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THE EFFECTS OF CRYSTAL METH ON THE LIVER AND KIDNEY FUNCTION PARAMETERS OF WISTAR ALBINO RATS

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ABSTRACT

The aim of this research is to evaluate the effects of administering crystal meth via oral, nasal and intraperitoneal routes on the liver function and renal toxicity parameters using wistar rats. A total of 35 wistar rats were split into 7 groups and administered two doses of the sample (40 and 1000mg/70kg) via 3 different routes for 21days. The animals were weighed 4 times before sacrifice and biochemical analysis. The group orally administered 1000mg/70kg showed the highest percentage reduction in weight 24.4% compared to day 1. The ALP and ALT levels showed significant elevations across all groups when compared to control. However, the group intraperitoneally administered 1000mg/70kg showed the highest increase in ALP activity levels when compared to control while the highest increase in AST activity levels were recorded in groups orally administered 1000mg/70kg. The total protein, serum creatinine and AST levels showed no significant fluctuations. The highest recorded increase in blood urea and serum creatinine levels were recorded in the group intraperitoneally administered 1000mg/70kg. The result showed that crystal meth increased the renal toxicity parameters and some liver function parameters especially in high doses, therefore can cause major damage to either or both organs if exposure prolonged.

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INTRODUCTION

The most abused substances in the world include hard drugs and psycho stimulants like opiates, cocaine, and cannabis (Rehm et al., 2010). Stimulants or psychostimulants are used to refer to many drugs including those that increase activity of the central nervous system or drugs that have effects that are sympathomimetic (Farzam et al., 2023). Methamphetamine is odorless and mostly without color; but exists in powder, paste and crystal form; and it could be administered via intranasal sniffing, oral ingestion, inhalation, intraperitoneal or intravenous injection (Rawson et al, 2007). Nigeria has emerged over the past decade as a significant producer of crystal meth (Mouhamadou, 2019). Since the National Drug Law Enforcement Agency's (NDLEA) first discovered of an illicitly concealed meth laboratory in Lagos in 2011; the proliferation of such labs and the trafficking of crystal meth has become exponential. Not less than 17 more meth labs have been dismantled elsewhere in the country since the first discovery with the amount of seized crystal meth rising from about 177 kg in 2012, to 1.3 tons in 2017 (Mouhamadou, 2019). The rate of crystal meth abuse is rising globally and in Nigeria, whereas exhaustive information of its effects

on the vital metabolic organs remains elusive. There is need therefore, to evaluate its effect on the kidney, liver by evaluating the kidney and liver function parameters. Some drugs like desoxyne possess crystal meth as an active ingredient and are prescribed legally; therefore it is necessary to investigate what such a prescription might affect the liver and the kidney of the patients.

MATERIALS AND METHODS

Materials: The equipments used were 3015 Single Chamber Waterjacketed Laboratory Incubator, Sectrophotometer (KJ – 721G China), Centrifuge (PEC – MEDICAL USA), Micro-hematocrit Centrifuge, Electronic Weighing, Balance (Model: Adam AFP 800L), Randox test kits The reagents / chemicals used were Chloroform (Tedia company INC, China), Water (Coca cola company, Nigeria), Randox reagents and all other reagents used are of analytical grade.

Methods

Site of Study: The study was carried out at Applied Biochemistry Laboratory of Nnamdi Azikiwe University, Awka, Anambra state.

Duration of Study: The study was completed in six weeks. Four weeks for the *in vivo* study, collection of results and statistical analysis.

Collection of Sample and Identification: Sample, crystal meth was acquired from the HQ National Drug Law Enforcement Agency (NDLEA) Kwata, Awka, Anambra state command.

Preparation of Crystal meth Stock Solution: Stock solutions 0.08mg/ml and 2mg/ml were prepared for the low and high doses of administrations respectively. Crystal meth (8mg) was weighed and dissolved in 100ml of distilled water by continuous stir in a bid to prepare the 0.08mg/ml stock solution; while 20mg (0.02g) was dissolved in 10ml of distilled water to prepare the 2mg/ml stock solution.

Animal Studies

Purchase, Acclimatization and Feeding of Animals: The animals were purchased from Onyebuchi Animal Farm and Research Laboratory, Ifite-Awka, Anambra state. A total of 62 adult albino wistar rats were purchased and allowed to acclimatize for seven days while being fed and given water *ad libitum*. Thirty eight animals were utilized for acute toxicity studies via intraperitoneal route using modified Lorke's method while thirty five were tested.

