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Acute Toxicity of a Novel Class of Hallucinogen "Voodoo" (Clinical and Experimental Study)

Marwa A. Abass[1] , Mohamad Z M Hassan[2], Manal R Abd Elhaleem[3], Hesham R Abd Elaziz and Rehab H. Abd-Allah[4]

[1] Departments of Forensic Medicine and Clinical Toxicology
[2] Departments of Histology and Cell Biology
[3] Departments of Pathology
[4] Departments of Phamacognosy of Pharmacy

Faculty of Medicine, Zagazig University, Al sharqia, Egypt.

Abstract **Background:** Voodoo is a newly emerged hallucinogenic substance in Egypt that target the youth aged 15-30 years causing many reported cases of acute toxicity. This made the Egyptian Ministry of Health in 2014 to list it in drug schedule 1 and warned traffickers and users that they are now under criminal penalties. **Aim**: this work was conducted to investigate the acute toxic effects of this hallucinogenic substance on human and experimental animals. **Methods**: this work included both clinical and experimental studies. The clinical study included 17 patients with acute Voodoo poisoning admitted to Poisoning Control Unit - Zagazig University Hospitals between July, 2015 and April, 2016. The experimental study included forty adult male albino rats were used for calculation of LD50 of Voodoo extract. The extract was prepared using Gas chromatography/ Mass spectrophotometry (GC/MS) to be given intra peritoneally to experimental animals. **Results**: the patients' main complaints were hallucination, disorientation and extreme fear of death, a picture resemble acute cannabis poisoning but with negative urinary screening test for cannabis and other common addictive substances. GC/MS analysis revealed the chief substance in the extract (54.54%) was a chemical analogue of PB 22; a designer synthetic cannabinoids. LD 50 of the extract was estimated to be 1334 mg/Kg. Liver, kidneys and brain were the most affected studied organs. The liver showed severe congestion and macro vesicular steatosis with diffuse intracytoplasmic esinophlic bodies. Kidneys showed focal vacuolar degeneration of renal tubules, accentuation of glomerular basement membrane, hyallinosis of renal tubules with large areas of epithelial necrosis. Brain cells were markedly shrunken and vacuolated. **Conclusion**: it could be concluded that Voodoo proved to have many toxic effects on human and experimental animals and further studies are warranted to evaluate other toxic effects of this substance.

Keywords Acute toxicity, Hallucinogen, LD 50, Synthetic cannabinoids, Voodoo extract.

Introduction

Cannabis sativa has been used medicinally and recreationally for centuries, and today, it remains one of the most abused drugs in the world (Tims et al., 2002). Africa is the second largest producer of herbal cannabis in the world. In Egypt, drug addiction is considered one of the serious problems to the community. It affects young people in the productive years. It may lead to many problems such as social problems, decreased works productivity and car accidents (Yassa et al., 2010).

The principal active and addictive constituent of marijuana, Δ-9-tetrahydrocannabinol (Δ-9-THC), induces its psychoactive properties through stimulation of cannabinoid CB1receptors, which are located primarily in the central nervous system (Svizenska et al., 2008).

Many other classical and nonclassical cannabinoids have been investigated for therapeutic use. Synthetic cannabinoids such as 1-naphthalenyl-1(1-pentyl-1H-indol-3-yl) methanone (JWH-018) and

(quinolin-8ly-1-pentyl-1-H-indole-3-carboxylate (PB 22) have recently emerged as new drugs of abuse and sold under brand names such as K2 and SPICE. These products are potent CB-receptor agonists, and recent clinical data provide evidence of development of anxiety (Schneir et al., 2011), tolerance (Zimmermann et al., 2009), and enhancement of psychoses (Every-Palmer, 2010) in individuals using these products. Voodoo is a concoction of herbs and spices that are sprayed with a synthetic compound that mimics the effects of THC.

Voodoo spice is marketed as a herbal incense. The Egyptian Ministry of Health warned against this drug that has recently spread in Egypt. This drug carries hidden danger to many people due to its great similarity to incense. The Egyptian Ministry of Health considered Voodoo as a highly hazardous addicting substance and listed it in schedule 1 and warned traffickers and users that they are now under criminal penalties (Egypt drug regulatory authority, 2014). This work was conducted to investigate the acute toxic effects of this hallucinogenic substance on human and experimental animals.

Methodology

I- Clinical Study:

A- Setting of the study

This study was a prospective study that included all consecutive patients presented with acute Voodoo poisoning, they were admitted to the Poisoning Control Unit (PCU)- Zagazig University Hospitals from July 2015 to April 2016. Patients and/ or their relatives gave history of either ingestion or smoking of a spice marketed as innocent herbal incense. Street name of this incense was Voodoo. Voodoo sold in metal-foil sachets, typically containing 3 gm of dried vegetable matter (Light green buds/herbal matter). The inclusion criteria included patients who reported single Voodoo poisoning. Exclusion criteria included any patient with positive history of preexisting hepatic renal or neurologic diseases or on other hepatoxic, nephrotoxic, or neurotoxic medication.

