Article

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Alterations in Serum Electrolytes in Cannabis and Amphetamine Use Disorders: A Comparative Clinical Study

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Abstract

Background: Amphetamine and cannabis use disorders are common due to recreational use and are linked to altered metabolomic and proteomic profiles. Chronic exposure to these substances may affect human physiological functions. Since serum electrolytes play a key role in these functions, this study aimed to investigate the effects of single or combined use of amphetamine and/or cannabis on serum electrolyte levels.

Methods: Blood electrolyte and kidney function parameters were measured using clinical chemistry and immunoassay techniques. Serum oxidative stress markers (catalase and superoxide dismutase (SOD)) were also analyzed. Participants were divided into four groups: controls, cannabis users, amphetamine users, and cannabis-amphetamine users.

Results: No significant differences in kidney function were observed across the groups. However, phosphorus levels were elevated in the cannabis (t = 4.748, $p <$ 0.001), amphetamine (t = 2.933, $p <$ 0.05), and cannabis-amphetamine (t = 3.364, $p <$ 0.05) groups compared to controls. Moreover, sodium (t = 3.931, $p <$ 0.01), chloride (t = 3.382, $p <$ 0.05), and calcium (t= 3.391, $p <$ 0.05) levels were significantly higher in the cannabis group compared to the control group. Patients with amphetamine (t = 4.311, $p <$ 0.001) or cannabis (t = 4.738, $p <$ 0.001) use disorder showed reduced catalase levels, with more pronounced reductions in those with combined use (t = 8.700, $p <$ 0.0001). As compared to controls, serum SOD levels were also lower in patients with amphetamine (t = 6.957, $p <$ 0.0001) or cannabis (t = 6.600, $p <$ 0.0001) use disorder, with the greatest reduction observed in the combined-use group (t = 12.11, $p <$ 0.0001).

Conclusions: Cannabis use disorder is associated with alterations in serum electrolytes while serum creatinine and urea levels remained within the measured range. Co-use of amphetamine and cannabis was associated with greater oxidative stress than the use of either drug alone. Further studies are needed to explore the molecular mechanisms involved in these changes.

Submitted: 11 June 2026 Revised: 17 August 2026 Accepted: 1 September 2026 Online First: 29 October 2026
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Keywords

amphetamine; cannabis; drug addiction; electrolytes; oxidative stress; mental health

Introduction

Amphetamine and cannabis use disorders are widespread due to the recreational use of such drugs, and are associated with altered metabolomic and proteomic profiles [1–3], including the neurobiological effects involved in the development of drug use disorder. Amphetamines can affect the levels of some neurotransmitters, such as elevating serotonin and dopamine, which can be potentiated with high body temperature [4]. *D*-amphetamine has been shown to be able to modulate plasma levels of sodium and potassium in hyperthyroid mice [5], and after a three-week treatment has been suggested to modulate plasma levels of magnesium and the calcium/magnesium ratio in hyperactive boys [6]. In *Caenorhabditis elegans*, amphetamine-induced changes in amine-gated chloride channels, an effect associated with behavioral changes [7].

Regarding cannabis, a growing number of studies have documented the long-term adverse effects of its use in humans. Evidence suggests that cannabis might cause several impairments in cognitive function [8], but the degree and duration varies depending on the user's age, quantity used, and duration of use. Cannabinoid exposure has also been shown to modulate sodium and calcium channels [9–11], and cannabidiol in particular has a potential to interact with voltage-gated sodium channels [9,10]. In addition, cannabis plants contain heavy metals [12], long-term exposure may affect electrolyte homeostasis; however, this hypothesis requires validation. Therefore, these mental health disabilities can affect the body functions and physiological parameters.

Accumulating evidence suggests that substance abuse may contribute to oxidative stress, which plays a key role in cellular damage and the pathophysiology of neurotoxicity and organ dysfunction [13]. Cannabis and amphetamines have been individually linked to elevated oxidative stress through the overproduction of reactive oxygen species (ROS) and impaired antioxidant defense systems, including reduced activity of key enzymes such as catalase and superoxide dismutase (SOD) [14]. However, limited studies have explored these effects in the context of co-abuse. Therefore, this study also assessed serum levels of catalase and SOD to determine whether cannabis and/or amphetamine use is associated with redox imbalance.

Electrolytes play an important role in human body functions [15]. Sodium and chloride are critical ions in extracellular compartments, including blood plasma, interstitial fluid, and transcellular fluid, and are key to a number of essential life processes [16]. Tight control of sodium levels is required because it influences cell membrane potential, which is required for nerve impulse transmission, muscle contraction, and cardiac function [17,18]. Moreover, chloride is linked to changes in blood pressure [19], acid-base balance or metabolic acidosis [20], and heart failure [21]. Magnesium is another important physiological cation, with both extracellular and intracellular functions [22]; more than 300 biochemical reactions in the body use it as an essential cofactor [23], especially systems that depend on adenosine triphosphate [24]. In addition, magnesium contributes to protein synthesis, cell membrane role, parathyroid hormone secretion, glucose metabolism and mitochondrial function [25–28], and uncontrolled magnesium levels commonly indicate issues in cardiovascular and neuromuscular function [29,30]. Without rapid treatment to correct magnesium level, life-threatening conditions can ensue, such as paralysis and cardiac arrhythmia. Similarly, calcium is a cation well-known for its involvement in biological functions such as muscle contraction [31,32], blood clotting [33,34], and bone mass [35].

