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Co-abuse of amphetamine and alcohol harms kidney and liver

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The prevalence of alcohol use disorder was found 75% higher among amphetamine dependent patients. Alcohol and amphetamine alone have nephrotoxicity and hepatoxicity. But, the degree of risk with coabuse of alcohol and amphetamine is unknown. The objective of this study was to assess toxic effects of amphetamine-alcohol co-abuse on the liver and kidney. he present study was a cross-sectional study conducted et al. Amal Hospital for Mental Health, Qassim region, KSA and include one hundred participants. Seventy-five participants were patients hospitalized for the treatment of abuse, and twenty-five participants, were healthy voluntaries, have no history of abuse. An experienced psychiatrist conducted patient interviews and assessed the patients using the DSM-5 criteria. The data from healthy participants were considered as a control. The abuse group was paired with the control group by age and lifestyle. Participants were split into: Group I: Control group (n = 25); Group II: Amphetamine (AMP) abuser group (n = 25); Group III: Alcohol abuser group (n = 25) and Group IV: Combined drug abuser group (AMP and alcohol) (n = 25). The socio-demographic data was collected. Complete medical examination, Body Mass Index and samples of blood and urine were collected from all participants for analytical tests; determination of alcohol and AMP levels, kidney functions and liver functions. The mean BMI values in groups II, III, and IV showed no significant change from the control group. The serum level of albumin and alkaline phosphatase showed significant decrease in all abuser groups. While, alanine transaminase (ALT), Aspartate transaminase (AST) and osteopontin levels showed significant increase in all abuser groups. Fasting blood sugar values showed significant increase in alcohol abusers. On the other hand, it revealed no significant change in AMP and combined groups. The mean values of urea showed no significant change in AMP and alcohol abusers and significant increase in combined drug abuser group. The serum creatinine and all abuser groups showed significant increase in Cystatin C. The alteration in the most of studied biochemical parameters were more than two folds in combined group compared with that of AMP or alcohol groups. Study reveals synergistic liver and kidney toxicity. Amphetamine-alcohol co-abuse significantly heightens kidney and liver toxicity.

Keywords Amphetamine, Alcohol, Abuse, Liver, Mental health, Kidney, Body mass

Substance usage and abuse are pervasive health problems. According to research by Stinson et al.[1], patients who are amphetamine addicted have a 75% greater prevalence of alcohol use disorder. For instance, according to a study, almost 60% of amphetamine users in New York City admitted to abusing the drug in addition to alcohol[2]. Amphetamine combined with THC and ethanol was implicated in 52% of deaths, according to Attafi et al.[3].

Co-abuse of substances poses significant health risks. Combination alcohol along with other substances of abuse are quite common among[4]. Public health has recently grown very concerned about the interaction of ethanol and other substances of abuse, including AMP, opiates, nicotine and γ-hydroxybutyric acid[5].

Amphetamine abusers frequently use alcohol and have a higher risk of reaching alcohol intoxication level[6]. Alcohol may slow the metabolism of amphetamine by lowering the amount of p-hydroxylated amphetamine metabolites in amphetamine abusers' urine. A higher blood concentration of amphetamine may result from this[7]. Additionally, amphetamine and its active metabolite were more widely absorbed and distributed when alcohol was consumed[8].

A study conducted in 2012 by Kirkpatrick et al.[9] showed that abusing alcohol and amphetamine together caused tachycardia, elevated mood, and changed sleep and performance. These results highlight the concerning possibility of co-abusing amphetamine and alcohol since amphetamine may conceal symptoms of alcohol intoxication, such as sedation and compensatory performance, enabling abusers to drink more alcohol at the

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risk of becoming toxic. Amphetamines in combination with alcohol abuse induces tachycardia and elevated myocardial[10]. A rat study found that drinking alcohol and amphetamine at the same time can have a synergistic effect on spatial memory impairment[11].

Alcohol and other drugs of abuse are frequently combined, possibly due to the stronger feelings of "high" than those experienced with each drug alone or the less strong experiences of alcohol's harmful consequences[12]. According to a 2018 Saudi Arabian survey, abuse of two or more substances, such as alcohol and AMP, is the most prevalent. 60% of people who abuse multiple drugs[13].

A prominent factor in the toxicity of AMP is excessive extracellular dopamine, norepinephrine, and serotonin. Secondary consequences include renal, hepatic, muscular, lung, and GI problems in addition to the core clinical problems substantial neurological and cardiovascular effects[14].

Acute liver injury that is clinically evident and occasionally severe or even fatal has been linked to AMP use. Amphetamines metabolized in the liver, primarily through the P450 system (CYP 2D6) and subsequently toxic metabolites are produced especially at high doses[15].

Acute kidney damage induced by AMP is known to occur through a number of pathways. Myoglobinuria-associated tubular damage related to rhabdomyolysis was discovered to be the most prevalent mechanism. Common nephrotoxic effects include necrotizing vasculitis, prerenal azotemia, hyponatremia, and malignant hypertensive nephropathy[16].

Alcohol abuse, especially in heavy drinkers, is a significant risk factor for a variety of toxic effects and, as a result, contributes significantly to the worldwide burden of illness. Alcohol actually contributes to the development of more than 30 diseases. Liver and renal disease, infectious illnesses, cancer, diabetes, neuropsychiatric diseases, cardiovascular disease, and unintentional and intentional harm are the most prevalent disease categories that are caused by alcohol usage[17].

Heavy drinking might result in the most tissue damage to the liver because it is the main location for the metabolism of alcohol. Steatosis, hepatitis, and fibrosis/cirrhosis are the most recognizable liver lesions that are caused by prolonged and heavy alcohol use[18].

The relationship between alcohol use disorder (AUD) and kidney damage is intriguing but debatable, and it is not well understood how alcohol may harm the kidneys at the molecular level. There is limited experimental data directly connecting alcohol use to kidney damage, and epidemiological research aiming to establish a link between AUD and renal disease have so far shown conflicting results. However, research focusing on other organs and tissues raises various hypotheses for how alcohol can contribute to kidney disease[19].

One potential explanation is oxidative stress brought on by increased reactive oxygen species generation, which can result in excessive free radical synthesis and subsequent tissue damage and inflammation. In addition, AUD appears to accelerate adverse pathological processes that are damaging to the kidneys in its effects on other important organs (liver, heart, intestines, and skeletal muscle) (Varga et al. 2017). It should be noted that these pathways have not yet been confirmed in kidney experiments. To determine if alcohol actually encourages kidney damage and the potential processes by which alcohol-induced kidney damage can occur, more research is required[19].

A study with a limited sample size and few animal studies focused on the toxic consequences of co-abuse of AMP and alcohol on the heart and brain, despite the fact that there is evidence that the prevalence of co-abuse of AMP and alcohol is considerable[19]. Limited studies on combined substance abuse toxicity were performed. Co-abuse is a serious threat to health as a result of negative interaction with one another and very common[20]. The aim from co-abuse is to have more euphoric highs. The most frequently included in co-abuse is alcohol. Although the situation is grave, it is unknown whether co-abuse of drugs studied, and no prior research has looked into the damaging consequences of both on the liver and kidneys. Prior research lacks focus on combined toxicity. Therefore, the present study plans to investigate the toxic effects of AMP co-abuse with alcohol on the liver and kidney. The objective of this study was to assess toxic effects of amphetamine-alcohol co-abuse on the liver and kidney.

