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Impact of Medicago Sativa (Alfalfa) Ethanol Leaf Extract on Renal Indices (Electrolytes) in Wistar Rats

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Abstract

Objective: This research investigates the impact of Medicago sativa (Alfalfa) ethanol leaf extract on renal indices in Wistar rats, exploring potential alterations in electrolytes, urea, and creatinine levels.

Materials and Methods: Fresh leaves of Medicago sativa were collected, identified, and authenticated. The ethanol leaf extract was prepared, yielding a dry extract, and then reconstituted to form doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg. Twenty-four male Wistar rats were divided into four groups and orally treated daily for 28 days. Renal indices were determined through biochemical analysis of blood samples.

Results: The study observed mild changes in bicarbonate ions without significant alterations in sodium, potassium, chloride, and calcium ions. Blood urea and creatinine levels showed no significant differences between treated groups and the control group.

Conclusion: Medicago sativa ethanol leaf extract, at the tested doses, did not induce substantial changes in renal indices in Wistar rats. These findings suggest a potential safety profile for the investigated doses of Medicago sativa ethanol leaf extract in the context of renal function.

Keywords: biochemical analysis; medicago sativa; medicinal plants; renal indices

Introduction

Chronic kidney disease (CKD) poses a significant global health burden, affecting millions of individuals worldwide [1,2]. As researchers strive to explore novel therapeutic agents with potential renoprotective effects, there is growing interest in the medicinal properties of various plant extracts. Medicago sativa, commonly known as Alfalfa, is a perennial legume that has been traditionally used in herbal medicine for its purported diuretic and anti-inflammatory properties [3]. Recent studies have suggested potential benefits of Alfalfa in various physiological systems, prompting investigation into its impact on renal function.

Alfalfa is rich in bioactive compounds such as flavonoids, saponins, alkaloids, and phenolic acids, which have been associated with antioxidant and anti-inflammatory properties [3]. This research aims to evaluate the impact of Medicago sativa ethanol leaf extract on renal indices in Wistar rats, shedding light on its potential as a natural therapeutic agent for kidney-related disorders.

Previous research has highlighted the antioxidant and anti-inflammatory capabilities of Alfalfa extracts. The presence of flavonoids and polyphenols in Alfalfa contributes to its ability to scavenge free radicals and mitigate oxidative stress, which are implicated in the pathogenesis of renal diseases [4,5]. Traditional uses of Alfalfa as a diuretic have been documented in ethnopharmacological studies. Diuretic properties may influence renal function by promoting the excretion of waste products and reducing fluid retention, potentially benefiting individuals with compromised kidney function [3,6].

Various plant extracts have demonstrated renoprotective effects in preclinical studies. These effects often involve modulation of oxidative stress, inflammation, and improvement in renal function parameters. Alfalfa's potential renoprotective properties warrant systematic investigation in animal models [7,8].

The phytochemical composition of Alfalfa, including its diverse array of bioactive compounds, has been extensively studied. Understanding the chemical constituents of Alfalfa will contribute to elucidating

its potential mechanisms of action on renal function [9,10].

While there is a growing body of evidence supporting the medicinal properties of Alfalfa, a comprehensive understanding of its impact on renal function in the context of CKD is lacking. This study aims to bridge this gap by systematically investigating the effects of Medicago sativa ethanol leaf extract on renal indices in a Wistar rat model, providing valuable insights into its potential as a therapeutic agent for kidney-related disorders.

Materials and Methods

Plant Collection and Identification

Fresh leaves of Medicago sativa (Alfalfa) were collected from farms at Obinze and Eziobodo, both in Owerri West Local Government Area of Imo State, Nigeria. The plant was identified and authenticated by Mr. Ibe Ndukwe, a taxonomist in the Department of Forestry, College of Environmental Sciences, Michael Okpara University of Agriculture, Umudike, Abia State and a sample specimen MOUAU/ZEB/21/009 was deposited in the University herbarium for reference.

Extraction of Plant Materials

The leaves were sliced into pieces, air-dried at room temperature and pulverized into powder using a Waring commercial blender. Eight hundred grams (800 g) of the coarse powder of M. sativa leaves were weighed using a sensitive digital weighing balance. The powder was soaked in a flask containing 80% ethanol (2.5 L w/v) and then placed on a shaker with occasional shaking for 48 hours at room temperature. The mixture formed was filtered using Whatman (No.1) filter paper, and the filtrate was concentrated using a rotary evaporator and dried on a water bath to give a yield of 30.45 g (4 % w/w) dry extract with a greenish colour. It was preserved in a refrigerator at 4°C for further analysis. The extract was later reconstituted in distilled water to give desired doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight. The percentage yield was calculated using the formula of Ezirim et al. [11] as stated below:

$$\%\ yield = \frac{\text{Weight(g)of residue (dry extract)}}{\text{Weight(g)of powdered material grinded}} \times \frac{100}{1}$$

Experimental Design

Twenty-four (24) male Wistar rats were used in this study. The animals were sourced from the Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka. They were kept in the animal house of the Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Abia State University, Uturu, Abia State. The animals were acclimatized for 14 days prior to the experiment. They were fed a standard diet (Ladokun feeds, Ibadan) and had access to water ad libitum. They were maintained under standard conditions of humidity, temperature and 12 hours light and 12-hour darkness cycle. The animals were used in accordance with the National Institutes of Health guide for the care and use of Laboratory Animals [12].

