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Effect of various level of MDMP-4en-PINACA Orally Gavaged to Wistar rats

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Abstract

This research studied effects MDMB-4en-PINACA on white Wistar rats' liver, kidney, and heart, as well as their biological and hematological parameters. For 14 days, 24 male albino rats received orally. First group was the control group; groups 2 and 3 received 5 and 50 mg/kg (bw) MDMB-4en-PINACA. Liver and kidneys deteriorated in treated rats. Small clusters of inflammatory cells, "cytoplasmic degeneration with karyolysis", "venous congestion with hemorrhage" and "edema", and "steatosis deterioration" were among the liver's histological abnormalities. Tubular deterioration increased, as did the appearance of tubular casts, and glomeruli suffered from atrophy followed by an increase in Bowman's gap in renal tissue. Tubular also showed signs of disintegration, including the formation of foam cells that seemed empty in the glomerulus. Myocardial fibers, the central disc nucleus, and endothelial cells were all healthy. The value of HGB, RBC, HCT and MCHC were higher in groups 2&3 and the values of MCV, MCH and WBC were decreased in groups 2&3. The activity of serum AST, ALT, ALP and Creatinine Kinase and the concentrations of total protein, Albumin, Triglyceride, Total Bilirubin, Direct Bilirubin, Urea, and Creatinine in number 2 group and 3 were greater than that of the control. and that of Cholesterol and HDL were lower in groups 2and 3. According to this research, synthetic cannabis' (MDMB-4en-PINACA) negative effects are worse on the liver and kidneys in group 3 than group 2.

Keywords: MDMB-4en-PINACA, designer drug, Forensic Science; Rat; Toxicity; histopathological

Introduction

A "designer drug" is a controlled substance functional or structural analog that is produced to mimic the pharmacological properties of the original substance in an effort to avoid being classified as illegal and/or being detected during standard drug testing [1].

Despite the fact that psychoactive ingredients and the recreational drugs (NPSs) have the potential to produce considerable morbidity and mortality, there is a scarcity of comprehensive data on acute drug/NPS toxicity in Europe [2]. MDMB-4en-PINACA is a "cannabinoid receptor agonist" that is synthesized. Same as the other artificial cannabinoids touted

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as a «legal» alternative to cannabis and controlled artificial cannabinoids. Synthetic cannabinoids, due to their great strength, can provide a significant risk of severe toxicity, which can be deadly in certain situations [3].

MDMB-4en-PINACA (Methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido) butanoate) has been reported as a plain solid in its pure form[4], and white powder[5]. It's also been called a tan and a yellow/brown powder[6]. Collected and sized MDMB-4en-PINACA White, yellow, orange and beige granules have been mentioned as samples[3] Contrary to other therapeutically accessible medications, little or no information on the pharmacokinetics and pharmacodynamics of MDMB-4en-PINACA is available to help prescribers. This research aims to determine the sub-acute toxicity of Synthetic Cannabinoid MDMB-4en-PINACA in rats blood samples

2.1 Drug preparation and Animals

MDMB-4en-PINACA was first dissolved in DMSO (to a final concentration of 5%), then utilizing maize oil was converted into final volume. DMSO and maize oil (being vehicle control) were used too. Twenty-four rats were randomized into three experimental groups at random. each of 8 rats. Groups 2 and 3 were given 5 and 50 mg/kg MDMB-4en-PINACA orally. Group 1 served as a control. All test rats had unrestricted access to water and a standardized pelleted meal (Saudi Grains Organization, Riyadh, Saudi Arabia). The temperature and humidity were kept under strict control for the animals' care. The lighting and darkness in the room were adjusted in 12-hour cycles.

All studies were carried out in compliance with the international norms for the treatment of animals in the experimant, as recommended by the Standing Committee for Scientific Research Ethics at Naif Arab University for Security Sciences. Rats were acclimated for a week before to the trial.

2.2. Chemicals and reagents

Blood samples were tested using biochemistry kits manufactured by Roche, Germany.

MDMB-4en-PINACA certified standards (purity 98%) utilized in this investigation were obtained from commercial providers, specifically "Cayman Chemical Company" (Ann Arbor, MI, USA). DMSO was purchased from Biotraxx Cyprus, and Corn oil from the local market. The assay kits (that were important apparatus for the experiment) ought to measure aspartate aminotransferases, alanine aminotransferases, alkaline phosphatase, Total protein, albumin, Cholesterol, Urea, and creatinine were bought from "United Diagnostics Industry".