Subjects and methods

This study was a cross-sectional study compares abuser groups with controls conducted et al. Amal Hospital for Mental Health, Qassim region, KSA and includes 100 participants. Seventy-five participants were patients hospitalized for the treatment of addiction and 25 participants were healthy voluntaries with no history of abuse and were defined as control group. The Control group was matched with abuse group by age and lifestyle. The medical history of all the participants was recorded. Abusers were interviewed by an experienced psychiatrist and evaluated according to the DSM-5 criteria. The data from healthy participants were considered as control. The samples were collected at first day of admission. The participants were divided into four equal groups (n = 25) as follows: Group I Control group (participants designated as control); Group II (AMP abuser group); Group III (Alcohol abuser group) and Group IV [Combined drug abuser group (AMP and alcohol)]. All attempts were made to exclude from study any subjects who abused other illicit substance and their combination with alcohol or amphetamine like opioids, cocaine, cannabis, etc., had past-history of diseases, taken any medications that could have an impact on the biochemical parameters and who are less than 18 years and more than 60 years old that would affect the measured parameters in this study.

Medical examination

The history of the patients was recorded from their medical record including age, sex, special habits, occupation, toxicological history, medical disease, and drug treatments. A complete physical examination was done including cardiological and neuropsychological examinations.

Determination of body mass index (BMI)
A carefully calibrated electronic balance (Camry EF711H, China) was used to measure the weight of each participant wearing light clothes without shoes. Each person's height was measured with their shoes off using a height measuring tape (Seca 206, USA). Weight divided by the square of height (kg/m^2) was used to measure the BMI[21].

Urine sample collection and storage
Under quality control and safety procedures, 25 cc of urine was collected at admission in a plastic container and stored at – 20 °C until analysis[22]. The initial day of admission saw the collection of the samples.

Blood sample collection and storage
Under strictly sterile settings, blood samples were taken by clean venipuncture with sterile, single-use syringes. Each patient, along with the controls, had approximately 5 ml of blood taken. A clean, dry test tube was filled with blood, which was then left to clot at room temperature. The serum was extracted and aliquoted into Eppendorf tubes after centrifuging at 1600 rpm for 5 min. Until they were used, serum samples were kept at − 80 °C in firmly covered tubes[21]. The initial day of admission saw the collection of the samples.

Determination of amphetamine in urine
A competitive binding-based chromatographic immunoassay using (model 8691C from Macherey–Nagel in Dueren, Germany). Amphetamines that might be in the urine sample compete with the drug conjugate for antibody binding sites. The urine sample moves upward during the test due to capillary action. Below 1,000 ng/mL of AMP did not saturate the antibody-coated particles' binding sites in the test strip. The immobilized AMP then caught the antibody-coated particles, conjugated them, and produced a visible colored line in the area of the test line. If the AMP concentration was greater than 1,000 ng/ml, the colorful line did not form in the test line area due to saturation of all the anti- AMP antibody binding sites. A colored line in the test line area was generated for a urine sample that tested positive for drugs, while a line was formed for a sample that tested negative for drugs or contained drug concentrations below the cut-off. A colored line always appears in the control line region as a procedural control, showing that the proper volume of sample has been introduced and membrane wicking has taken place[23].

Blood alcohol concentration
In order to determine blood ethyl alcohol concentration, two tubes of venous blood must be drawn as soon as patients arrive. Following the use of benzalkonium chloride to disinfect the venepuncture site, matched samples were collected into unmarked blood collection tubes. The data management system kept track of the collecting time. Following immediate delivery to the laboratory, samples were centrifuged for 10 min at 3000 × *g*. For both specimens, measurements were made immediately following centrifugation. Using the Beckman-Coulter Olympus AU400 auto analyser and a Synchron Systems Ethanol Assay kit (A-E 474,947), blood ethanol concentration was determined (Beckman Coulter Inc., Melville, USA). The conversion of ethanol and NADH into acetaldehyde catalyzed by alcohol dehydrogenase and NADH during this process. To determine the amount of ethanol present in the sample, the rate of absorbance was recorded at 340 nm[24].

Determination of serum albumin level
The serum albumin concentration was determined in accordance with the approach of Bowers and Wong[25] using commercial kit supplied by Diamond (Egypt).

Determination of serum aspartate aminotransferase (AST) activity
Serum Aspartate aminotransferase was determined spectrophotometrically (Model 6305. Bibby Scientific Ltd, Staffordshire, United Kingdom) according to manufacturer's instructions, using Transaminase kit (Sigma–Aldrich, Saint Louis, U.S.A.)[26].

Determination of serum alanine aminotransferase (ALT) activity
Serum Alanine Aminotransferase was determined according to Modified Reitman & Frankel Method spectrophotometrically (Model 6305. Bibby Scientific Ltd, Staffordshire, United Kingdom) according to the manufacturer's instructions, using Transaminase kit (Sigma–Aldrich, Saint Louis, U.S.A.)[26].

Determination of serum alkaline phosphatase (ALP) activity
Serum Alkaline Phosphatase was analyzed according to King & King Method spectrophotometrically (Model 6305. Bibby Scientific Ltd, Staffordshire, United Kingdom) according to the manufacturer's instructions, using kits (Sigma–Aldrich, Saint Louis, U.S.A.)[27].

Determination of serum osteopontin (OPN)
The OPN ELISA kit from Sigma-Aldrich uses the quantitative sandwich enzyme immunoassay method. An OPN-specific monoclonal antibody has been pre-coated on the microtiter plate. The wells of the microtiter plate were then filled with standards or samples, and any OPN that was present bound to the antibody-coated wells. A standardized preparation of a polyclonal antibody coupled to horseradish peroxidase (HRP), specific for OPN, was added to each well in order to "sandwich" the OPN immobilized on the plate in order to quantify the amount of OPN present in the sample. After the microtiter plate has been incubated, any unbound components were removed by thoroughly washing the wells. Substratum solutions were then poured into each well. In a brief incubation period, the enzyme (HRP) and substrate were allowed to react. Only the wells with enzyme-

conjugated antibody and OPN showed a color shift. The enzyme–substrate reaction was halted by the addition of a sulphuric acid solution, and the color change was detected spectrophotometrically. In a nutshell, the needed number of coated wells were placed in the holder, and the corresponding well received 50 L of Standards (each standard's bottle was gently shaken by hand before being pipetted up and down three times before adding) or samples. In the empty control well, 50 L of PBS (pH 7.0-7.2) was added. 50 mL of samples were filled with 5 l of balance solution, and the mixture was thoroughly stirred. To each well, 100 mL of conjugate were added. It was thoroughly jumbled. At 37° C, the plate was covered and incubated for an hour. A wavelength of 450 nm was used to measure the color shift[28].

Determination of fasting blood sugar

With the use of the Glucose Assay Kit, Enzymatic-Colorimetric technique, serum glucose levels were assessed (GOD-PAP; Glucose oxidase-phenol, and four aminophenazone)[29].

Determination of serum creatinine

When creatinine was combined with picric acid in an alkaline solution, a red tautomer of creatinine picrate was created, and its intensity can be quantified spectrophotometrically[30].

Determination of serum urea

Acidic reaction nearly at 100 °C occurs when urea and diacetyl monoxime react, resulting in a red product that may be quantified colorimetrically[25].