Ethical Approval: All experimental protocol were approved by aREC, Nnamdi Azikiwe University Awka, Anambra state, Nigeria. The approval number obtained is NAU/AREC/2023/00064.

Administration: Syringes were obtained and used to administer different doses of the substance orally and intraperitoneally from the same stock solution. However, a digestion flask, pipe, a bunsen burner and a partially airtight container were used to administer the substance via inhalation. Measured volumes of the stock solution was poured into the digestion flask and then cocked with pipe channeled into the container where the animals were chambered. As heat was applied to the digestion flask, the contents vaporized into the chamber where the animals were kept. The animals inhaled the vapors.

Acute Toxicity Study (LD₅₀): The median lethal dose (LD₅₀) was determined using modified lorke's method as described by Sani et al. (2020). It was carried out in three phases; the initial phase which had four groups of three rats each and was administered 5, 15, 25 and 40mg/70kg of crystal meth. The animals were monitored within the first four hours for signs of toxicity such as sniffing, restlessness, hyperactivity, leaking of paws, defecation, stretching and protrusion of the eye balls, calmness and death. The symptoms highlighted above were nonexistent so the study proceeded into the second phase. In this phase, there were four groups with three rats each. These were administered 80, 150, 250 and 350mg/70kg of the sample and monitored as well. The absence of the highlighted symptoms necessitated the third and final phase with five groups of three rats each. These were administered 600, 1000, 2000, 2500, and 3500 mg/70kg of the crystal meth and monitored. Casualties were recorded and the LD₅₀ was calculated by obtaining the geometric mean of the Highest Non Lethal Dose and the Lowest Lethal Dose that caused death.

Grouping of Animals: The rats were divided into five groups of five rats each. A total of twenty five rats were used for this study, and for the duration of 28days. The groups were as enumerated below:

Group A (Control group 0mg/70kg), Group B (Orally administered 1.5% of the LD_{50} at 40mg/70kg, Group C (Orally administered 38% of the LD_{50} at 1000mg/70kg, Group D (Intraperitoneally administered 1.5% of LD_{50} at 40mg/70kg), Group E (Intraperitoneally administered 38% of LD_{50} at 1000mg/70kg), Group F (Inhaled 40mg/70kg of Crystal meth), and Group G(Inhaled 1000mg/kg of Crystal meth).

Indices of growth parameters measured: The initial weights of the animals were taken after acclimatization. The weights were taken four more times in a five days interval using electronic weighing balance.

Sacrifice and blood collection: The animals were anaesthetized with chloroform and blood was collected via closed cardiac puncture. The blood samples for hematological analysis were placed in EDTA bottles while those for other analysis will be collected using universal bottles and allowed to clot then centrifuged for 15 minutes at 4000rpm to separate the serum.