B- Study Groups

1- Patient Group: according to inclusion and exclusion criteria only 17 patients were enrolled in this study.

2- Control group: consisted of 20 apparently healthy individuals who are age and sex-matched admitted to the hospital for unrelated clinical conditions.

Informed consents from all participants were obtained and the Ethical Committee for Research, Faculty of Medicine, Zagazig University approved the study protocol to assume confidentiality and privacy.

C- Methods

1- History taking

Thorough history taking was done for all patients including age, sex, dose, route, and stated time of voodoo administration and whether they abuse it or not. Patients were asked about their main complaints following poisoning, and if any medical intervention was given to them before being admitted to PCU.

2- General examination

General examination for all cases included assessment of consciousness level using Galsgow coma scale, vital signs (pulse, blood pressure, respiratory rate and temperature), pupil (size and reactivity), and chest and heart examination.

3- Investigations

Routine investigations were done for all patients including arterial blood gases, blood glucose level, liver and kidney function tests. ECG was recorded with a paper speed of 25mm/s for patients with cardiovascular manifestations.

2 ml arterial blood samples were obtained from every patient for analysis of arterial blood gases. Arterial blood gases were performed using blood gas analyzer, Bayer 855. 3 ml venous blood samples were obtained from every patient for analysis of blood glucose level, liver and kidney function tests. Blood glucose level was measured using diamond Diagnostic kit (Germany), liver and kidney function tests were measured using Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim (Germany).

All investigations were performed in Zagazig University Hospitals laboratories. Urine samples were collected for urinary screening test using EIA kit (Immunalysis Corporation, USA), DRI®.

2-Experimental Study

A- Gas chromatography/ Mass spectrophotometery analysis (GC/MS)

1- Sample preparation for GC/MS analysis

Voodoo extract was prepared from Voodoo sachets, which was brought, after taking permission from the Ministry of Justice, to be given to animals. Each sachet contains 3 gram of voodoo. It contains dried vegetable matter (Light green buds/herbal matter), when opened it has a solvent/chemical smell inside.

Voodoo extract was prepared by crushing the buds/herbal matter in the sachets. Each 3 gram was extracted three times with equal mixture of diethyl ether and light petroleum (40-60) giving 0.58 gm green residue (Obata and Ishikawa, 1960). This residue was subjected to GC/MS analysis. Identification of the components was based on the fragmentation pattern in the resulted mass spectra using mass spectral data base.

2- GC/MS analysis

The GC analysis was carried out using Shimaduz GC gas chromatograph (GC-17 ver.3) system interfaced with a Shimadzu model QP-5000 mass spectrometric detector and a Shimadzu AOC-20i auto-injector module (Japan). Simultaneous auto injection was done on a duplicate of the same operational conditions. Identification of the extract components was carried out by comparing their relative retention times with those of authentic samples or by comparing their relative retention indices (RRI). The later were computed using a mixture of a continuous series of n-alkane hydrocarbons (C4-C28) run on SLB-5ms (non-polar) column. The components of the extract were fully identified by their mass spectral fragmentation patterns with those reported in computerized MS-data bank spectral libraries (Akutsu, 2017).

B- Calculation of lethal dose 50 (LD50) for intra peritoneal Voodoo extract.

1- Experimental animals

In this part of the study, 40 male albino rats were obtained from the animal house, Faculty of Veterinary Medicine, Zagazig University, after taking approval for the study design from Ethical Committee for Research, Faculty of Medicine, Zagazig University. They were about 12 weeks old; their weight was between 120 and 140 gm at the beginning of the study. Rats were housed in groups in clean suitable cages (five per cage) under standard laboratory conditions including good aerated room with suitable temperature (22C±2) and at a12-hour light/dark cycle. Standard rodent's food (bran and grinded maize) and water were available ad libitum.

Rats were left to acclimatize to the experimental conditions for 1 week before commencing the experiment. All rats received human care in compliance with the guidelines of the Ethical Committee for Research, Faculty of Medicine of Zagazig University, Egypt.

2- Study design

An approximate LD50 can be determined by so called "up and down' or the staircase method' using gradually increased doses according to the response (Gad and Weil, 1989). The rats were randomly divided into eight groups of five each. Every group was housed in separate cages.

The first group was considered the negative control group and the second group' positive control administered 1 ml of dimethyl sulfoxide (DMSO) the vehicle of the extract intra peritoneal (IP). Voodoo extract was given IP in increasing doses to rats from the third group to the last group.

The third group was given the extract in a dose equal to 162 mg/kg; this dose is the minimal dose for weak toxins. Dose was then multiplied as follows: the fourth group was given the extract in dose equal to 323 mg/kg; the fifth group was given the extract in dose equal to 646 mg/kg; the sixth group was given the extract in dose equal to 1292 mg/kg; the eighth group was given the extract in dose equal to 2584 mg/kg. Another group (seventh group) was selected to take a dose equal to 1936 mg/kg because there was a high difference in the effects between dose of 1292 and 2585mg/kg. The animals were observed for 24 hours; for any toxic signs.