Another key physiological ion is inorganic phosphorus, which is involved in many physiological compositions such as phosphoproteins and nucleic acids, the last being specifically required for mitochondrial function [36]. Due to its role in generating 2,3-diphosphoglycerate in the blood cells, phosphorus is also crucial in maintaining the integrity of cell structures, bone health and acid–base homeostasis [37–39]. As an electrolyte, phosphorus also either influences or is critical to many physiological functions such as minerals transfer, contractility of the muscles, certain biochemical processes and neurological behaviors. Notably, hyperphosphatemia is associated with decreased improvement in patients with sepsis [40].

In the current work, we explored the role of cannabis and/or amphetamine addiction on modulating serum levels of essential electrolytes, namely sodium, potassium, phosphorous, chloride, magnesium, and calcium. In addition, we investigated the effects of amphetamine and/or cannabis addiction on kidney function assessed by determining the levels serum creatinine and urea. This study will provide information regarding whether the effects of single and co-exposure amphetamine and/or cannabis on serum electrolytes may be attributable to kidney dysfunction or to other causes.

Actas Esp Psiquiatr 2026;1–13. https://doi.org/10.62641/aep.2390 | ISSN:1578-2735
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Materials and Methods

Study Design and Blood Collection

This study was conducted at Eradah (Al Amal) Mental Health Complex, Riyadh, Saudi Arabia. Participant recruitment and biological sample collection were initiated after obtaining ethical approval on 14 April 2020. Blood samples were collected from eligible participants during the recovery period, and the duration of the recovery period was determined according to each participant's clinical status, based on the diagnosis and the rehabilitation center's guidelines. The laboratory analyses of serum electrolytes, kidney function parameters, and oxidative stress biomarkers were performed between 2021 and 2023. In the current work, 40 subjects were classified into four groups (Fig. 1), each comprising 10 subjects as follows: Group 1, the healthy (control) group; Group 2, patients with cannabis use disorder; Group 3, patients with amphetamine use disorder; and Group 4, patients with both cannabis and amphetamine use disorders. Diagnosis of cannabis and/or amphetamine use disorders was carried out with respect of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [41]. Baseline demographic and clinical characteristics were collected from participants through medical records and direct interview at the time of enrollment. The demographic and clinical background of participants is provided in Table 1. Subjects all had no medical history of renal diseases, blood disorders, infectious diseases, psychotic disorders, or obesity. Peripheral blood samples were collected, when they started the recovery period, by venipuncture in the early morning, before breakfast, to avoid the influence of breakfast on electrolyte serum levels (fasting for at least 8 hours). Serum separator tubes were employed after collection. Amphetamine and cannabinoids were detected in body at the time of blood collection. The presence of amphetamine and/or cannabinoids was confirmed using an immunofluorescence-based screening assay at the time of blood collection. No other drugs of abuse were detected in the participants included in the amphetamine and/or cannabis use disorder groups.

Lipid Biomarkers Analysis

Lipid profile of different subjects was obtained by using Reflotron Plus (03012573001, Roche, Mannheim, Germany). Levels of total cholesterol and triglycerides were measured in whole blood with specific stripes available from the Roche. All the tests were done on the day of blood samples collection. A lipid profile was included as part of the routine biochemical assessment performed for all study participants.

Sample Centrifugation

Serum was collected from clotted blood samples by centrifuging them for 10 min at 1000 × *g*, as previously described [2]. Aliquots were stored at –20 °C for electrolytes (sodium, chloride, potassium, calcium, phosphorous and magnesium) and kidney function parameters (creatinine and urea) analysis by clinical biochemistry and immunoassay system.

Determination of Kidney Function and Serum Electrolytes

The ARCHITECT c4000 (02P2401, Abbott Laboratories, Lake County, IL, USA) clinical biochemistry and immunoassay system was used to measure kidney function (serum creatinine and urea) and serum essential electrolytes (sodium, chloride, potassium, calcium, phosphorous, and magnesium), normal levels of which are listed in Table 2.

Data Description

MetaboAnalyst software (version 6.0; Xia Lab, McGill University, Montreal, Québec, Canada) was used to qualitatively explore patterns in the electrolyte data across study groups. Data description included the main visualization of the heatmap analysis.

Heatmap Analysis

A heatmap was generated using the group-wise mean values of electrolyte concentrations to identify visual clustering and variation trends among the control, cannabis, amphetamine, and cannabis-amphetamine groups.

Biochemical Analysis (Catalase and SOD)

Catalase and SOD activities were measured using commercially available assay kits, as previously described [42]. In brief, diluent and catalase standards or serum were mixed and prepared. Transfer 10 μL from the prepared mixtures to 96-well plate. Working reagents and assay buffer, were added to each well. Then, H_2O_2 and dH_2O (ECAT-100; BioAssay Systems, Hayward, CA, USA) was prepared and added to the wells. The incubation period then was started, and the readings were measured at 30 minutes. The standard curve was then created and the samples catalase concentrations were determined (ECAT-100; BioAssay Systems, Hayward, CA, USA).