Biochemical Analysis

Liver Function Tests: The blood samples were collected with universal bottles and centrifuged at 4000rpm for 15mins to obtain the serum which was used for the liver function test. The Aspartate Aminotransferase (AST) was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2,4dinitrophenylhydrazine at 546nm. According to Limdi and Hyde (2003), an aliquote of the serum (0.1ml) was mixed with 0.5ml of Randox AST R1 buffer containing 100mmol phosphate buffer, 100mmol L-aspartate and 2mmol α-oxoglutarate. This was allowed to stand for 30mins at room temperature followed by the addition of 0.5ml of 2mmol 2, 4 - dinitrophenylhydrazine. After 20mins, 5ml of 0.4M NaOH was added and the absorbance was taking at 546nm after 5min. the concentration of AST in the serum was calculated from the standard values given by Randox. The Alanine Aminotransferase was determined by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nm. As reported by Limdi and Hyde (2003), an Aliquote of the serum (0.1ml) was mixed with 0.5ml of Randox ALT R1 buffer containing 100mmol phosphate buffer, 200mmol L-alanine and 2mmol a-oxoglutarate. This was allowed to stand for 30mins at room temperature followed by the addition of 0.5ml of 2mmol 2,4-dinitrophenylhydrazine. After 20mins, 5ml of 0.4M NaOH was added and the absorbance was taking at 546nm after 5min. the concentration of ALT in the serum was calculated from the standard values given by Randox. Alkaline phosphatase (ALP) hydrolyses p-nitrophenylphosphate to producephosphate and p-nitrophenol. In the assay conducted using the method reported by Limdi and Hyde (2003), 20µL of the serum was mixed with 1ml of 10mmol/L p-nitrophenylphosphate in 1mol/l Diethanolamine buffer. The initial absorbance was read immediately with Axiom 752 UV-VIS spectrophotometer at 405nm, and then the absorbance was taken again after a minute, 2minutes and 3minutes. The ALP activity was calculated as follows: ALP (U/L) = 2760 ΔA_{405} nm/min. Where ΔA_{405} = change in absorbance at 405nm. The albumin test was conducted using the method reported by Limdi and Hyde (2003). This is based on its quantitative binding to the indicator 3, 3', 5, 5' - tetrabromo - m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample. An Aliquote of the serum (10µL) was mixed with 3ml of BCG reagent. This was allowed to stand for 5mins at 30°C. The absorbance was read at 578nm. Total protein was determined using Biuret method (Tonog, P. and Lakhkar, D.A. 2022). The serum (0.1ml) was dilute in 0.9ml of normal saline. Blank Biuret reagent (5ml) was added to samples and were mixed well and allowed to stand for 20 min at room temperature 27°C. Absorbance was read for one test and standard against a blank at 540 nm.

Renal Toxicity Assay: The blood was centrifuged at 4000rpm for 15mins and the serum was used to assay for the renal function enzymes. The renal toxicitytest was determined spectrophotometrically according to the method of Limdi and Hyde (2003). The kidney function parameters assayed were serum creatinine and serum urea. Creatinine was determined based on its reaction with picric acid to form a coloured complex. An aliquot of the serum (50µL) was mixed with 0.5ml of randox reagent containing 35mmol/l picric acid and 0.32mol/l NaOH. This was read with autoanalyzer and the concentration of creatinine displayed on the machine was recorded. For serum urea, an aliquot of the serum $(5\mu L)$ was mixed with 50µL of Randox reagent containing 116mmol/L sodium nitroprusside and 6mmol/L urease. It was allowed to stay for 10mins at 37°C after which 1.25ml of 120mmol/l phenol and 27mmol/l sodium hypochlorite was added and allowed to stay for

 $15 \mathrm{mins}$ at $37^{\mathrm{o}}\mathrm{C}.$ The concentration of urea was then recorded with auto analyzer.

Statistical Analysis

The findings; from the discrepancies in weight, to those of biochemical parameters; are presented in tables after being analyzed with SPSS (Statistical Package for Social Sciences) version 25 to facilitate interpretation.

RESULTS

The results obtained from the investigations are presented below in figures and tables.

Acute Toxicity Test: The result of a 24 hour acute toxicity test of intraperitoneally administered crystal meth is shown in Figure 1. In the first phase, the groups administered 5, 15, 25 and 40mg/70kg did not show any signs of toxicity. So also did the groups; in the second phase, administered 80, 150, 250 and 350 mg/70kg; not show any signs of toxicity. In the third and final phase, the groups administered 2000mg/70kg showed signs of mild toxicity, groups administered 2500mg/70kg reported a 67% mortality rate within 24 hour while those administered 3500 mg/70kg showed 50% mortality. The lethal dose (LD₅₀) of crystal meth was calculated and found to be 2645.8 mg/70kg.

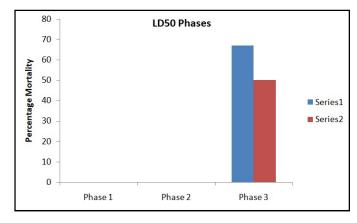


Figure 1. Result of the acute toxicity study (LD₅₀)

an increase in weight with the groups intraperitoneally administered 1000mg/70kg gaining approximately 20g with the highest increase percentage of 15.9% as shown in Figure 2 and Table 1.