A total of twenty-four male rats were weighed and grouped into 4 groups of 6 rats in each group for this study. The animals were grouped based on three different treatment doses of the leaf extract and one control (distilled water) group. The rats were orally treated daily with Medicago sativa ethanol leaf extract at doses of 200, 400 and 600 mg/kg, and distilled water for 28 days. At the end of the 28-day treatment period, the rats were deprived of pellets but had free access to drinking water for 24 hours before being sacrificed under halothane anaesthesia. Blood samples were collected through the orbital puncture into non-heparinized containers for biochemical analysis.

Determination of Renal Indices

Serum levels of electrolytes, urea and creatinine were determined according to the methods of Abali et al., [13].

Results

The results indicate changes in sodium, potassium, chloride, bicarbonate, and calcium concentrations. Statistical analysis was performed, and a significant difference ($P<0.05$) was observed in the bicarbonate levels at 400 mg/kg and 600 mg/kg of M. sativa compared to the control (Table 1). The results further show changes in total calcium, anion gap, urea, and creatinine concentrations. Statistical analysis revealed no significant differences in these parameters between the treatment groups and the control (Table 2).

Table 1: Effect of the ethanol leaf extract of M. sativa on electrolytes in rats.

Treatment Groups	Sodium (mmol/ L)	Potassium (mmol/ L)	Chloride (mmol/ L)	Bicarbonate (mmol/ L)	Calcium (mmol/ L)

Control	145.33±0.92	5.88±0.55	101.83±0.98	19.33±0.61	1.22±0.04
200 mg/kg of M. *sativa*	143.67±0.95	5.27±0.18	101.67±0.84	17.50±0.62	1.19±0.05
400 mg/kg of M. *sativa*	143.17±0.95	5.13±0.29	101.13±0.37	16.00±1.79[a]	1.10±0.11
600 mg/kg of M. *sativa*	143.00±0.86	5.12±0.15	98.50±0.50	15.67±1.20[a]	1.02±0.06

One-way ANOVA + Dunnett's post hoc test (n=6). a P<0.05 compared to control.

Table 2: Effect of the ethanol leaf extract of M. sativa on electrolytes in rats.

Treatment Groups	Total Calcium (mmol/L)	Anion Gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Control	2.16±0.172	8.50±1.61	5.05±0.31	66.63±2.96
200 mg/kg of M. *sativa*	2.42±0.192	5.33±0.61	5.77±0.26	59.78±4.44
400 mg/kg of M. *sativa*	2.57±0.072	5.50±0.67	4.87±0.23	72.58±9.05
600 mg/kg of M. *sativa*	2.56±0.092	5.67±1.20	5.52±0.35	65.22±3.78

One-way ANOVA + Dunnett's post hoc test (n=6).

Discussion

The present study investigated the impact of Medicago sativa (Alfalfa) ethanol leaf extract on renal indices in Wistar rats, specifically focusing on electrolyte levels, blood urea, and creatinine. The results revealed significant changes in bicarbonate ions across all treated rats, while there were no significant alterations in sodium, potassium, chloride, calcium ions, blood urea, and creatinine levels in comparison to the control group.

The statistically significant decrease in bicarbonate ions with increasing doses of the extract is noteworthy. However, non-significant changes observed in sodium, potassium, chloride, and calcium ions, as well as blood urea and creatinine levels among the treated groups compared to the control is an indication of a lack of renal toxicity. Notably, the anion gap exhibited a slight decrease in the treated groups, although it was not statistically significant.

While there is limited direct research on the impact of M. sativa ethanol leaf extract on renal indices in rats, the observed decrease in bicarbonate ions is consistent with studies reporting alkalizing effects of alfalfa in different contexts. Alfalfa has been historically used in traditional medicine for its diuretic properties and potential influence on electrolyte balance [14]. Similarly, the findings of our study align with those of Johnson et al. [15], who reported significant changes in bicarbonate ions with Medicago sativa treatment but no significant alterations in other electrolytes.

Furthermore, studies exploring the effects of herbal extracts on renal function have reported varying outcomes. For instance, a study by Smith et al. [16] demonstrated that certain herbal extracts caused alterations in electrolyte levels in animal models. However, the specific effects on bicarbonate ions, as observed in our study, may vary depending on the plant extract and experimental conditions.

The absence of significant changes in blood urea and creatinine aligns with findings from studies investigating the renal safety of other herbal extracts. Li et al. [17] reported similar results in a study evaluating the impact of a different plant extract on renal function in rodents. These findings collectively suggest that M. sativa ethanol leaf extract may not exert detrimental effects on renal indices in the studied rat model.

Conclusion

The present study provides valuable insights into the impact of M. sativa ethanol leaf extract on renal indices in Wistar rats. The observed changes in bicarbonate ions, along with the stability of other electrolytes, blood urea, and creatinine levels, contribute to our understanding of the potential renal effects of this herbal extract. Comparisons with existing literature underscore the need for further research to elucidate the underlying mechanisms and establish the safety profile of M. sativa in the context of renal function.

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