Diluent and SOD standards or serum were mixed and

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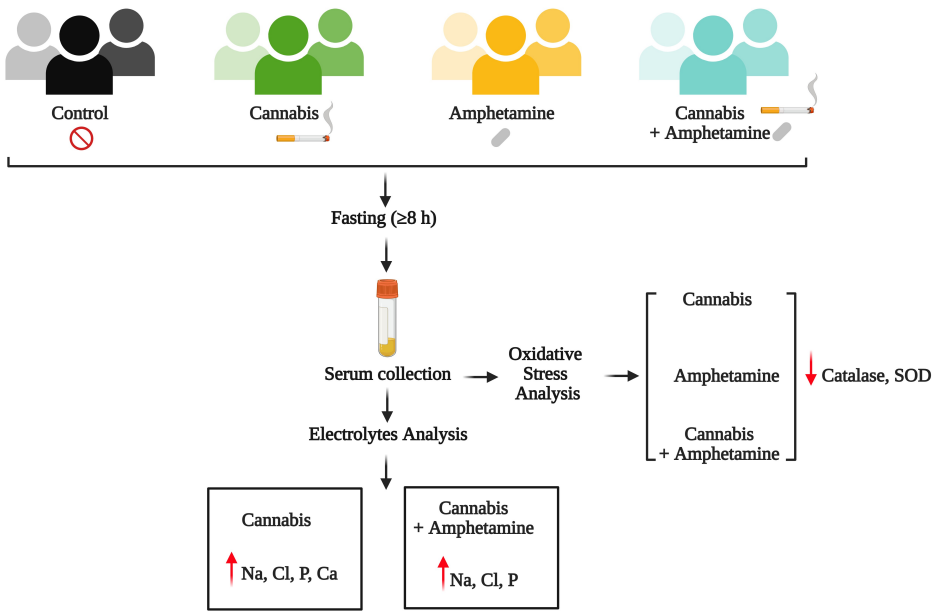


Fig. 1. Schematic diagram of the study design. SOD, superoxide dismutase; Na, sodium; P, phosphorus; Cl, chloride; Ca, calcium. The 40 subjects were divided into four groups: control (n = 10), cannabis group (n = 10), amphetamine (n = 10), and cannabis-amphetamine (n = 10). Both groups underwent fasting for at least 8 h, before serum collection. The collected samples underwent electrolytes and kidney function analysis compare with the control group. The figure was created with BioRender®.

Table 1. Demographic information of subjects participated in the study.

Variable	Control group	Cann group	Amph group	Cann-Amph group
No. of patients	10	10	10	10
Gender	10 M, 0 F	10 M, 0 F	10 M, 0 F	10 M, 0 F
Age (Mean ± SD)	29.30 ± 5.19	27.10 ± 7.50	31.6 ± 7.01	30.9 ± 6.89
Infectious diseases (HIV, HCV, TB)	Negative	Negative	Negative	Negative
Substance dosage use	None	Smoking	Oral tablets	Smoking (Cann) Oral tablets (Amph)
Substance use history				
1–4 years	None	2 patients	3 patients	5 patients
5–9 years	None	4 patients	3 patients	4 patients
10–14 years	None	3 patients	1 patient	None
≥15 years	None	1 patient	3 patients	1 patient

SD, standard deviation; Amph, amphetamine; Cann, cannabis; F, female; HCV, hepatitis C virus; HIV, human immunodeficiency virus; M, male; TB, tuberculosis.

prepared. Transfer 20 μL from the prepared mixtures to 96-well plate. Working reagents, assay buffer, xanthine and water-soluble tetrazolium salt-1 (WST-1) were added to each well. Then, xanthine oxidase was prepared and added to the wells. After incubation, the readings were measured at 450 nm at 0 and 60 minutes. The standard curve was then created and the samples SOD concentrations were determined after subtracting 0 minute' absorbance data from 60 minutes' absorbance data (ESOD-100; BioAssay Systems, Hayward, CA, USA).

Statistical Analysis

Analysis of the levels of the electrolytes (sodium, chloride, potassium, calcium, phosphorus and magnesium), cholesterol, triglycerides, kidney function parameters (creatinine and urea) and oxidative stress markers were performed using GraphPad prism software (version 6.01; GraphPad Software, Inc., La Jolla, CA, USA). One-way Analysis of Variance (ANOVA) analysis was employed to detect the significant changes on the levels of electrolytes

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Table 2. Serum parameters detected in participants and corresponding normal value ranges.

#	Biomarker	Normal value
1	Creatinine	50–110 μmol/L
2	Urea	2.9–8.9 mmol/L
3	Sodium	133–146 mmol/L
4	Chloride	95–108 mmol/L
5	Potassium	3.50–5.30 mmol/L
6	Calcium	2.20–2.60 mmol/L
7	Phosphorous	0.80–1.50 mmol/L
8	Magnesium	0.70–1.00 mmol/L

Table 3. Blood cholesterol and triglycerides in cannabis and/or amphetamine use disorder patients. Data is shown as median (interquartile range).