Determination of serum cystatin C (CYS-C)

Measurement of Cystatin C by ELISA applies the immunoassay technique by Spectrophotometer (Bibby Scientific Ltd, Staffordshire, United Kingdom, Model 6305). A specific anti-cystatin C antibody that was coated on latex particles attaches to the sample and induces agglutination. The level of cystatin C in the sample directly correlates with the optical measurement of the agglutination-induced turbidity. Bringing all of the reagents to room temperature was the first step in getting ready for the experiment. The experiment was carried out at room temperature. Sated wells included, sample, standard, and blank wells. To the matching standard wells, 50 µl of Standard (S1, S2, S3, S4, S5, S6) was added. Each sample well received a 50 µl addition of sample. It was added HRP-conjugate reagent (100 µl) the standard and sample wells. The plate was sealed with a plate membrane and heated to 37 °C for 60 min. All wells, including the blank wells, were washed four times in total. Each well received equal amounts of chromogen solution A and B (50 µl). The mixture was gradually incorporated. The Plate was incubated at 37 °C in the dark for 15 min. Then all wells received 50 µl of stop Solution. After applying stop solution, the optical density was recorded at 450 nm with an ELISA reader after 15 min[31].

Statistical analysis

Data were gathered, collated, and statistically evaluated using one-way analysis of variance (ANOVA) to look for differences that were statistically significant between the abuse groups and the controls and combined group. Results were presented as means ± SEM, and a p value of less than 0.05 was used to determine statistical significance. The relationships between the study groups in each of the parameters determined using Pearson correlation coefficients. For all statistical analysis, the SPSS Version 21.0 (IBM Corp., Armonk, NY, USA) software package was utilized.

Results

Socio-demographic characteristics

Age

In this study, the participants were aged between 21 and 59 years old, with mean ages 36.04 ± 1.40, 37.72 ± 1.38, 36.52 ± 1.70 and 37.12 ± 1.76 years in groups I, II, III and IV respectively. Table 1 revealed that, the majority of patient's ages ranged between 31 and 40 years old represented 36%, 36% and 36% in group II, group III and group IV respectively. There were no significant changes between the four studied groups (I, II, III and IV) as regards the mean age ($p > 0.05$).

Marital status

Table 2 showed that, the majority of participants were married in groups I, II, III and IV with percentages 76, 48, 76 and 48 respectively.

Educational level

The present study showed that, the highest percentages of patients were high education with percentages 40, 36, 48 and 32 in groups I, II, III and IV respectively. While, the lowest percentages were for patients who post graduated in all abuser groups, as shown in Table 3.

Occupational status

This study showed that, the highest percentage of participants were unemployed participants, which were 56, 60, 32 and 56% in groups I, II, III and IV respectively. On the other hand, participants had a job with percentages 32, 32, 56 and 28% in groups I, II, III and IV respectively (Table 4).

Duration of intake

The present study demonstrated that, the duration of AMP, alcohol or alcohol plus AMP intake in the study patients ranged from 3 years as a minimum to a maximum of 15 years with a mean duration 8.13 ± 4.93,

	Group I (n = 25)			Group II (n = 25)			Group III (n = 25)			Group IV (n = 25)		
Age groups (years)	No	%	Mean ± SEM	No	%	Mean ± SEM	No	%	Mean ± SEM	No	%	Mean ± SEM
20- 30	3	12	36.04 ± 1.40	10	40	37.7 ± 1.38	2	8	36.52 ± 1.70	3	12	37.12 ± 1.76
31- 40	12	48		9	36		9	36		9	36	
41- 50	4	16		5	20		9	36		6	24	
51- 60	6	24		1	4		5	20		7	28	

Table 1. Distribution of the control, amphetamine, alcohol and combined alcohol-amphetamine abuser groups (n = 100) according to their ages (years). Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined drug abuser group (amphetamine and alcohol). Values are expressed as mean ± SEM. *Significant at $p < 0.05$ level.

Marital status	Group I (n = 25)		Group II (n = 25)		Group III (n = 25)		Group IV (n = 25)	
	No	%	No	%	No	%	No	%
Single	6	24	11	44	6	24	9	36
Married	19	76	12	48	19	76	12	48
Divorced	0	0	2	8	0	0	4	16

Table 2. Distribution of the control, amphetamine, alcohol and combined alcohol-amphetamine abuser groups (n = 100) according to their marital status. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined drug abuser group (amphetamine and alcohol).

Educational levels	Group I (n = 25)		Group II (n = 25)		Group III (n = 25)		Group IV (n = 25)	
	No	%	No	%	No	%	No	%
Primary School	6	24	5	20	3	12	9	36
Intermediate school	5	20	11	44	0	0	6	24
High School	10	40	9	36	12	48	8	32
University	4	16	0	0	8	32	2	8
Post- graduated	0	0	0	0	2	8	0	0

Table 3. Distribution of the control amphetamine, alcohol and combined alcohol-amphetamine abuser groups (n = 100) according to their educational levels**.** Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined drug abuser group (amphetamine and alcohol).

Occupational status	Group I (n = 25)		Group II (n = 25)		Group III (n = 25)		Group IV (n = 25)	
	No	%	No	%	No	%	No	%
Yes	8	32	8	32	14	56	7	28
No	14	56	15	60	8	32	14	56
Retired	3	12	2	8	3	12	4	16

Table 4. Distribution of the control, amphetamine, alcohol and combined alcohol-amphetamine abuser groups (n = 100) according to their occupational status. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined drug abuser group (amphetamine and alcohol).

8.06 ± 4.56 and 9.00 ± 3.19 years in groups II, III and IV respectively. This study showed that, there were no significant changes between the three studied groups of the study (II, III and IV, $p > 0.05$) (Table 5).

Alcohol and AMP level

The mean alcohol level was 130.10 ± 4.30 and 129.80 ± 4.48 mg/dl in groups III and IV respectively. This study demonstrated that, the AMP levels were 1489.60 ± 139.48 and 1381.70 ± 140.98 (ng/mL) for groups II and IV (Table 6).

Body mass index (BMI)

The current study demonstrated that, the mean BMI of the studied groups were 42.30 ± 0.85, 43.66 ± 1.55, 42.79 ± 0.85 and 44.36 ± 1.47 kg/m^2 in groups I, II, III and IV respectively. Figure 1 showed that, the mean BMI showed no significant change in groups II, III and IV compared with control and combined groups ($p < 0.05$).

Liver function tests

Albumin

This study demonstrated that, the mean albumin levels of the studied groups were 4.20 ± 0.05, 3.84 ± 0.08, 3.07 ± 0.06 and 2.84 ± 0.09 g/dL in groups I, II III and IV respectively. Figure 2 showed that, the mean albumin levels were significantly reduced in groups II, III and IV compared with control group ($p < 0.05$). Also, the mean albumin levels in groups II and III showed significant elevation compared with group IV ($p < 0.05$).

Serum alkaline phosphatase

This study demonstrated that, the mean alkaline phosphatase levels of the studied groups were 84.40 ± 3.14, 72.36 ± 3.05, 64.00 ± 2.30 and 68.00 ± 2.98 U/L in groups I, II III and IV respectively. Figure 3 showed that, the mean alkaline phosphatase levels were significantly decreased in groups II, III and IV compared with control

Duration of intake (years)	Group II (n = 25)		Group III (n = 25)		Group IV (n = 25)	
	No	%	No	%	No	%
No	0	0	0	0	0	0
1–5	6	24	8	32	1	4
6–10	6	24	7	28	11	44
11–15	6	24	2	8	6	24
16–20	2	8	3	12	1	4
21–25	3	12	2	8	2	8
26–30	2	8	3	12	4	16

Table 5. Distribution of amphetamine, alcohol and combined alcohol-amphetamine abuser groups (n = 75) according to the duration of Amphetamine, Alcohol or Amphetamine plus alcohol intake (years). Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined drug abuser group (amphetamine and alcohol).