By the end of the 24th hour, the number of deceased animals in each group was counted and the percentage of mortality (percent of dead animals) was calculated according to the method of Miller and Tainter (1944). Log-probit table was used to determine the probit for each mortality percent in each used dose. Graphical presentation was used for calculation of LD50 of the extract. For each used dose the log dose was obtained then each log dose was plotted versus probit values obtained from log-probit table. The dose corresponding to probit5 i.e, 50% was estimated to be the LD50 of the extract.

C- Histopathological examination

By the end of the study, organs were dissected from the dead animals (brain, liver, kidney, heart and testicles) and fixed in 10% formol saline and processed to prepare 5µm thick serial paraffin sections for haematoxylin &eosin staining to verify the histological details according to the method described by Wilson and Gamble (2002).

Statistical analysis

Data for all groups were expressed as mean± standard deviation (X±SD). The data obtained were subjected to SPSS program, version 15 (Norusis, 1997). Statistically significant difference was determined by one-way analysis of variance (ANOVA), followed by the post hoc test for multiple comparisons between different groups. The probability values (P) less than 0.05 was considered significant.

RESULTS

1- Clinical Study

Patient group was consisted from 14 males and 3 females, with their age range (17-32 years).There was a non-significant difference between poisoned patients and controls regarding the personal data (Table 1). The most common route of poisoning was smoking of Voodoo cigarettes (in nine cases), followed by oral ingestion of 1-2 Voodoo sachets (in six cases), and then smelling of Voodoo incense (in two cases). Patients presentation included variable signs and symptoms; most frequently were hallucination (100% of cases with a characteristic hallucination of picking imaginary objects from the surroundings), feeling of extreme anxiety (70%), drowsiness (29%), fear of death (88%), dry mouth (53%), nausea& vomiting (100%), tremors (35%), sinus tachycardia (82%), hypertension (41%), dilated reactive pupils (100%) .

Conventional treatment included gut decontamination via gastric aspiration, lavage and activated charcoal for patients admitted within four hours of ingestion. Patients with acute psychosis or panic attack were treated with diazepam. Patients with persistent nausea and vomiting were given intravenous fluid replacement and antiemetics. Urinary screening tests for all patients were negative for cannabis and other addictive substances.

There was a statistically significant increase in the heart rate, systolic blood pressure between the patient group and the control group. There was no statistical significant difference of diastolic blood pressure, temperature and respiratory rate between the patient group and the control group (Table 2). Regarding results of arterial blood gases (ABG), there was only a statistically significant difference in pH results (Table 3). Although random blood sugar were lower in patient group compared to controls but it was not statistically significant difference, also there was no statistical significant difference in liver and kidney function tests between the patient group and the control group (Table 4).

2- Experimental Study

2-1-GC/MS analysis of the extract

Voodoo extract revealed 166 compound with the chief compound was ethyl 1,2,3,4- tetrahydro-2,2,4- trimethylquinolin-6 carboxylate (54.54%) (Figure1). This

compound was identified as a chemical structure analogue of PB 22; a designer synthetic cannabinoids. The extract also contains trace amounts of hydrocarbons with the highest concentration for toluene (9%).

2-2- Toxic Signs recorded during the experiment

Initially doses of 162 mg/kg and 323 mg/kg didn't produce any significant effects on central nervous system (CNS), while rest of doses produce wide range of signs of CNS stimulation. The animal exhibited chewing, vomiting, sweating, arching, tail twisting, coarse tremors, hyperesthesia, and generalized convulsion (figure 2). Finally animals showed labored breathing, exhaustion, gasping, cyanosis and death (figure 3).

2-3- Determination of percentage mortalities and probits for calculation of LD50

The percent of died animals at each dose and the corresponding probit was determined (table 5). The probit values were plotted against log dose and then the log dose corresponding to probit 5 i.e., 50% was found to be 3.2. The equivalent dose for this log dose was 1334 mg/kg, which represent the Ld50 of the extract (figure 4).

2-4-Histopathological examination

Brain

In control groups, the cortical part of the brain consists of multiple layers which are molecular layer, outer granular layer, outer pyramidal layer, inner granular layer, inner pyramidal layer and polymorphic layer. The cytoplasm of large pyramidal cells is basophilic and contains large coarse basophilic granules. The nucleus is large, rounded, and central with prominent nucleus (Figure 5). In treated male albino rats, it shows shrunken vacuolated cells with dense nuclei and cytoplasm. The cells are surrounded by irregular wide spaces (Figures 6,7).

Liver

In control groups, the hepatocytes are polygonal in shape, arranged in cords radiating from the central veins, with a rounded vesicular nucleus.The cords of cells are separated by blood sinusoids that are lined with endothelial cells (Figure 8). In treated male albino rats, the liver shows severe congestion and macro vesicular steatosis with diffuse intracytoplasmic esinophlic bodies. (Figures 9,10,11).