#	Biomarker	Control	Cann	Amph	Cann + Amph
1	Total cholesterol (mg/dL)	179 (42)	182 (50)	174 (62.5)	154.5 (68)
2	Triglycerides (mg/dL)	70 (25.1)	91.3 (69.2)	105 (58.75)	131.5 (77.1)

Amph, amphetamine; Cann, cannabis.

and kidney function parameters in the four groups. Bonferroni post hoc analysis was also employed to further investigate whether there were any significant changes in parameters of interest between the four groups. Age was presented as mean ± standard deviation (SD). Other demographic and participant characteristics were reported descriptively, with categorical variables presented as frequencies. To account for multiple comparisons, the Benjamini–Hochberg False Discovery Rate (FDR) correction was applied to all biomarker analyses. All statistical analyses were performed using two-sided tests, with a significance level set at $p < 0.05$.

Results

Blood Cholesterol and Triglycerides in Cannabis and/or Amphetamine Use Disorder Patients

One-way ANOVA analysis did not reveal any significant differences among the studied groups on the total cholesterol ($F(3, 35) = 1.028$, $p = 0.3922$) or triglycerides ($F(3, 35) = 0.1902$, $p = 0.9024$) (Table 3). However, triglycerides levels were increased in both cannabis or amphetamine disorder patients as compared to control group, an effect associated with higher triglycerides levels in co-abuse as compared to other groups.

Kidney Function Parameters in Cannabis and/or Amphetamine Use Disorder Patients

We estimated the effects of cannabis, amphetamine, and cannabis-amphetamine use disorder on selected biomarkers of kidney function, namely serum creatinine and urea. No significant difference in creatinine was identified among the four groups or in relation to controls ($F(3, 36) = 1.234$, $p = 0.3115$) (Fig. 2A). Similarly, urea levels were not significantly different among the groups ($F(3, 36) = 0.3358$, $p = 0.7995$) (Fig. 2B).

Serum Sodium and Chloride Levels in Cannabis and/or Amphetamine Use Disorder Patients

We detected serum sodium level in patients diagnosed with cannabis and/or amphetamine addiction. One-way ANOVA revealed significant changes in serum sodium levels ($F(3, 36) = 5.258$, $p < 0.01$) and chloride ($F(3, 36) = 4.068$, $p < 0.05$) levels among the groups. The post hoc analysis revealed serum sodium to be significantly higher in the cannabis group as compared with the control group ($t = 3.931$, $p < 0.01$) (Fig. 3A). We further determined serum chloride level in the cannabis, amphetamine, and cannabis-amphetamine groups relative to controls. Here, statistical analysis revealed that the cannabis group had significantly higher serum chloride levels as compared with the control group ($t = 3.382$, $p < 0.05$) (Fig. 3B).

Serum Potassium and Calcium Levels in Cannabis and/or Amphetamine Use Disorder Patients

We also determined serum potassium level in patients diagnosed with cannabis and/or amphetamine addiction. The one-way ANOVA test did not reveal any significant between-group difference in serum potassium ($F(3, 36) = 0.8594$, $p = 0.4709$) (Fig. 4A). We further estimated the

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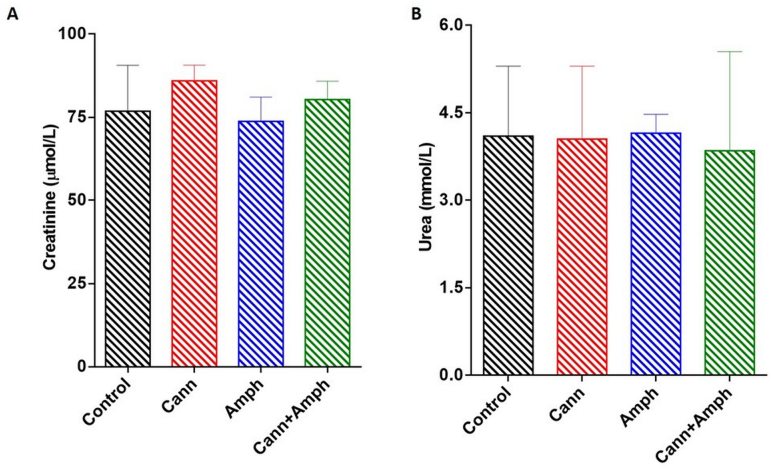


Fig. 2. Bar chart shows kidney function parameters in cannabis and/or amphetamine use disorder patients. Amph, amphetamine; Cann, cannabis. (A) One-way ANOVA on creatinine levels did not show significant differences among groups. (B) The same analysis on urea levels did not show any significant differences among groups. Data are shown as median with interquartile range.