Items	Group II (n = 25)	Group III (n = 25)	Group IV (n = 25)
Amphetamine level (ng/mL)	1489.60 ± 139.48	Nil	1381.70 ± 140.98
Alcohol level (mg/dl)	Nil	130.10 ± 4.30	129.80 ± 4.48

Table 6. Amphetamine and alcohol levels for amphetamine, alcohol and combined alcohol-amphetamine abuser groups (n = 75). Group II: Amphetamine abusers and Group IV: Combined drug abuser group (amphetamine and alcohol). Values are expressed as mean ± SEM. *Significant at P < 0.05 level.

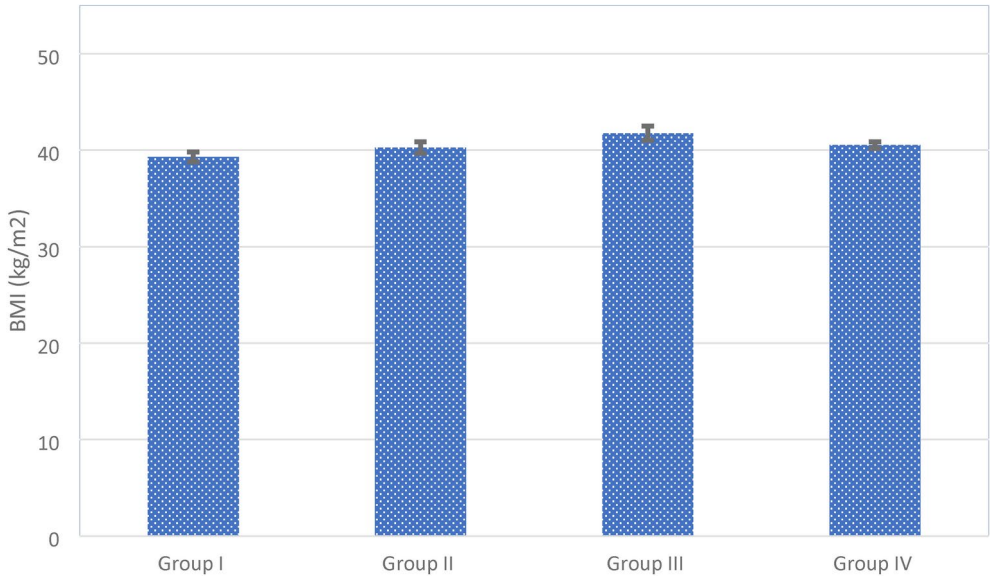

Fig. 1. Body Mass Index (BMI) values for control, amphetamine, alcohol and combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at p < 0.05 level.

group (p < 0.05). On the other hand, there the mean alkaline phosphatase levels showed no significant change in groups II and III compared with group IV (p < 0.05).

Serum alanine aminotransferase

The present study demonstrated that, the mean alanine aminotransferase levels of the studied groups were 19.12 ± 0.64, 25.62 ± 0.89, 43.10 ± 1.80 and 60.51 ± 1.83 U/L in groups I, II III and IV respectively. Figure 4 showed that, the mean alanine aminotransferase levels were significantly elevated in groups II, III and IV

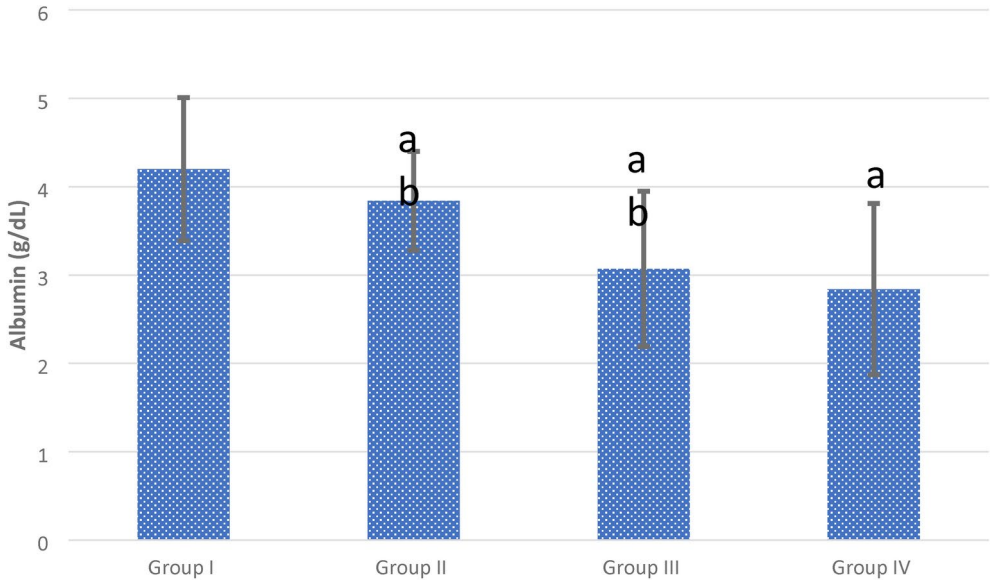

Fig. 2. Albumin level for control, amphetamine, alcohol and combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

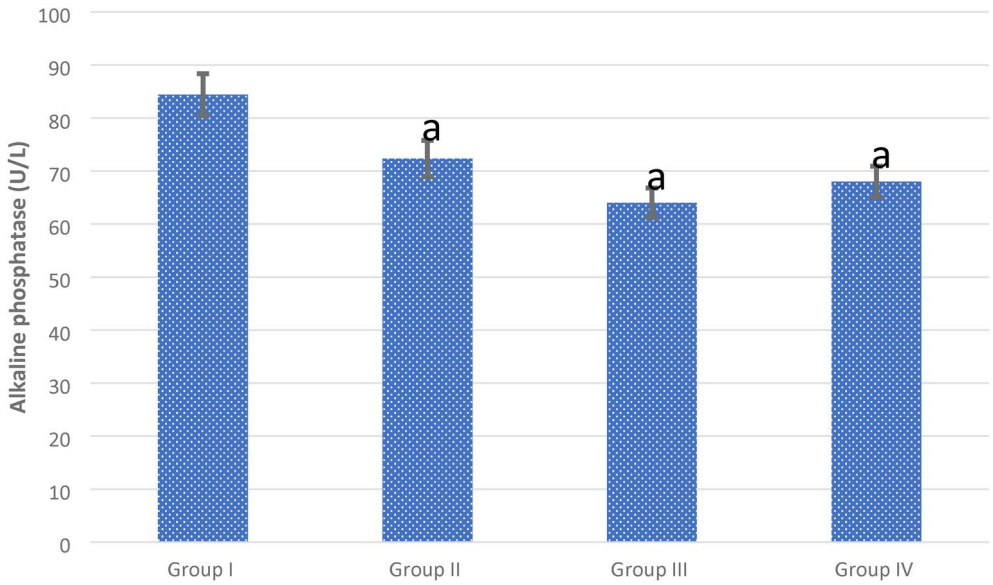

Fig. 3. Alkaline phosphatase level for control, amphetamine, alcohol and combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

compared with control group ($p < 0.05$). On the other hand, the mean alanine aminotransferase levels were significantly decreased in groups II and III compared with group IV ($p < 0.05$).