Effects of Crystal Meth on the Hepatic Parameters of Rats: The liver function parameters of the wistar rats which include the Aspartate-amino Transferase (AST), Alanine-amino Transferase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP) and Serum Albumin (ALB) are presented in figure 3 and table 2. The ALT level of the control group falls within the reference range of <56 as reported by Clevland Clinic (2021). The results also show that all values obtained for ALT among the groups were significantly (p<0.05) higher than the control. The highest value among the test samples of 31.70 ± 2.60 (U/L) was obtained for 1000mg/70kg orally administered while the least value of 18.83 ± 1.79 (U/L) was obtained for groups administered 40mg/70kg administered through inhalation. This shows that the substance has the most pronounced effect on the ALT when administered orally than through other routes at the dosage of 1000mg/70kg. The AST range of the control group all fell within the reference range of 5 to 33 as reported by Kalas et al. (2021). The result shows no significant alteration (p>0.05) in the aspartate aminotransferase (AST) levels of the rats orally, nasally and intraperitoneally administered 40mg/70kg and 1000mg/70kg of the aqueous crystal meth solution when compared to the control. This signifies that the substance has no effect on the AST levels when administered through any of the three routes and at both low and high doses.

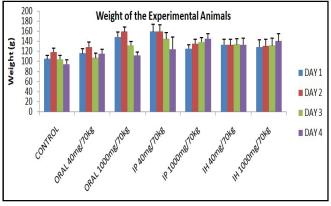


Figure 2. Weight profile of the Animals administered 40mg/70kg and 1000mg/70kg Crystal meth

Table 1. Percentage Difference when compared to Day 1

SN	NORMAL	ORAL 40mg	ORAL 1000mg	IP 40mg	IP 1000mg	IH 40mg	IH 1000mg
Day 2	12.93%	11.41%	7.22%	0.11%	7.91%	0.12%	1.17%
Day 3	40%	7.62%	10.90%	9.08%	10.38%	0.67%	2.06%
Day 4	9.7 <mark>%</mark>	0.09%	24.5%	21.7%	15.9%	0.6%	8.99%

%Percentage Decrease

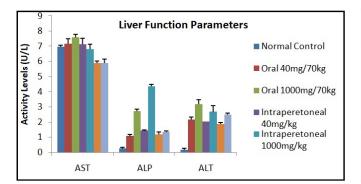
%Percentage Increase

IP Intraperitoneal IH Inhalation

Body Weight Profile: The average body weights of the rats administered 40mg/70kg and 1000mg/70kg doses of crystal meth via oral, nasal and intraperitoneal routes are expressed in Table 2. The results show that there were no significant alteration (p>0.05) in the weight of the animals administered 40mg/70kg of Crystal meth. However; besides those orally administered 1000mg/70kg of crystal meth which showed significant decrease (p<0.05) of 26.9g and 46.9g respectively in days 3 and 4 when compared to day2, the results show no significant alteration (p>0.05) in the body weight of the animals administered 1000mg/70kg of crystal meth as shown in Figure 2. The highest reduction in weight was recorded in groups orally administered 1000mg/70kg of the crystal meth solution which reduced by a value of 36.15g and with a reduction percentage of 24.4%, followed by those intraperitoneally and orally administered 40mg/70kg of the crystal meth solution with reduction percentages of 21.7% and 0.09% respectively. However the other groups showed

The ALP range of the control group all fell a little below the reference range of 0.44 to 1.74 as reported by Cleveland Clinic (2023). All test groups however showed varying increase in the alkaline phosphate (ALP) activity when compared with the control but only those orally and intraperitoneally administered 1000mg/70kg of the crystal meth solution were statistically significant (p<0.05) when compared to the control. The highest value of 4.35 ± 0.74 was obtained for the groups' intraperitonally administered 1000mg/70kg of the substance while those orally administered 1000mg/70kg had a statistically significant value of 2.72 ± 0.32 . It means that the substance affects the ALP only at a dose of 1000mg/70kg administered via oral and intraperitoneal routes. The TP range of the control group all fell within the reference range of 6.0 to 8.3 as reported by Novkovic (2021). The total protein (TP) test result reveals no significant alteration (p>0.05) in the total protein concentration of the test groups administered 40mg/70kg and 1000mg/70kg of the crystal meth solution via all the routes when

compared with the control. Therefore, it is correct to say that the substance does not affect the TP levels when administered through any of the three routes and at either dose. The ALB range of the control group all fell within the reference range of 3.5 to 5.0 as reported by Devaraj (2022). The albumin levels of the test groups administered 40mg/70kg and 1000mg/70kg of the solution via all the routes showed no significant (p<0.05) alterations when compared with the control. This also indicates that the substance does not affect the ALB concentration when administered at either dose and through any of the routes as shown in Figure 3 and Table 2.