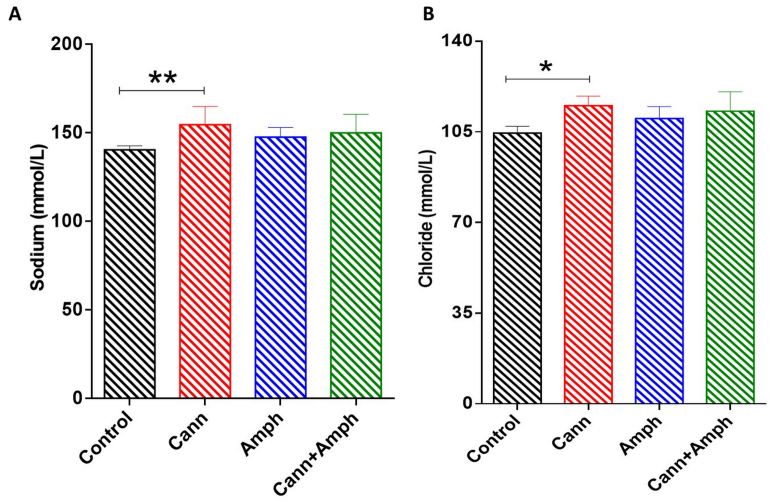


Fig. 3. Bar chart shows serum sodium and chloride levels in cannabis and/or amphetamine use disorder patients. Amph, amphetamine; Cann, cannabis. (A) One-way ANOVA followed by Bonferroni multiple comparisons (Bonferroni post hoc) analysis on sodium levels showed an increase in serum sodium levels in cannabis group as compared to controls. (B) The same analysis on serum chloride levels showed higher chloride levels in cannabis group as compared to controls. Data are shown as median with interquartile range. *$p < 0.05$, **$p < 0.01$.

serum calcium levels in the cannabis, amphetamine, and cannabis-amphetamine groups relative to controls, and one-way ANOVA revealed significant differences among the groups ($F(3, 36) = 4.286$, $p < 0.05$). Bonferroni post hoc test identified the cannabis group as having significantly higher serum calcium than the control group ($t = 3.391$, $p < 0.05$) (Fig. 4B).

Serum Phosphorous and Magnesium Levels in Cannabis and/or Amphetamine Use Disorder Patients

We also investigated serum phosphorus in patients with cannabis and/or amphetamine addiction. One-way ANOVA revealed significant changes in serum phosphorous levels among the groups ($F(3, 36) = 7.976$, $p < 0.001$). The post hoc analysis revealed that that serum phosphorus was increased in the cannabis ($t = 4.748$, $p < 0.001$), amphetamine ($t = 2.933$, $p < 0.05$) and cannabis-amphetamine

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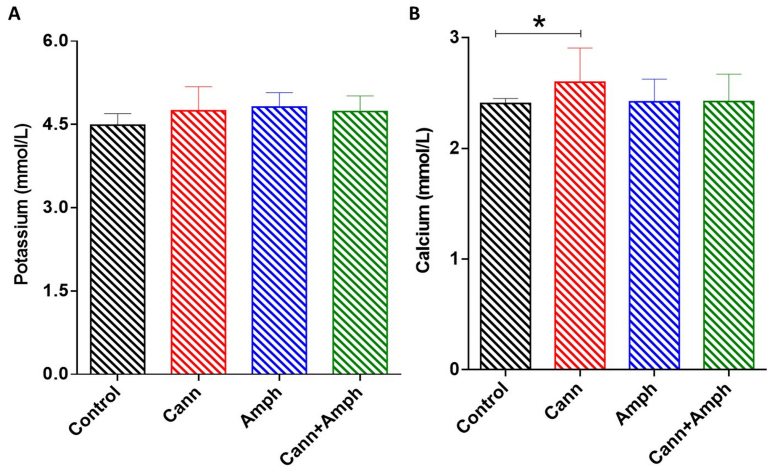


Fig. 4. Bar chart shows serum potassium and calcium levels in cannabis and/or amphetamine use disorder patients. Amph, amphetamine; Cann, cannabis. (A) One-way ANOVA analysis on serum potassium levels did not show significant differences among groups. (B) One-way ANOVA test followed by Bonferroni multiple comparisons (Bonferroni post hoc) analysis on calcium levels showed an increase in serum calcium levels in cannabis group as compared to controls. Data are shown as median with interquartile range. *$p <$ 0.05.

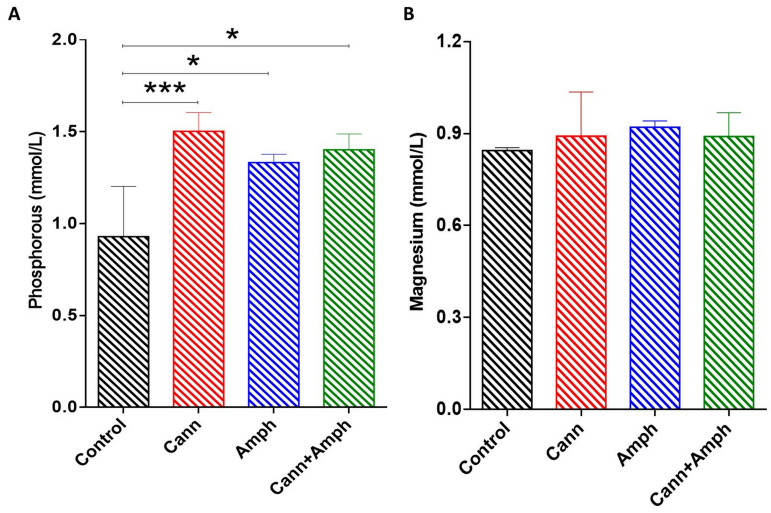


Fig. 5. Bar chart shows effects serum phosphorous and magnesium levels in cannabis and/or amphetamine use disorder patients. Amph, amphetamine; Cann, cannabis. (A) One-way ANOVA analysis followed by Bonferroni multiple comparisons (Bonferroni post hoc) analysis on phosphorous levels showed an increase in serum phosphorous levels in cannabis, amphetamine and cannabis-amphetamine groups as compared to controls. (B) One-way ANOVA analysis on serum magnesium levels did not show significant differences among groups. Data are shown as median with interquartile range. *$p < 0.05$, ***$p < 0.001$.