Kidney

In control groups, the kidney consists of the cortex and medulla. The renal cortex is characterized by the presence of proximal and distal convoluted tubules in addition to the renal corpusles. The renal corpusles are spherical bodies formed of a glomerulus and a Bowman's capsule. The glomerulus is an invagination of the Bowman's capsule by a vascular tuft of capillaries (figure 12). Kidneys showed in treated rats focal vacuolar degeneration of renal tubules, accentuation of glomerular basement membrane, hyallinosis of renal tubules with large areas of epithelial necrosis (figures 13,14).

Table 1: Statistical analysis of personal data among the patient and control groups.

	Control group N= 20 Mean ± SD	Patient group N= 17 Mean ± SD	p-value	Statistical significance
Age (years)	22.5±8.5	20.7±9.9	>0.05	NS
Sex (M/F)	15/5	13/4	>0.05	NS

(NS) = non significant.

Table 2: Student t test comparing vital signs between the control and patient groups.

	Control group N= 20 Mean ± SD	Patient group N= 17 Mean ± SD	P value	Student t value
Pulse (beat/min)	74.6±5.0	112.0±10.0	0.0001*	4.44
Systolic BP (mmHg)	110.5±10.8	140.5±6.5	0.03*	2.28
DiastolicBP(mmHg)	75.0±5.3	80.6±8.5	0.568^{NS}	0.577
Temperature (ºC)	37.0±0.0	36.0±0.9	0.235^{NS}	1,21
Respiratory rate (/min)	12.2±4.2	14.1±2.0	0.702^{NS}	0.386

*NS= non significant *= significant*

Table 3: Student t test comparing arterial blood gases parameters between the control and patient groups.

	Control group N= 20 Mean ± SD	Patient group N= 17 Mean ± SD	P value	Student t value
pH	7.40± 0.04	7.30± 0.06	0.0001*	6.05
PCO2 mmHg	36.1 ± 3.1	36.2 ± 2.9	0.982^{NS}	0.023
PO2 mmHg	102.8±8.1	100.5± 8.4	0.846^{NS}	0.196
SaO2 %	98.9 ± 5.6	98.6±6.6	0.972^{NS}	0.035
HCO3 (mmol/L)	24.7± 2.2	25.7± 1.6	0.724^{NS}	0.356

*NS= non significant *= significant*

Table 4: Student t test comparing random blood glucose level, liver and kidney function tests between control and patient groups.

	Control group N= 20 Mean ± SD	Patient group N= 17 Mean ± SD	P value	Student t value
Random blood glucose (gm/dl)	92.7± 12.2	85.7± 11.6	0.684^{NS}	0.411
serum AST (IU/L)	49.5±3.5	48.2±3.0	0.778^{NS}	0.284
serum ALT (IU/L)	48.9± 4.5	46.2 ± 2.9	0.607^{NS}	0.519
Blood urea nitrogen (mg/dl)	22.8±6.1	24.5± 6.4	0.849^{NS}	0.192
Serum creatinine (mg/dl)	0.98 ± 0.06	0.96±0.08	0.840^{NS}	0.203

NS= non significant

Table 5: Mortality percent, probit and the logarithm of the dose to calculate the LD50

	Dose mg/kg	No. of animals	Log dose	No of dead animals	Mortality %	Probit
1st group (control)	-	5	0	0	0	0
2nd group (DMSO)	-	5	0	0	0	0
3rd group	162	5	2.21	0	0	0
4th group	323	5	2.51	0	0	0
5th group	646	5	2.81	1	20%	4.10
6th group	1292	5	3.11	2	40%	4.75
7th group	1936	5	3.29	3	60%	5.25
8th group	2584	5	3.41	4	80%	5.54

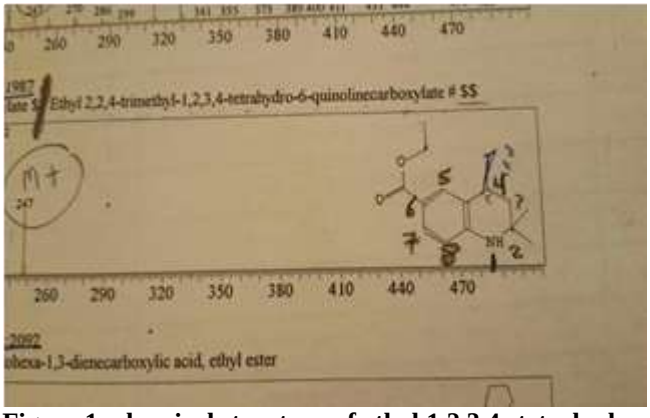

Figure 1: chemical structure of ethyl 1,2,3,4- tetrahydro-2,2,4- trimethylquinolin-6 carboxylate the chief compound in the extract.

Fig. (2): a photograph showing rat from 6th group showing sweating, arching of the back, tail twisting and coarse tremors in left hand.

Fig. (3): a photograph showing rat from 7th group showing generalized convulsion.