Kidney Function Parameters: The mean value for blood urea levels of the control group; 12.48 ± 0.39 ; falls within the reference range as reported by Hosten (1990). The results of the urea levels show a significant increase (p<0.05) in all the groups administered 40mg/70kg of the crystal meth solution when compared to control group except for those orally administered. The highest value among the test samples with a value of 24.47 ± 1.99 was obtained for groups intraperitoneally administered 1000mg/70kg, while the lowest statistically significantly value was obtained in groups

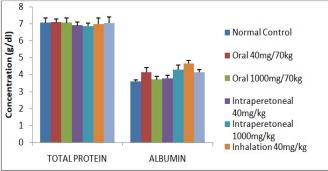


Figure 3. The effect of crystal meth on the AST, ALP and ALT levels

Figure 4. The effect of crystal meth on the Total Protein and Serum Albumin

Table 2.	Percentage Difference when compared to Control

Groups (mg/70kg)	AST	ALT	ALP	TP	ALB
Control	6.98±1.0U/L	1.70±0.79U/L	0.25±19U/L	7.05±0.87U/L	3.59±0.36U/L
Oral 40mg/70kg	2.87%	1.2×10 ³ %	328%	40%	15.04%
Oral 1000mg/70kg	8.88%	1.8×10 ³ %	988%	0.14%	3.06%
IP 40mg/70kg	2.15%	1.1×10 ³ %	468%	2.13%	5.01%
IP 1000mg/70kg	2.44%	1.1×10 ³ %	1640%	2.7%	19.78%
IH 40mg/70kg	15.76%	1.0×10 ³ %	376%	0.99%	29.81%
IH 1000mg/70kg	15.47%	1.4×10 ³ %	436%	0.14%	15.32%
Reference Range	5-33	< 56	0.44 - 1.74	6.0 - 8.3	3.5 - 5.0

%Percentage Decrease

%Percentage Increase

IP Intraperitoneal IH Inhalation

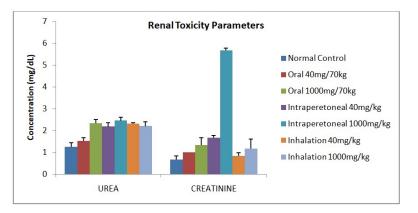


Figure 5. Renal Function Profile after Crystal meth Administration

Table 3. Percentage Difference when compared to Control

Groups	Urea	Creatinine
Normal Control	12.48mg/dL	0.67mg/Dl
Oral 40	22.68%	49.25%
Oral 1000	87.66%	98.51%
IP 40	74.60%	149.25%
IP1000	96.07%	746.27%
IH 40	86.3%	23.88%
IH 1000	75.96%	74.63%
Reference Range	5.0-20mg/dL	0.74-1.35mg/dL

intraperitoneally administered 40mg/70kg with a value of 21.79 \pm 2.87. Also, significant increase (p<0.05) was also recorded in the urea levels of all the groups administered 1000mg/70kg of the solution when compared to the control group. The serum creatinine levels of the control group with a mean value of 0.67 ± 0.29 falls within the reference range as reported by Mayo Clinic (2023). However, except for the groups intraperitoneally administered 1000mg/70kg, the creatinine levels of all the groups administered crystal meth showed no significant alterations (p<0.05) when compared with the control group. The creatinine levels of the groups administered 1000mg/70kg of the crystal meth solution orally and through inhalation showed no significant alteration as well when compared to the control group. The group intraperitoneally administered 1000mg/70kg of the crystal meth solution showed a significant increase (p<0.05) in their levels of creatinine when compared to the control group. Among the test groups, the highest value of 5.67 ± 1.17 was obtained for groups intraperitoneally administered 1000mg/70kg while the lowest value of 0.83 ± 0.29 was obtained for groups administered 40mg/70kg via inhalation. It is therefore evident that high dose intraperitoneal administration has the most effects the creatinine levels.