(t = 3.364, $p < 0.05$) groups relative to the control group (Fig. 5A). We also determined serum magnesium levels in cannabis, amphetamine and cannabis-amphetamine groups relative to controls. For this parameter, no significant changes were observed (F(3, 36) = 1.321, p = 0.2827) (Fig. 5B).

Data Description Using Heatmap Analysis

We further analyzed the obtained electrolyte data using heatmap analysis. This analysis qualitatively revealed that cannabis use disorder patients had the most abnormal in the serum electrolyte concentrations compared to other group of patients (Fig. 6).

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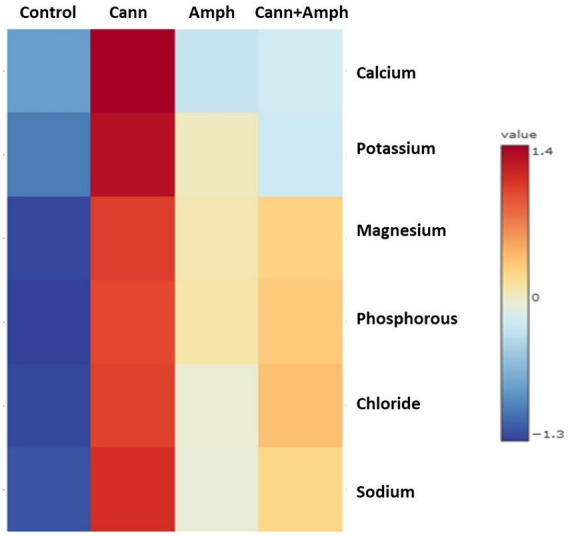


Fig. 6. Heatmap analysis of serum electrolyte concentrations across the four study groups. Amph, amphetamine; Cann, cannabis. The heatmap was generated based on the average values of each electrolyte from all subjects within each group.

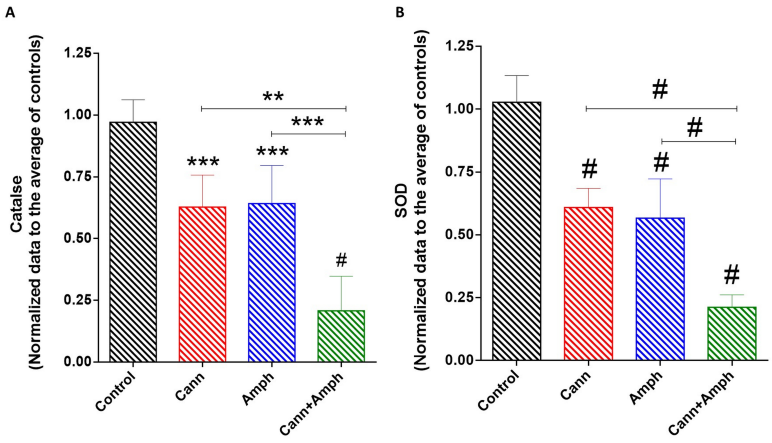


Fig. 7. Bar chart shows serum catalase and SOD levels in cannabis and/or amphetamine use disorder patients. SOD, superoxide dismutase; Amph, amphetamine; Cann, cannabis. (A) One-way ANOVA analysis followed by Bonferroni multiple comparisons (Bonferroni post hoc) analysis on catalase levels showed a decrease in catalase levels in cannabis, amphetamine and cannabis-amphetamine groups as compared to controls. (B) The same analysis on serum SOD levels showed a reduction in SOD levels in cannabis, amphetamine and cannabis-amphetamine groups as compared to controls. Data are shown as median with interquartile range. $^{**}p < 0.01$, $^{***}p < 0.001$, # $p < 0.0001$.

Serum Catalase and SOD Levels in Cannabis and/or Amphetamine Use Disorder Patients

Additionally, we evaluated the effects of cannabis and/or amphetamine abuse on the serum oxidative stress, particularly catalase and SOD. One-way ANOVA revealed significant changes in serum catalase levels among the groups ($F(3, 36) = 25.31$, $p < 0.0001$). Statistical analysis revealed that that serum catalase was decreased in the cannabis ($t = 4.738$, $p < 0.001$), amphetamine ($t = 4.311$, $p < 0.001$) and cannabis-amphetamine ($t = 8.700$, $p < 0.0001$) groups as compared to controls, moreover, significant reduction in catalase was observed in the serum of cannabis-amphetamine groups relative to the cannabis ($p < 0.01$) or amphetamine ($p < 0.001$) groups (Fig. 7A). Similarly, One-way ANOVA revealed significant changes in serum SOD levels among the groups ($F(3, 36) = 49.26$, $p < 0.0001$). Statistical analysis revealed that that serum

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Table 4. Benjamini-Hochberg adjusted *p* value using FDR method.