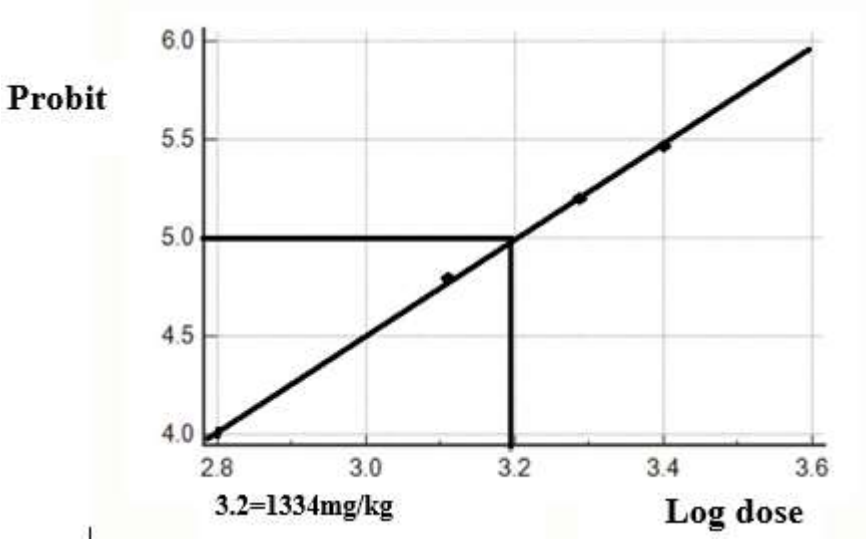

Fig. (4): plot of log doses versus profit for calculation of the LD50 of the extract.

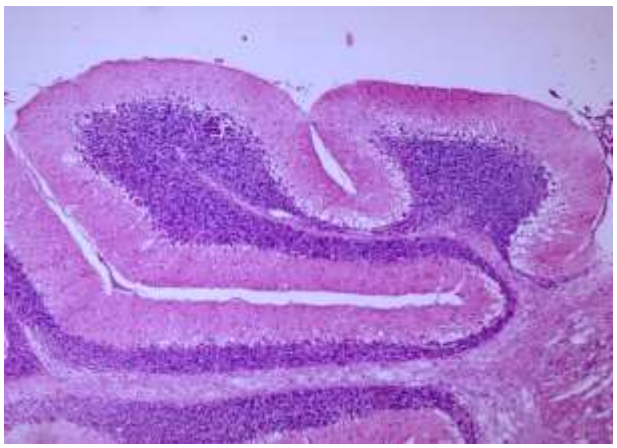

Fig. (5): a photomicrograph of rat brain from the negative control group (1st group) showing the normal architecture of the brain H&E x 100.

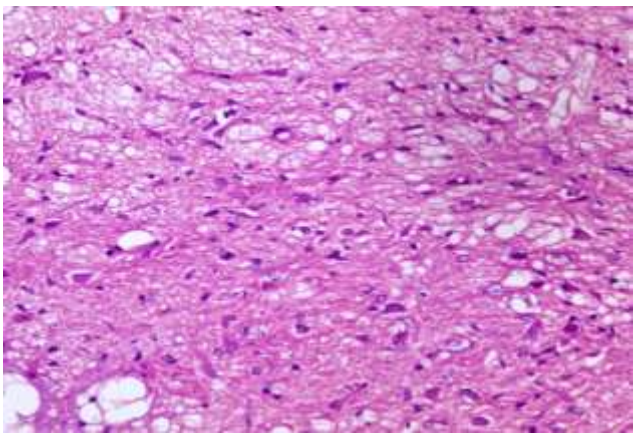

Fig. (6): a photomicrograph of rat brain from the negative control group (1st group) showing the normal brain cells H&E x 400.

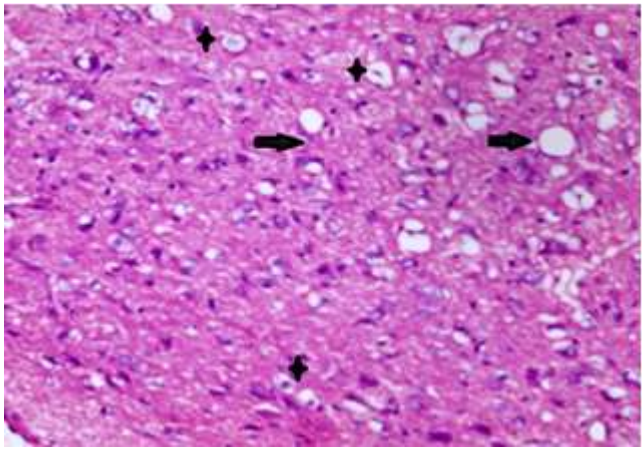

Fig. (7): a photomicrograph of rat brain from the treated group (7th group) showing shrunken brain cells (asterix) and vacuolation (arrows) H&E x 400.