Serum aspartate aminotransferase

The present study demonstrated that, the mean aspartate aminotransferase levels of the studied groups were 25.76 ± 1.16, 58.63 ± 1.08, 89.05 ± 1.50 and 226.27 ± 2.15 U/L in groups I, II, III and IV respectively. Figure 5 showed that, the mean aspartate aminotransferase levels were significantly elevated in groups II, III and IV

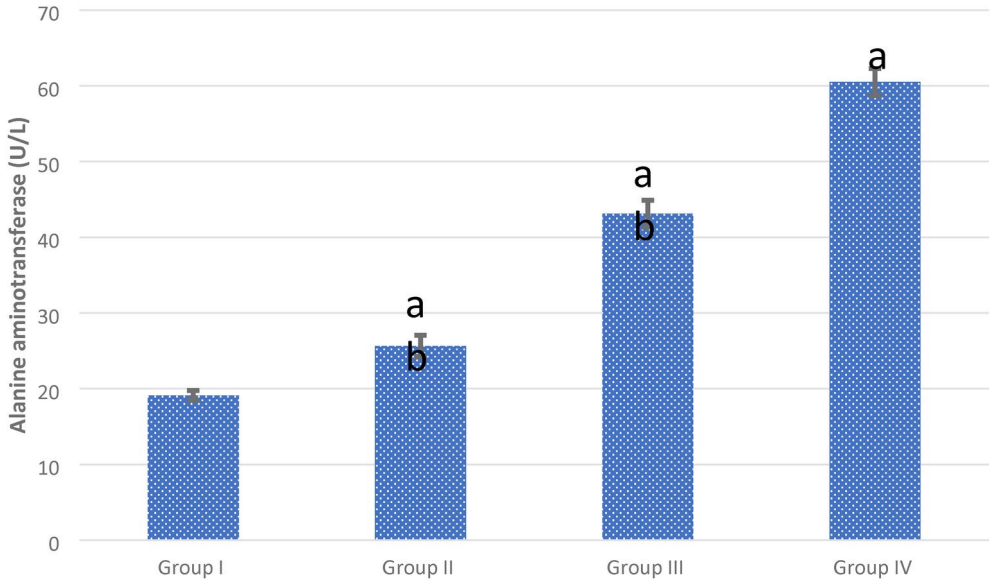

Fig. 4. Alanine aminotransferase level for control, combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

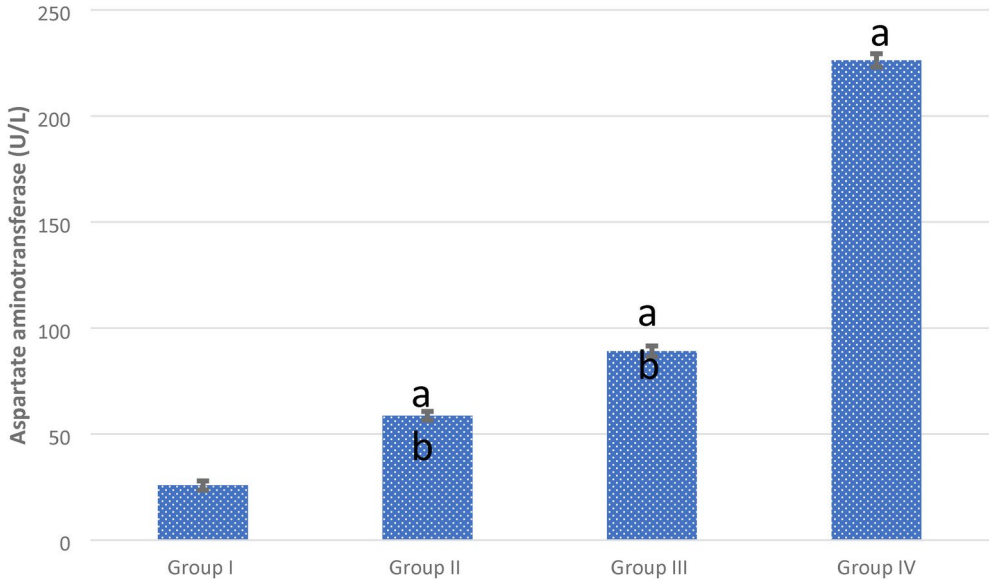

Fig. 5. Aspartate aminotransferase level for control, combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

compared with control group ($p < 0.05$). On the other hand, the mean aspartate aminotransferase levels were significantly decreased in groups II and III compared with group IV ($p < 0.05$).

Fasting blood sugar

This study reported that, the mean fasting blood sugar levels of the studied groups were 4.66 ± 0.20, 4.82 ± 0.21, 5.17 ± 0.21 and 4.70 ± 0.20 mmol/L in groups I, II III and IV respectively. Figure 6 showed that, the mean fasting blood sugar levels showed no significant changed in groups II, III and IV compared with control group ($p < 0.05$). Also, the mean fasting blood sugar levels showed no significant change in groups II and III compared with group IV ($p < 0.05$).

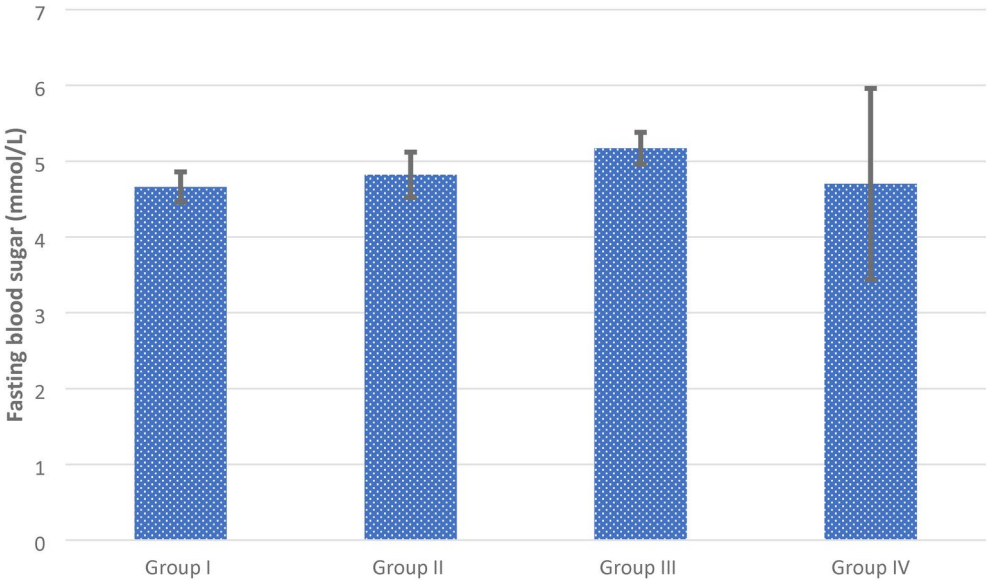

Fig. 6. Fasting blood sugar level for control, combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

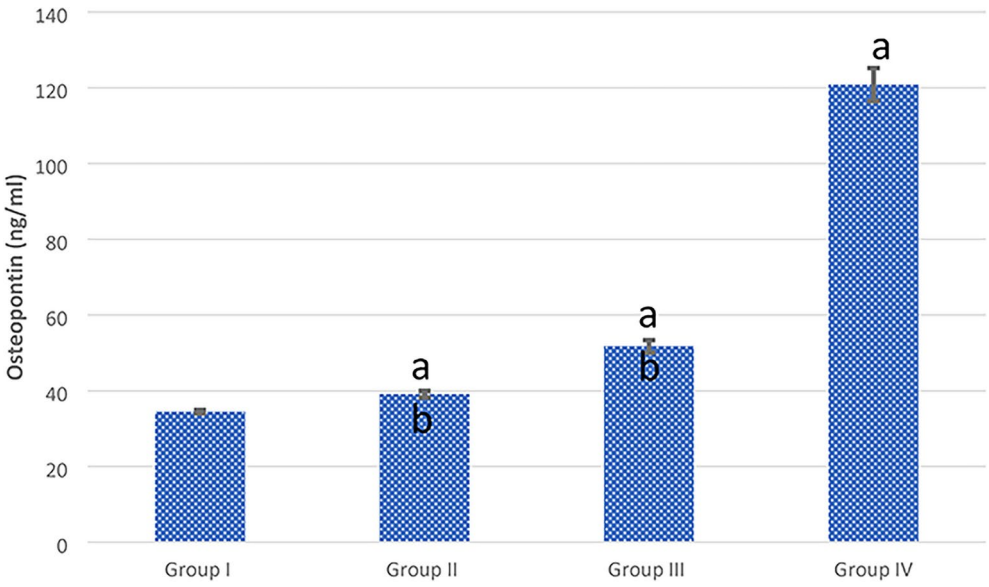

Fig. 7. Osteopontin level for control, combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

Osteopontin

The current study demonstrated that, the mean osteopontin levels were 34.45 ± 0.0.48, 39.10 ± 0.87, 51.75 ± 1.08 and 120.85 ± 4.47 ng/ml in groups I, II and III respectively. Figure 7 showed that, the mean osteopontin levels were significantly elevated in groups II, III and IV compared with control group ($p < 0.05$). On the other hand, the mean osteopontin levels were significantly reduced in groups II and III compared with group IV ($p < 0.05$).