Rank	Variable	Original *p* value	Benjamini-Hochberg adjusted *p* value	Significant using an FDR of 0.05?
1	SOD	7.9514×10^{-13}	9.5417×10^{-12}	Yes
2	Catalase	5.5168×10^{-9}	3.3100×10^{-8}	Yes
3	Phosphorous	0.0003	0.0012	Yes
4	Sodium	0.0041	0.0123	Yes
5	Calcium	0.0110	0.0220	Yes
6	Cholesterol	0.0110	0.0220	Yes
7	Chloride	0.0138	0.0237	Yes
8	Magnesium	0.2827	0.4153	No
9	Creatinine	0.3115	0.4153	No
10	Urea	0.7995	0.9024	No
11	Potassium	0.8594	0.9024	No
12	Triglycerides	0.9024	0.9024	No

SOD, superoxide dismutase; FDR, False Discovery Rate.

SOD was decreased in the cannabis ($t = 6.600$, $p < 0.0001$), amphetamine ($t = 6.957$, $p < 0.0001$) and cannabis-amphetamine ($t = 12.11$, $p < 0.0001$) groups as compared to controls, moreover, significant reduction in SOD was observed in the serum of cannabis-amphetamine groups relative to the cannabis ($p < 0.0001$) or amphetamine groups ($p < 0.0001$) (Fig. 7B).

FDR Analysis

Because multiple biomarkers were analyzed, *p*-values were additionally adjusted using the Benjamini–Hochberg False Discovery Rate (FDR) procedure. All comparisons that were statistically significant in the primary analysis remained significant after FDR correction (Table 4).

Discussion

In the present study, we investigated the effects of cannabis and/or amphetamine addiction on serum levels of electrolytes. Our data indicate that cannabis use disorder patients was associated with increased serum levels of sodium, calcium, chloride, and phosphorous without evidence of impaired renal function based on the measured serum creatinine and urea levels. In addition, elevated phosphorous levels were also observed in the amphetamine and cannabis-amphetamine groups as compared to controls. Importantly, catalase and SOD serum levels were decreased in cannabis, amphetamine and cannabis-amphetamine groups compared to control group. Interestingly, significant reduction of catalase and SOD in cannabis-amphetamine group as compared to amphetamine or cannabis group.

Our results suggest that both cannabis use disorder is associated with altered serum essential electrolytes while serum creatinine and urea levels remained within the measured range; this suggests that cannabis may modulate the function or expression of the proteins that regulate these electrolytes, however, this hypothesis needs to be validated. For one, we found here that serum sodium was increased in individuals exposed to cannabis when compared with the control group. Cannabidiol has been reported to bind to and block sodium ion channels [9], inhibiting ion translocation and so transmembrane signal transduction. These findings were further confirmed *in vitro* [10]. In addition, cannabidiol has been shown to inhibit the skeletal muscle sodium channel Nav1.4 via modulating elasticity of the surrounding membrane and blocking the channels pores [43]. This suggests that cannabidiol in particular might be responsible for the observed modulation of sodium levels. However, serum electrolyte concentrations are influenced by multiple physiological factors, including hormonal regulation, hydration status, dietary intake, acid-base balance, and renal handling [44]. Therefore, the observed findings should not be interpreted as evidence of a direct ion channel-mediated effect. The suggested mechanisms of ion channels should therefore be considered as potential targets based on previous experimental evidence, and this hypothesis should be validated in the future in patients with substance use disorder.

We also found that patients with cannabis (but not amphetamine or cannabis-amphetamine) use disorder had higher serum calcium than did controls. A previous report found that Δ9-tetrahydrocannabinolic acid (THCA), a compound in cannabis plants, is able to modulate T-type calcium channels in cell lines [45]. Similarly, exposure to cannabidiol and Δ9-tetrahydrocannabinol (THC) has been demonstrated to inhibit T-type calcium channels, also

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termed CaV3 channels, in HEK293 cells [11]. These data indicate that cannabinoids, including cannabidiol, THCA, and THC, have modulatory effects on T-type calcium channels. The absence of a significant difference in serum calcium levels in the co-use group should not be interpreted as evidence of a cancellation effect or attenuation of the effect of cannabis by amphetamine. Further studies are needed to clarify this observation. Future work is also warranted to investigate the molecular effects of cannabinoids on calcium channels and correlate the findings to serum calcium levels.

Our results also indicated that serum chloride and phosphorus are elevated in patients with cannabis use disorder, while amphetamine and cannabis-amphetamine groups had higher serum phosphorus levels as compared to controls. Notably, activation of cannabinoid receptors is associated with decreased glomerular filtration rate [46], and imbalances in these electrolytes are associated with kidney diseases [47,48]. It is important to note that endocannabinoid system is located in renal system [49], which might be affected by cannabis exposure. Future research should explore the effects of cannabis compounds on proteins that regulate serum levels of phosphorous and chloride.

