the group-2, group- 5 respectively. On the other hand, group-1, group-3, group-4, group-6 doesn't have any significant difference".

## SOD

From ANOVA Table 11 of the case SOD we can say that there is a significant difference among the groups as the p -value is lesser than 0.05 .

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean differences and their significance values are tabulated in Table 12.
"From Table 12 it is justifiable that there is a significant difference of means of group-1 with respect to the group-2, group- 3 respectively. On the other hand, group-4, group-5, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-2 with respect to the group-1, group-4, group-6, group-7. On the other hand, groupgroup-3, group-5 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-3 with respect to the group-1, group-4, group-6, group-7 respectively. On the other hand, group-2, group-5 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-4 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-1, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-5 with respect to the group-4, group-6, group-7, respectively. On the other hand, group-1, group-2, group-3 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-6 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-1, group-4, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-7 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-1, group-4, group-6 doesn't have any significant difference".

## Glutathione

From ANOVA Table 13 of the case Blood urea nitrogen we can say that there is a significant difference among the groups as the p - value is lesser than 0.05 .

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean differences and their significance values are tabulated in Table 14.
"From Table 14 it is justifiable that there is a significant difference of means of group-1 with respect to the group-2, group-3, group-5, group-6 respectively. On the other hand, group-4, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-2 with respect to the
group-1, group-3, group-4, group-5, group-6, group-7.
From the above table it is justifiable that there is a significant difference of means of group-3 with respect to the group-1, group-2, group-4, group-6, and group-7 respectively. On the other hand, group-5 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group- 4 with respect to the group-2, group-3 and group-5 respectively. On the other hand, group1, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group- 5 with respect to the group-1, group-2, group-4, group-6, group-7, respectively. On the other hand, group-3 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-6 with respect to the group-1, group-2, group-3, group-5 respectively. On the other hand, group-4, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-7 with respect to the group-2, group-3, group-5 respectively. On the other hand, group-1, group-4, group-6 doesn't have any significant difference".

## Malondialdehyde

"From ANOVA Table 15 of the case malondialdehyde, we can say that there is a significant difference among the groups as the p-value is lesser than $0.05^{\prime \prime}$.

Later on to evaluate the mean difference between the groups post-hoc turkey test is performed. The obtained mean differences and their significance values are tabulated in Table 16.
"From the Table 20 it is justifiable that there is a significant difference of means of group-1 with respect to the group-2, group-3 respectively. On the other hand, group-4, group-5, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-2 with respect to the group-1, group-4, group-6, group-7. On the other hand, group3 , group- 5 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-3 with respect to the group-1, group-6, group-7 respectively. On the other hand, group-2, group-4, group-5 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group- 4 with respect to the group-2, respectively. On the other hand, group-1, group-3, group-5, group-6, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is no a significant difference of means to group-5. From the above table it is justifiable that there is a significant difference of means of group-6 with respect to the group-2, group-3 respectively. On the other hand, group-1, group-4, group-5, group-7 doesn't have any significant difference.

From the above table it is justifiable that there is a significant difference of means of group-7 with respect to the group-2, group-3 respectively. On the other hand, group-1, group-4, group-5, group-6 doesn't have any significant difference".

## DISCUSSION

The nephroprotective efficacy of a hydroalcoholic extract of T. ammi leaves and C. paradisi fruits against prednisoloneinduced ADPKD in experimental rats was evaluated to identify the extract's usefulness in preventing or slowing disease development.

The study's findings revealed that combining the hydroalcoholic extracts of $T$. ammi leaves and C. paradisi fruits was efficient in decreasing the pathological alterations exhibited in prednisolone-induced ADPKD mice.

Renal function indices including urea and creatinine clearance were improved, and serum creatinine levels were lowered, thanks to the extract. The extract had a reparative impact on the renal parenchyma, as shown by the enhancement of renal histology.

The extract was observed to lower serum creatinine, urea, uric acid, \& creatinine clearance levels in rats. It was also shown to lower oxidative stress indicators such as malondialdehyde, nitric oxide, and protein carbonyl while increasing Enzymes that neutralise free radicals include catalase and superoxide dismutase.

These results of this research show that a combination of the hydroalcoholic extracts of T. ammi leaves and C. paradisi fruits may be useful in avoiding or slowing the development of ADPKD in rats given prednisolone.

The findings suggest that the extract might be effective as a nephroprotective agent in ADPKD. However, further research is required to determine the extract's safety and effectiveness in people and its long-term impact on ADPKD.

More research should be done to establish the specific mechanism of action of the extract in slowing the course of ADPKD.

## Clinical implications

The clinical implications of this study provide evidence that these natural extracts may provide some beneficial effects in treating ADPKD, a chronic and progressive kidney disease. The results suggest that these natural extracts may help reduce kidney damage, inflammation, and function. In addition, these natural extracts may also help to slower the disease's development, improve quality of life, and reduce the need for medications or treatments. Based on the findings of this research, an important origin for further research into the potential therapeutic usage of these natural extracts in the treatment of ADPKD.

## CONCLUSION

Results showed that the extracts significantly reduced creatinine, urea, uric acid, and proteinuria in a dose-dependent manner. Histopathological studies also showed that the extracts
reduced damage to the renal tubules caused by prednisolone. The findings of this study suggest that hydroalcoholic extracts of T. ammi leaves and C. paradisi fruits have nephroprotective activity against prednisolone-induced ADPKD in experimental rats (Table 21).

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