Kidney function tests

Serum urea level

This study demonstrated that, the mean urea levels of the studied groups were 3.68 ± 0.09, 4.62 ± 0.19, 4.17 ± 0.19 and 19.29 ± 1.46 mmol/L in groups I, II III and IV respectively. Figure 8 showed that, the mean urea levels were

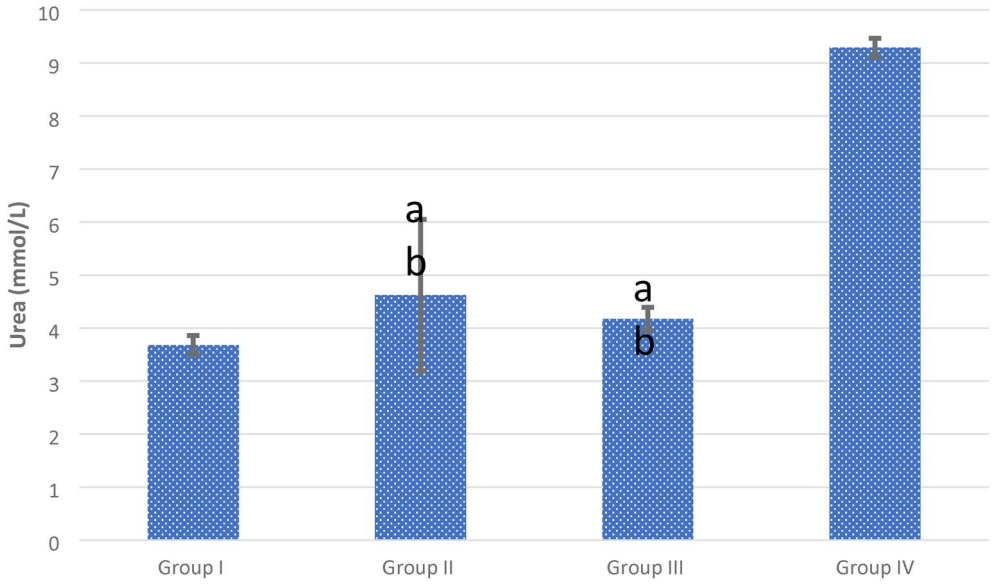

Fig. 8. Serum urea level for control, combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

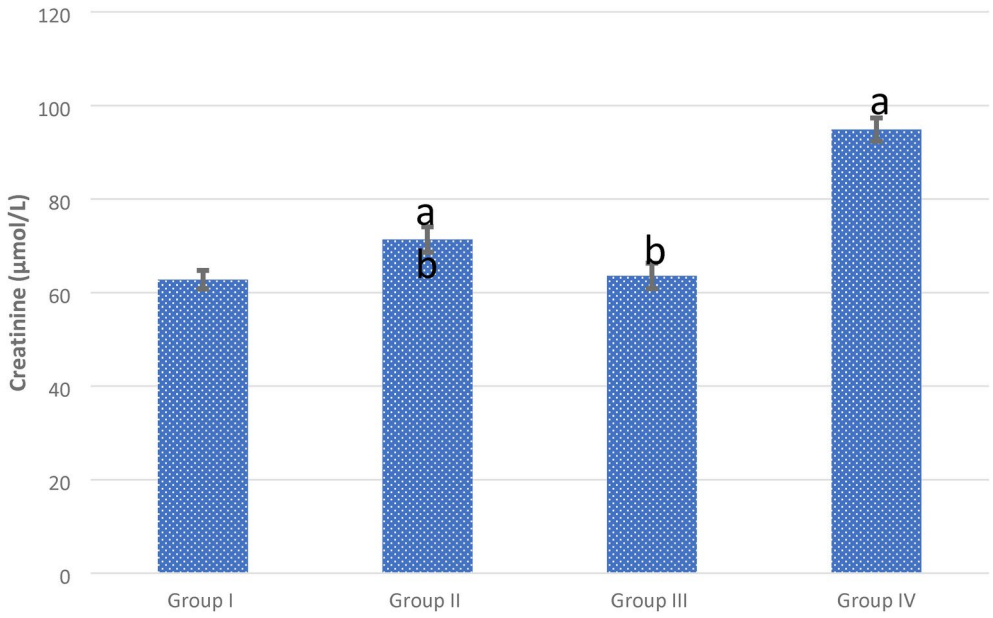

Fig. 9. Serum creatinine level for control, combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

significantly elevated in groups II, III and IV compared with control group ($p < 0.05$). The mean urea levels showed significant reduction in groups II and III compared with group IV ($p < 0.05$).

Serum creatinine level

This study demonstrated that, the mean creatinine levels of the studied groups were 62.77 ± 2.03, 71.31 ± 2.80, 63.56 ± 2.79 and 94.85 ± 2.55 µmol/L in groups I, II, III and IV respectively. Figure 9 showed that, the mean creatinine levels were significantly elevated in groups II and IV compared with control group ($p < 0.05$). On the other hand, the mean creatinine levels showed no significant change in group III compared with control group

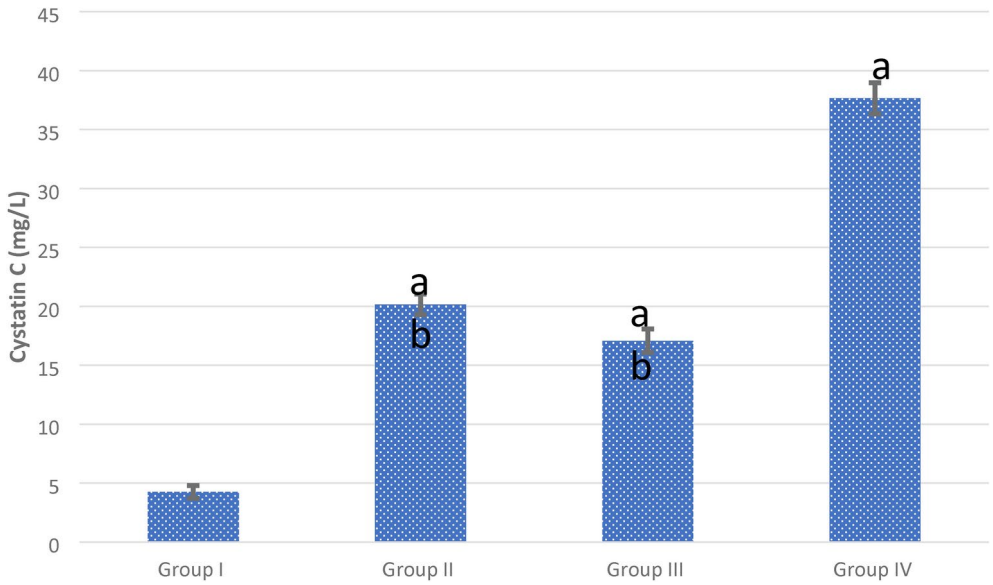

Fig. 10. Serum cystatin C level for control, combined alcohol-amphetamine abuser groups. Group I: Control group; Group II: Amphetamine abusers; Group III: Alcohol abusers and Group IV: Combined alcohol-amphetamine abuser group. N = 100. Values are expressed as mean ± SEM. [a]Significant compared with control group, [b]Significant compared with combined alcohol-amphetamine abuse group, Significant at $p < 0.05$ level.