2.3. Experimental design

OECD 407 standards for subacute toxicity research (14-day consecutive oral dosage) [7]. Rats were randomly split into three groups: Group 1 (G1): (Normal group) control group; groups 2 and 3 received 5 and 50 mg/kg (bw) MDMB-4en-PINACA.

Until the experiment/trail ended rats were checked for mortality at least twice a day. They were studied for clinical indicators, onset, and duration. Before dosage, once weekly throughout treatment, and on sacrifice day, all rats' body weights were recorded. After 2 and 4 weeks of therapy, rats were retro-orbitally bled for hematological and biochemical tests.

2.4. Hematological parameters

Heparinized blood was used to investigate hematological traits including the WBC count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and Hb concentration (Hematology Analyzer cellular analysis system DXH 600).

Biochemical Parameters

Aspartate aminotransferase (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), Creatine Kinase (CK), total protein, albumin, cholesterol, HDL, Triglycerides, urea, creatinine, Total bilirubin and Direct bilirubin were analyzed by using biochemistry Analyzer cobas 6000, Roche.

Histopathology

Death and morbidity were evaluated twice daily until the experiment finished. Clinical indicators, start timing, and duration were noted. Diethyl ether was used to put every group's surviving rats to sleep, and they were all put to death after two weeks. When

animals were killed, blood samples were taken. All rats were inspected at necropsy to check for obvious lesions, and samples of the heart, liver, and kidneys were processed for histopathology after being fixed in 10% neutral buffered formalin.

Statistical Analysis

The mean ± standard error mean (SEM) was used to depict the trial outcomes. Student's t-test analyzed experimental data for statistics and correlations. Values of $P \leq 0.05$ qualified as significant. The main tool for assessing the empirical values was SPSS 22 (SPSS, Chicago, IL, USA). [8].

Result

Histopathological changes

After two weeks of treatment, no changes in the control group was observed (group 1). Small clusters of inflammatory cells, cytoplasmic degeneration with karyolysis, vascular constriction with hemorrhage and edema, and steatosis degeneration were among the acute histopathological alterations. Tubular degeneration intensified, as did the existence of tubular casts, and glomeruli damaged from atrophy accompanied by an increase in Bowman's gap in the renal tissue. Cell tubule deterioration was also visible, as was the formation of foam cells that appeared hollow in the glomerulus. Cardiac muscle fibers 14 days after therapy Male rats given low and high dosages of MDMP-4en-PINACA had healthy features such as cardiac fibers, the central disc nucleus, and endothelial cells were showed in figures (1-4).

Hematological changes

Table 1 contains the data. After 2 weeks of therapy, the values of HGB, RBC, HCT, and MCHC in groups 2&3 were greater than in the control. MCV, MCH, and WBC levels were lower ($P<0.05$) in groups 2&3 compared to controls.

Serobiochemical changes

These data which presented in Table.2 After 2 weeks of treatment, the activity of serum AST, ALT, ALP and Creatinine Kinase and the concentrations of total protein, Albumin, Triglyceride, Total Bilirubin, Direct Bilirubin, Urea, and Creatinine in groups 2 and 3 were higher ($P<0.05$) than control. and that of Cholesterol and HDL were lower ($P<0.05$) in groups 2 and 3 than control.

Effect of various level of MDMP-4en-PINACA orally gavaged to Wistar rats

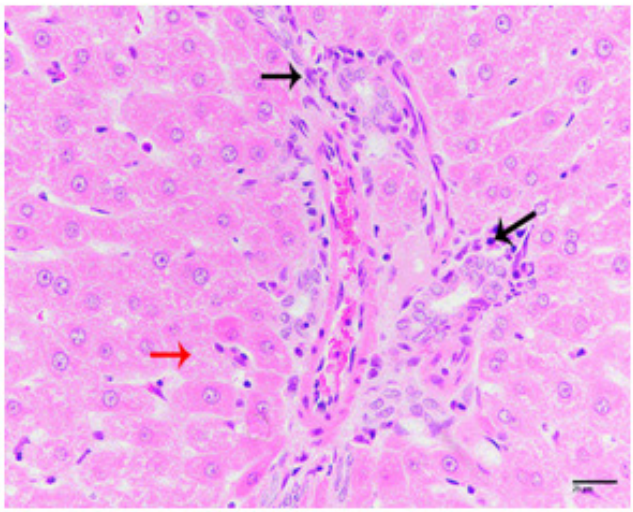

Fig.2: Photomicrograph of rat's liver treated with (50 mg/kg) of MDMP-4en-PINACA group2 showing inflammatory cells (black arrows), cytoplasmic degeneration with karyolysis (red arrow). (H&E-400X)