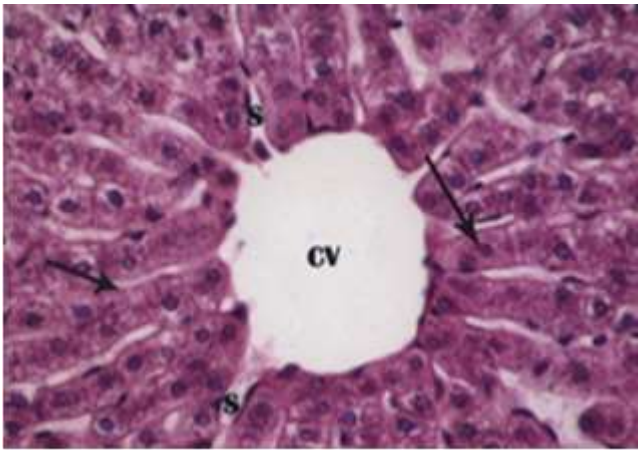

Fig. (8): Photomicrograph of rat liver from the negative control group (1st group) showing regular liver morphology with blood sinusoids (s) among hepatic cords. Regular hepatic cords (arrows) diverging from the central vein (cv) H&E x400

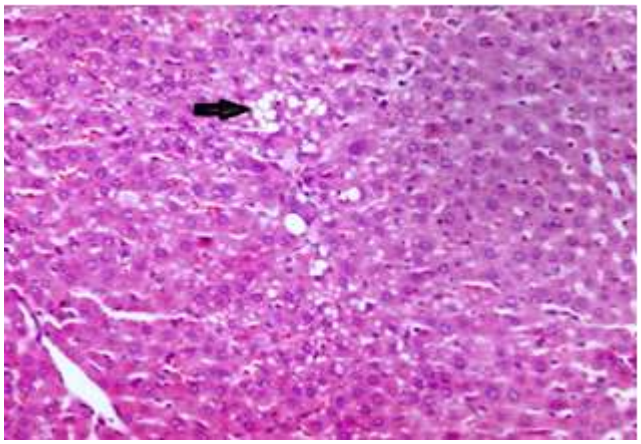

Fig. (9): Photomicrograph of rat liver of the treated group (6th group) showing macro vesicular steatosis (arrow) with diffuse intra lobular nuclear debris, H&E x 400

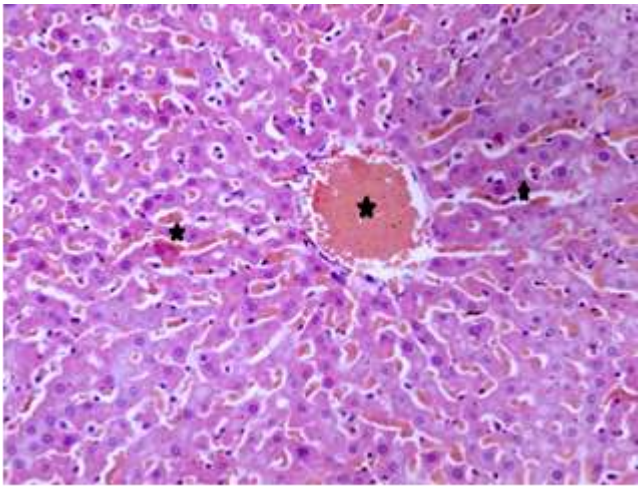

Fig. (10): Photomicrograph of rat liver of the treated group (5th group) showing severe congestion in central vein and hepatic sinusoids (asterix), H&E x 400

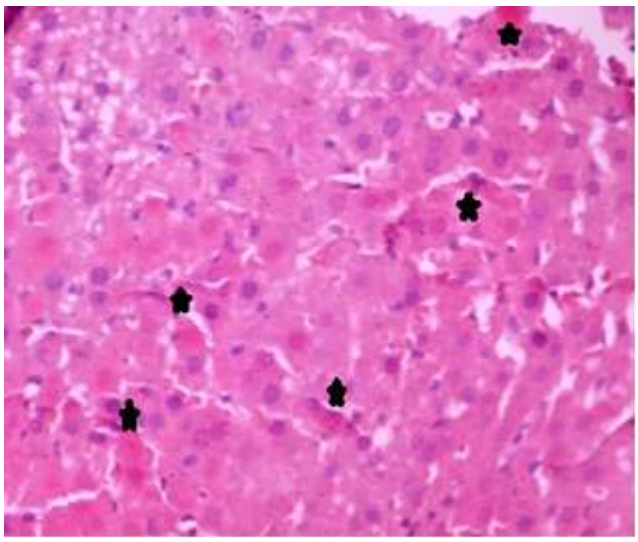

Fig. (11): Photomicrograph of rat liver of the treated group (8th group) showing intra cytoplasmic esinophilic bodies (asterix), H&E x 400.

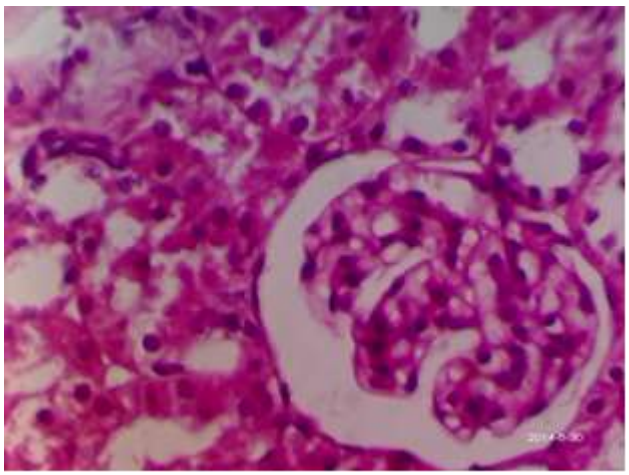

Fig. (12): Photomicrograph of rat kidney of the negative control group (1st group) showing normal renal glomeruli (G) and tubules (PT), (DT) H&E original magnification x 400.