DISCUSSION AND CONCLUSION

The acute toxicity study revealed the median lethal dose to be 2645.8mg/70kg (37.797mg/kg) via intraperitoneal administration. The LD_{50} result contradicts Funahashi *et al.* (1988) with a lethal dose report of 95mg/kg for orally administered Crystal meth. The discrepancy might be as a result of the difference in the route of administration and the significance of first pass effect. The first pass effect is a phenomenon of drug metabolism that causes a reduction in the concentration of the active drug, specifically when administered orally (Maheshwari et al., 2018). Therefore a higher oral dose is needed to illicit the same effects when compared to other routes of administration that bypasses the first pass phenomenon. According to Lorke (1983), substances and extracts with an LD₅₀ value below 5g/kg are declared toxic whereas those with an LD₅₀ above are absolutely nontoxic. Therefore, it can be said that crystal meth is toxic at high doses. The reduction percentage in weight was highest in groups administered high oral doses (1000mg/70kg) at 29.6%, followed by those intraperitoneally administered 40mg/70kg with a reduction percentage of 21.7%. The 29.6% reduction is statistically significant confirming that oral consumption of the substance reduced the weights of these animals more than those administered nasally or intraperitoneally. This observation agrees with Yasaei and Saadabadi (2022) who states a gross reduction in the weight of crystal meth users as one of the long term effects of crystal meth consumption. According to American Addiction Centers Editorial Staff (2023), crystal meth users experience a severe loss of appetite and a sped up metabolism leading to significant weight loss.

Crystal meth has also shown to be able affect organ-system functions. Although the Aspartate Transaminase (AST), Albumin levels and Total Protein (TP) levels remained normal, the Alkaline Phosphatase (ALP), and Alanine Transaminase (ALT) levels showed significant differences when compared to the control. According to Lala et al, 2022, abnormal levels of ALP in the blood might be a sign of a wide range of health maladies, including liver disease, chronic kidney disease and bone disorders. Therefore the significant increase noticed in groups administered 1000mg/70kg might be attributed to the onset of one of those health conditions. Recall that it was only the oral and intraperitoneal routes that showed the significant difference in ALP levels. Although Hassan et al. (2018) reports that the normal Alanine Transaminase (ALT) range for rats is 10U/L to 30U/L, the ALT levels of the samples remained significantly (p < 0.05) high when compared to the control group. This results agrees with Kerrigan et al.(2016) who reports that crystal meth abuse increased ALT and AST levels. Also, Lasker et al. (2019) noted the normal ALT levels to be 6U/L to 8U/L in his yogurt syndrome and oxidative stress induced experiment with stressed ALT levels at 17U/L. In summary it can be hypothesized that crystal meth stresses the liver and if prolonged, might damage them irrevocably.

Significant increase in renal toxicity parameters observed especially in groups administered high doses (1000mg/70kg) shows that the kidney is affected when the substance is consumed, especially in high doses. The significant increase seen in the blood urea levels of all the test groups when compared to the control is indicative of renal toxicity. This finding agrees with Kala et al. (2007) who reports an increase in urea levels of chronic crystal meth abusers when compared to the control subjects. According to Bishop et al (2010), elevated levels in the blood of byproducts of protein metabolism such as urea and creatinine; signals the onset of uremia. Only groups intraperitoneally administered 1000mg/70kg displayed a statistically significant (p<0.05) elevation in the serum creatinine level when compared to the control group. Therefore high intravenous or intraperitoneal administrations can be said to pose the most threat against the kidney; especially in high doses. This is partly in agreement with the report of Torrecilla et al. (2018) who recorded increases in the creatinine levels of exposed test groups when compared to the control group. However, the result affirms that the least nephrotoxic route is via oral administration because low doses did little to significantly alter the kidney function parameters. This gives a benign nod to those drugs like Desoxyn which is are crystal meth hydrochloride tablets. Also, drugs like adderall, vyvanse, ridlin and concerta; with amphetamine ingredients, have also been produced and is currently in circulation.

CONCLUSION

The results show that crystal meth deteriorates the liver and the kidney of abusers and users by elevating the liver and renal toxicity parameters. It has also shown to be able to cause a loss in the body weight especially if orally administered and in high doses, so users and abusers can be identified with such symptoms. It can be assumed that if abuse is prolonged, the substance can and will damage either organs, or both. It is evident from the result that low doses taken orally is the least nephrotoxic, however prescription drugs with crystal meth active ingredients like dexoxyne should remain strictly regulated. Summarily, this research reveals that notwithstanding reason, consumption of crystal meth does more to harm the body than it does to aid it.

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