We did not observe any significant difference in the serum levels of tested electrolytes among patients with amphetamine use disorder alone, except for phosphorous. Previous research has shown that in hyperthyroid mice, *d*-amphetamine exposure is able to decrease plasma sodium and increase plasma potassium [5]. Similarly, in hyperactive boys, three-week *d*-amphetamine treatment is associated with increased magnesium plasma levels and decreased calcium/magnesium ratio as compared with controls [6]. Interestingly, a study using the model organism *Caenorhabditis elegans* also reported amphetamine to modulate amine-gated chloride channels and produce behavioral changes [7]. Taken together, these findings suggest that factors such as genetic background and the duration and quantity of amphetamine exposure may significantly impact the effects of amphetamine on serum electrolyte levels. Elevated serum phosphorus levels were observed in both the amphetamine and co-use groups, suggesting an association between amphetamine exposure and altered phosphorus homeostasis. However, this hypothesis needs further investigations. Given the relatively small sample size, larger and adequately powered studies are needed to confirm these findings and improve the ability to detect smaller between-group differences. A previous study found that cannabis smoking was not associated any changes on serum level of sodium, chloride and potassium in participants [50]. However, sodium and potassium serum levels were reduced in cannabis smokers in a population from another city in the same country [51]. Although we found that cannabis smoking increased serum sodium and chloride levels in Saudi population, genetic and ethnicity factors might play critical role on the effects of cannabis smoking on serum electrolyte levels.

We also found that serum levels of the antioxidant enzymes catalase and SOD were significantly reduced in cannabis, amphetamine and cannabis-amphetamine groups, with more significant reductions in the co-abuse group as compared to single drug use groups. These findings are in line with earlier reports showing reduced antioxidant activity and elevated oxidative stress in individuals with chronic substance use, including cannabis and amphetamines [14,52,53]. The more pronounced alterations observed in the combined-use group warrant further investigation in larger studies designed to evaluate potential interactions and underlying mechanisms.

Limitations: This study has a few notable limitations. First, the sample size was relatively small, which may limit the generalizability of the findings. Second, all participants were adult males, as female participants were not available during recruitment. As a result, the influence of gender on serum electrolyte alterations could not be assessed. Future studies with larger, gender-diverse populations are recommended to validate these findings and explore potential sex-specific effects. Potential confounding factors, including behavioral factors, sleep, medical history, and medication use, were not fully controlled and may have influenced the measured biomarkers. Detailed substance-use characteristics, cumulative exposure, frequency and exact time since last use, were not systematically available for all participants. Consequently, these potentially important confounding factors could not be incorporated into the present analyses and should be considered in future prospective studies. The present study was not designed to evaluate statistical interactions between cannabis and amphetamine exposure. However, studies are needed in the near future to explore the synergistic or additive biological effects of the two drugs among gender-diverse populations.

Conclusions

Patients with cannabis use disorder exhibited significantly altered serum levels of various ions without evidence of impaired renal function based on the measured serum creatinine and urea levels. However, patients with amphetamine use disorder showed comparable ionic profiling to control healthy group. These findings suggest that mechanisms other than kidney dysfunction may underlie the observed electrolyte alterations. However, both cannabis and

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amphetamine use disorders were associated with alterations in serum oxidative stress markers, and the co-use group exhibited significantly greater alterations than either the cannabis-only or amphetamine-only group. These observations suggest the exposure to these compounds may be accompanied by alterations in serum markers that are linked to other diseases at the beginning of recovery period. Accordingly, more studies are needed to investigate the role of cannabis use disorder on modulating proteins regulating the serum concentrations of these ions. For instance, sodium and potassium channels in the circulatory system may be modulated at genetic or protein levels after chronic drug exposure. More research is warranted to find out the effects of amphetamine derivatives such as methamphetamine on the investigated parameters. This study describes alterations in serum electrolyte profiles associated with amphetamine and/or cannabis use disorder adult male patients. Further studies with larger, adequately powered cohorts are warranted to confirm the present findings.

Availability of Data and Materials

Data of this study are available upon request from the corresponding authors.

Author Contributions

SA and FA led the conception and design of the study; MMA, WHA, and II drafted the manuscript; data acquisition was carried out by MMA, AMH, AFA, and FA; while KA, AMH, WAQ, and AFA handled analysis and interpretation; SA, IAA, SIA, WQ, and AMA prepared the figures; funding support was provided by SA. All remaining authors critically revised the manuscript for important intellectual content. All authors reviewed and approved the final version and agree to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The research ethics committee at Eradah (Al Amal) Mental Health Complex, Ministry of Health, Riyadh, Saudi Arabia approved the present research study (H-01-R-063-10). This research work was conducted according to the requirements of the Declaration of Helsinki of 1975 and updated amendments. Consent forms including the objective of the study were agreed to and signed by participants.

Acknowledgment

The authors would like to thank Eradah (formerly Al Amal) Mental Health Complex, Riyadh, Saudi Arabia, for their assistance and cooperation in facilitating this study and participant recruitment.

Funding

The authors declare that no specific funding was received for this study.

Conflict of Interest

Authors declare no conflict of interest.

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