Items	Amphetamine level			Age			Duration of addiction		
	Group II	Group III	Group IV	Group II	Group III	Group IV	Group II	Group III	Group IV
BMI (kg/m^2)	0.0451	0.231	0.8213**	0.850**	0.798**	0.809**	0.778**	−0.078	−0.082

Table 7. Pearson correlation coefficients between Amphetamine level, Age and duration of Addiction, Body Mass Index (BMI) in amphetamine, alcohol and combined alcohol-amphetamine abuser groups. Group II – Amphetamine abusers; Group III – Alcohol abusers; Group IV- Combined drug abusers (amphetamine and alcohol).; BMI- Body Mass Index. *Correlation was statistically significant at 0.05 level (2-tailed). **Correlation was statistically significant at 0.01 level (2-tailed).

($p < 0.05$). The mean creatinine levels showed significant reduction in groups II and III compared with group IV ($p < 0.05$).

Cystatin C level

The current study demonstrated that, the mean cystatin C levels of the studied groups were 1.69 ± 0.05, 20.16 ± 1.32, 17.07 ± 0.95 and 37.66 ± 0.88 mg/L in groups I, II, III and IV respectively. Figure 10 demonstrated that, the mean cystatin C levels were significantly elevated in groups II, III and IV compared with control group ($p < 0.05$). The mean cystatin C levels demonstrated significant reduction in groups II and III compared with group IV ($p < 0.05$).

Correlations of the studied parameters:

Body mass index (BMI)

Table 6 revealed that, there were no significant correlation between BMI and AMP level in groups II and III and there was significant positive correlation between BMI and AMP level in group III. Although, the present study revealed that, there was significant positive correlation between BMI and Age of groups I, II and III. The present study revealed that, there was significant positive correlation between BMI and duration of addiction in group I. On other hands, there were no significant correlations between BMI and duration of addiction in groups II and III (Table 7).

Liver function tests

Albumin level

The present study demonstrated that, albumin level had no significant correlation in abuser groups with the AMP level. On the other hand, Albumin level had a significant positive correlation with the age of participants and duration of addiction in abuser groups (Table 8).

Items	Amphetamine level			Age			Duration of addiction		
	Group II	Group III	Group IV	Group II	Group III	Group IV	Group II	Group III	Group IV
Albumin (g/L)	0.331	0.187	0.146	0.639**	0.790*	0.777**	0.842**	0.539**	0.659**
ALP (U/L)	0.225	0.046	0.022	0.577**	0.721**	0.871**	0.812*	0.557**	0.586**
ALT (U/L)	0.217	0.098	0.024	0.592*	0.671**	0.876**	0.784**	0.592**	0.621**
AST (U/L)	0.019	0.044	0.033	0.586**	0.743**	0.608**	0.712*	0.566**	0.436**
FBS (mmol/L)	0.226	0.244	0.190	0.735**	0.581**	0.746**	0.863**	0.535**	0.363*
Osteopontin (ng/ml)	0.413	0.322	0.265	0.801**	0.644**	0.598**	0.822**	0.714**	0.523*

Table 8. Pearson correlation coefficients between Amphetamine level, age, duration of addiction, liver function tests in amphetamine, alcohol and combined alcohol-amphetamine abuser groups. Group II – Amphetamine abusers; Group III – Alcohol abusers; Group IV- Combined drug abusers (amphetamine and alcohol).; BMI- Body Mass Index. *Correlation was statistically significant at 0.05 level (2-tailed). **Correlation was statistically significant at 0.01 level (2-tailed).

Items	Amphetamine level			Age			Duration of addiction		
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
Urea (mmol/L)	0.178	0.098	0.514	0.402**	0.380**	0.612**	0.684**	0.413*	0.831**
Creatinine (µmol/L)	0.344	0.014	0.078	0.543**	0.619**	0.703**	0.144	0.342	-0.573
Cystatin C (mg/L)	0.428	0.123	0.098	0.770**	0.689**	0.781**	0.054	0.210	0.228

Table 9. Pearson correlation coefficients between Amphetamine level, Age, duration of Addiction, urea and creatinine levels in amphetamine, alcohol and combined alcohol-amphetamine abuser groups. Group II – Amphetamine abusers; Group III – Alcohol abusers; Group IV- Combined drug abusers (amphetamine and alcohol).; BMI- Body Mass Index. *Correlation was statistically significant at 0.05 level (2-tailed). **Correlation was statistically significant at 0.01 level (2-tailed).

ALP level
This study demonstrated that, ALP level had no significant correlation with the AMP level in abuser groups. On the other hand, ALP level had a significant positive correlation with the age and duration of addiction in abuser groups (Table 8).

ALT level
Table 8 revealed that, ALT level had no significant correlation with the AMP level in abuser groups. On the other hand, ALT level had a significant positive correlation with the age and duration of addiction in abuser groups at the baseline.

AST level
Table 8 revealed that AST level had no significant correlation with the AMP level in abuser groups. On the other hand, AST level had a significant positive correlation with the age and duration of addiction in abuser groups.

FBS level
Table 8 revealed that FBS level had no significant correlation with the AMP level in abuser groups. On the other hand, FBS level had a significant positive correlation with the age and duration of addiction in abuser groups.

Osteopontin level
Table 8 revealed that, Osteopontin level had no significant correlation with the AMP level in abuser groups. On the other hand, Osteopontin level had a significant positive correlation with the age and duration of addiction in abuser groups at the baseline.

Kidney function tests

Serum urea level
Table 9 revealed that, Urea level had no significant correlation with the AMP level in abuser groups. On the other hand, Urea level had a positive significant correlation with the age and duration of addiction in abuser groups.

Serum creatinine level
Table 9 revealed that creatinine level had no significant correlation with AMP level and duration of addiction in abuser groups. On the other hand, creatinine level had a positive significant correlation with the age of abuser groups.

Cystatin C level

Table 9 revealed that, cystatin C level had no significant correlation with AMP level and duration of addiction in abuser groups. On the other hand, cystatin C level had a significant positive correlation with the age of abuser groups.

Discussion

According to this study, all the patients were men. This might be as a result of the fact that men have more freedom than women to leave the house and remain out late, to visit rest spots, and to travel with their peers more readily than women. SUD is seen as a serious issue in Saudi Arabia, and research linking it to psychiatric illnesses, a number of diseases, significant losses in educational and occupational opportunities, as well as the resulting socioeconomic burden, is well- established[32].

This study indicated that the majority of abusers were between the ages of 31 and 40. There has been a surge in SUD among Saudi citizens at this range of ages, according to several reports[33]. An analysis of patients admitted to the Buraidah psychiatric rehabilitation clinic was conducted in the Al-Qassim district. Most abusers were between the ages of 20 and 40, unable to finish their high school education, and thus dropped out. Additionally, 60% of the study's subjects were found to be heavy drug users, primarily of alcohol and AMP. This study found that most abuser groups had married marital status. According to a study, married AMP users were bodybuilders[34].