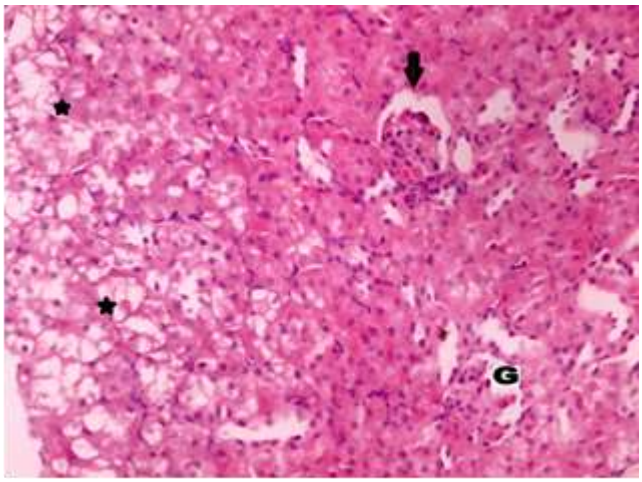

Fig. (13): Photomicrograph of treated rat kidney (8th group) showing focal vacuolar degeneration of renal tubules (star), widening of the bowman's capsular spaces (arrows), bifid glomeruli (G) are also seen H&E x 100.

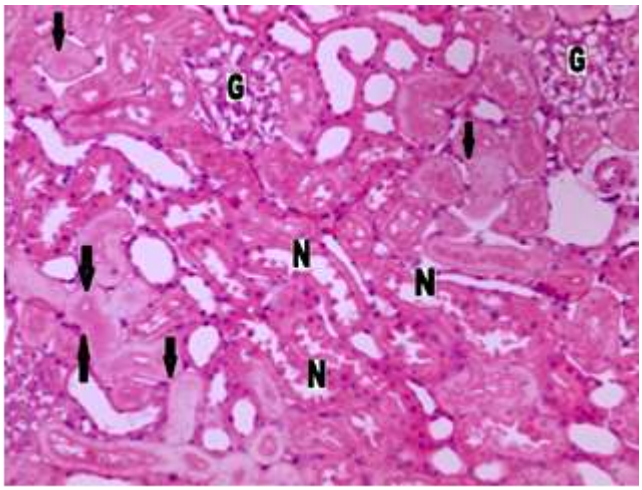

Fig. (14): Photomicrograph of treated rat kidney (8th group) showing accentuated glomerular basement membrane, hyalinosis of renal tubules (arrows) with large areas of epithelial necrosis , Glomeruli (G) shows vacuolation of their cells and marked narrowing of the glomerular bowman's space (edema of the glomeruli) H&E X 400.

Discussion

Several synthetic cannabinoids compounds are recently found to be active in rodent behavior assays like JWH-018, UR-144, PB-22 and 5F-PB-22. Each compound produced dose-dependent depression of the locomotor activity similar to that produced by Δ9-THC. The synthetic cannabinoids were all at least as potent as Δ9-THC, and some were as much as 100-fold more potent than it like PB-22 and 5F-PB-22 (Wiley et al., 2013). On March 2011, synthetic cannabinoids were listed as Schedule I substances, making their possession and use illegal in the United States; nevertheless, it has been found that the popularity of synthetic cannabinoids is increasing among young people (ONDCP, 2012).

In the current study the newly emerged hallucinogenic substance "Voodoo" was investigated for its toxic effects on human and experimental animals. GC/MS extraction revealed that it was a mixture of 166 compound with the most abundant one is a structure chemical analogue of PB 22; a new synthetic cannabinoid.

In the current study acute Voodoo intoxicated patients presented to PCU with a wide variety of manifestations including neurological manifestations resemble acute psychosis (Hallucination, feeling of extreme anxiety, fear of death), dry mouth, numbness and tingling, nausea& vomiting, drowsiness, tremors, tachycardia, hypertention, dilated pupils. Laboratory

investigations revealed slight metabolic acidosis. Random blood sugar was statistically insignificantly lower in patients compared to controls, also there was no statistical significant difference in liver and kidney function tests between patients and the controls.

It is well documented in the literature that the use of synthetic cannabinoids has been reported to cause agitation, anxiety, nausea, vomiting, tremors, seizures, hallucinations, and paranoid behavior (Fattore and Fratta, 2011).

Experimental study proved the above mentioned results as it revealed that the most affected organ was the brain. Brain affection was in the form of irregular shrunken cells with dense nuclei and vacuolated cytoplasm.

Brain is the organ mostly affected by Δ-9-THC because the main effects of it are mediated through the cannabinoid receptors CB 1 that densely distributed in brain regions. CB1 receptors have very high densities in the basal ganglia and in the cerebellum. In the limbic forebrain, CB1 receptors are found particularly in the hypothalamus and in the anterior cortex. The hippo-campus also contains a high density of CB1 receptors (Iversen, 2003).