The most commonly abused substances among Saudis are amphetamines, heroin, alcohol, and cannabis, and a majority of abusers are addicted to multiple substances. The substance that appears most frequently in many polydrug usage repertoires is alcohol. It is essential to be aware of the dangers of combining stimulants and depressants. The blend of these substances can have perilous, even lethal, results[32]. In recent years, combining alcohol with other addictive substances like AMP has become a big public health concern. The perception of amplified euphoric and pleasurable effects and diminished negative subjective effects may contribute to drug dependence. Antioxidant enzyme levels may decline as a result of co-abuse of alcohol and psychostimulants. Alcohol also stimulates inhibitory GABAergic and OPergic neurons while inhibiting excitatory gluergic neurons. Additionally, alcohol may alter the liver's CYP enzymes, which altered the plasma level of medications including AMP. Greater organ toxicity could result from AMP and alcohol exposure. Increased formation of reactive oxygen species (ROS) that change signal transmission or oxidative stress-related damage to cellular macromolecules such lipids, proteins, and DNA, the latter of which results in altered gene expression, may be the cause of this interaction[35,36].

Alcoholism can lead to disorders of the liver as cirrhosis, hepatitis, and fatty liver[34]. The liver performs the majority of the metabolism of alcohol. Alcohol-related negative health effects are primarily liver-related. Alcohol damages the liver in a wide variety of ways. Acetaldehyde is produced when alcohol is metabolized and both substances are harmful to hepatocytes. Due to strong reactivity of acetaldehyde, it can induce a variety of protein and DNA adducts[37,38]. Damaged hepatocytes then emit molecular patterns associated with danger, which attract innate and adaptive immune cells and continue liver damage by causing sterile inflammation[34].

Alcohol can harm the liver and subsequently alters drug metabolism. So, alcoholics are more vulnerable to drug poisoning e.g. AMP. In addition, alcohol causes depletion of glutathione (a hepatoprotective compound) storage brought on by alcohol, a person is more vulnerable to the toxicity of medicines e.g. AMP[34]. Amphetamine can also induce toxic effects on the liver. Amphetamine-induced liver damage might occur due to direct dose-dependent liver damage or auto-immune hepatitis-like injury, which resolves on its own upon drug withdrawal[39,40]. Many cases may be asymptomatic and go undetected[41]. Numerous studies have revealed how AMP affects all the body's organs by increasing free radical generation and oxidative stress[42]. In this study AMP, alcohol and combination groups demonstrated significant increase in alanine aminotransferase and aspartate aminotransferase an indicative of hepatotoxicity and significant decrease in alkaline phosphatase activities. A study reported that alcohol abusers had significantly lower levels of ALP and higher levels of AST, GGT and bilirubin[43]. Our results also confirmed earlier reports in which the authors paid attention to the toxic effects of drugs and alcohol on the liver[44], these results agree with, who observed significantly elevated ALT and AST of alcoholic consumptions to control subjects[45].

The current study revealed that, AMP significantly increases hepatic AST and ALT. This study confirmed earlier reports in which the authors paid attention to the toxic effects of AMP and alcohol on the liver[46]. The study was consistent with the results of our study. They discovered that AMP users had better biochemical liver functioning values than alcoholics[28].

Alcohol abuse is regarded as a potential risk factor for the development of type 2 diabetes mellitus. However, drinking alcohol leads to the dysregulation of a number of metabolic processes, including a failure in the insulin-mediated glucose function of adipocytes and a liver-specific impairment of insulin action. Additionally, the impacts of impaired glucose homeostasis and insulin resistance, which are impacted by changed appetite that controls peptides and neurotrophic factors, are linked to the neuronal profiles of alcoholism[47]. Alcoholism has been linked to increased glucose levels, which raises the risk of metabolic syndrome and diabetes. These data agree with the study's findings. Amphetamine and its derivatives have been shown in numerous investigations to significantly lower blood glucose levels in experimental mice. Another study found that, in part because of a direct impact on the pancreas, AMP increased the secretion of insulin in rats and mice[48]. The present study's findings corroborate past studies about the drop in blood glucose levels. However, our research does not back up the assertion that administering AMP to animals resulted in a momentary increase in blood sugar levels.

The serum albumin levels of the alcohol group significantly decreased, according to the current study. Our findings are consistent with earlier studies that showed acute alcohol consumption in humans significantly decreased plasma total protein and albumin levels[49]. According to data, drinking alcohol may prevent the production of proteins, particularly in heavy drinkers[50]. Despite the fact that AMP poisoning cases have been

documented in the literature. Our findings demonstrated that the AMP group's serum albumin level was much lower, and Nabavizadeha et al.[44] corroborated these findings.

Osteopontin (OPN) is a predictor in a variety of hepatic illnesses. OPN concentrations are substantially correlated with hepatocellular carcinoma, hepatic insufficiency, fibrosis stage and portal hypertension. OPN plays a role in the etiology of numerous chronic hepatic illnesses, including drug-induced liver injury, alcoholic and non-alcoholic steatohepatitis, and viral hepatitis. OPN correlates with alcoholic hepatitis, hepatic inflammation and hepatic fibrosis, as has already been shown in alcoholic patients[51]. These results supported ours and showed that patients with alcoholic hepatitis had higher OPN levels.

There is debate over whether drinking excessively leads to kidney damage. The suggested mechanism is oxidative stress, which causes an excessive amount of free radical production, increasing inflammation and causing tissue damage[52]. The combined abuse of alcohol and AMP may severely elevate the liberation of free radical and induce impairment of kidney function tests[53]. Our investigation established a link between the disordered use of alcohol and AMP and abnormal kidney function tests.

Patients with liver impairment may benefit from using Cystatin C, a novel serum biomarker that increases in the presence of kidney disease, as a better surrogate marker to measure kidney function[9]. These data support our findings.

Hyperpyrexia and fibrinolysis are linked to the renal side effects of AMP use disorder. Renal failure can also be brought on by microvascular blockage brought on by myoglobinuria, systemic hypotension, or hyperpyrexia. Amphetamine use may result in increased oxidative stress or mitochondrial malfunction, which can lead to tubular damage in kidney tissue[54,55]. Combined abuse causes greater biochemical alterations.

Conclusion

The outcome of this study revealed that the addiction of alcohol, AMP and combined AMP plus alcohol was associated hepatotoxicity and nephrotoxicity. Amphetamine-alcohol co-abuse significantly heightens kidney and liver toxicity. Addiction of combined AMP plus alcohol had a synergistic hepatotoxicity and nephrotoxicity**;** so therapeutic plan should be focusing on how to manage this synergistic hepatotoxicity and nephrotoxicity. Understanding the dangers implied can assist people with pursuing informed choices and look for suitable assistance and treatment.

Data availability

Data is provided within the manuscript file.

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Author contributions

All authors contributed to the study conception and design. Material preparation, collection of samples and laboratory analysis was performed by Sharifah Alharbi and Ashraf Mahmoud Emara. Analysis and interpreted of patient's data was carried out Maha A Aldubayan, Ahmad H Alhowail, Yasser S. Almogbel and Ashraf Mahmoud Emara. The first draft of the manuscript was written by Ashraf Mahmoud Emara and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval
The current study was approved by the College of Pharmacy, the University Ethics Committee and the Research Ethics Committee at Ministry of Health, Saudi Arabia (approval ID No. 1442–156546), and was conducted in line with the Declaration of Helsinki.

Consent to participate
Written informed consent was obtained from each participant after explaining to them the purpose of the study.

Consent to publish
All authors approved publication of this article.

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