Different mechanisms were postulated for toxic effects of THC on the brain cells. Sànchez et al. (2008) explained the effect of marijuana on brain. It induces apoptosis in glioma cells, as determined by DNA fragmentation and loss of plasma membrane asymmetry. THC stimulated sphingomyelin hydrolysis in glioma cells.

The liver was also markedly affected in the form of severe congestion and macro vesicular steatosis with diffuse intracytoplasmic esinophlic bodies It is well known that the liver is a major site of metabolism and so the vulnerability of the liver to toxic agents is high. Factors affecting this vulnerability are due to its primary role in the metabolism and disposition of foreign substances, and the concentration of foreign chemicals in the liver (Yassa et al., 2010).

The kidney affection was in the form focal vacuolar degeneration of renal tubules, accentuation of glomerular basement membrane, hyallinosis of renal tubules with large areas of epithelial necrosis Susceptibility of the kidney to toxicity is due to its role in conversion of drugs and other compounds into products that are more easily excreted than the potent compound through kidney (Atici et al., 2005).

Similar effects were explained by Athanasiou et al. (2007), found that the THC causes morphological changes characteristic of apoptosis. In rat mitochondria, it causes significant decreases in oxygen consumption and mitochondrial membrane potential. THC causes significant increases in mitochondrial hydrogen peroxide production and hence changes in integrated mitochondrial function even in the absence of cannabinoid receptors.

CONCLUSION

It could be concluded that Voodoo proved to have many toxic effects on human and experimental animals and further studies are warranted to evaluate other toxic effects of this substance.

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الملخص العربى

السميه الحاده لفئة جديدة من المهلوسات "الفودو" (دراسة سريرية وتجريبية)

مروة أحمد عباس ومحمد زايد حسن ومنال رضا عبد الحليم و هشام عبد العزيز ورحاب عبد الله

الفودو عبارة عن مادة مهلوسة ظهرت حديثا في مصر تستهدف الشباب الذين تتراوح أعمارهم بين 15-30عاما مما تسبب في حدوث العديد حالات التسمم الحاد .مما جعل وزارة الصحة المصرية في عام 2014 لادراجه ضمن الجدول الاول لقائمة المخدرات، كما حذرت التجار والمستخدمين انهم الآن تحت طائلة القانون .وقدأجري هذا العمل لدراسة الآثار السمية الحادة لهذه المادة المهلوسة فى الإنسان وحيوانات التجارب .وشمل هذا العمل كل من الدراسات السريرية والتجريبية .وشملت الدراسة السريرية 17مريضا يعانون من التسمم الحاد للفودو ممن تم علاجهم بوحدة علاج التسمم –مستشفيات جامعة الزقازيق فى الفترة بين يوليو 2015وأبريل 2016. أما الدراسه التجريبية فقد شملت الدراسة أربعين جرذ من ذكور الجرذان البيضاء البالغة لحساب LD50 للفودو .وقد تم تحضير مستخلص الفودو باستخدام الكروماتوجرافى الغازى/القياس الطيفي الكتلي .كانت الأعراض الرئيسية لدى حالات التسمم بالفودو هى الهلوسة، والارتباك والخوف الشديد من الموت، فى صورة مشابهة للتسمم الحاد للحشيش ولكن مع سلبية اختبار فحص البول له او لغيره من المواد الادمانيه الشائعه.وقد كانت نتائج التحليل بالكروماتوجرافى الغازى/القياس الطيفي الكتلي أن المكون الرئيسى لهذا المستخلص (بنسبه 54.54)٪ هو مركب يتماثل كيميائيا مع مركب22 PB؛ أحد مركبات الحشيش الجديدة المخلقه صناعيا .وقدرت LD 50من المستخرج ب 1334 ملغم /كغم .وكانت الكبد والكلى والمخ هى الانسجه الأكثر تضررا .أظهر الكبد الاحتقان الشديد فى الاوعية الدمويه وتنكس دهني حويصلي ، أما الكلى فقد ظهر نخر فجوي فى الأنابيب الكلوية، وتضخم فى الغشاء القاعدي الكبيبي، ومساحات واسعة من نخر الظهارية .وقد كانت خلايا المخ متقلصة ومجوفة بشكل ملحوظ . ومن هذا يمكن استنتاج أن الفودو ثبت ان له العديد من الآثار السامة على الإنسان وحيوانات التجارب ولذا يوصى بإجراء المزيد من الدراسات لتقييم الآثار السامة الأخرى لهذه المادة.

[1] قسم الطب الشرعى والسموم الإكلينيكية- كلية الطب البشرى - جامعة الزقازيق
[2] قسم الهستولوجيا وبيولوجيا الخلية- كلية الطب البشرى - جامعة الزقازيق
[3] قسم الباثولوجيا- كلية الطب البشرى - جامعة الزقازيق
[4] قسم الصيدلنيات، كلية الصيدلة - جامعة الزقازيق