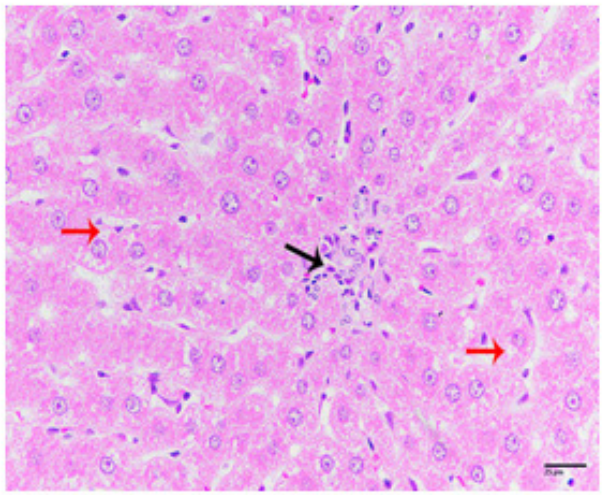

Fig.1: photomicrograph of rat's livre treated with (5 mg/kg) of MDMP-4en-PINACA group1 showing small aggregation of inflammatory cells (black arrow), cytoplasmic degeneration (red arrows). (H&E-400X)

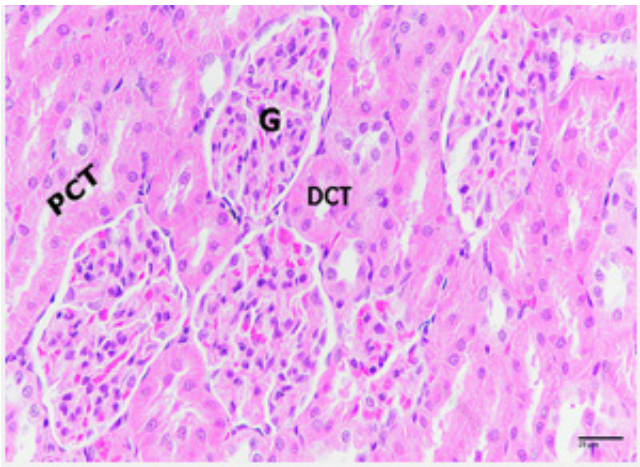

Fig.3: Photomicrograph of rat's kidney treated with (5mg/kg) of MDMP-4en-PINACA group1 showing healthy renal tissue glomerulus (G), proximal tubule (PCT), distal tubule (DCT). (H&E-400X)

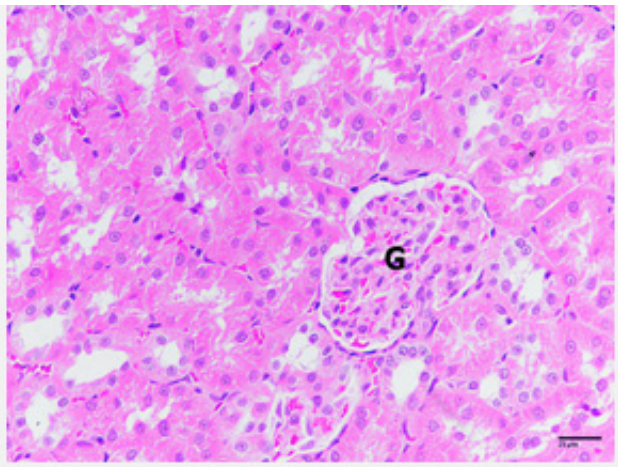

Fig.4: Photomicrograph of rat's kidney treated with (50mg/kg) of MDMP-4en-PINACA group 2 showing healthy renal tissue glomerulus (G). (H&E-400X)

Table. 1 Hematological changes in rats given various levels of (MDMB-4EN-PINACA) orally for 2 weeks

No	Parameters	Control	MDMB-4en-PINACA (5mg/kg)	MDMB-4en-PINACA (50mg/kg)
1	HGB	95.33±5.3	139.33±1.3*	97.16±2.32
2	RBC (10^6/UL)	4.51±0.31	7.9±0.13*	5.38±0.11*
3	HCT (%)	32.11±2.17	42.7±0.52*	35.68±0.67*
4	MCV (fl)	60.73±2.35	53.9±0.89*	56.66±0.33*
5	MCH (pg)	19.28±0.86	17.56±0.18*	17.71±0.24*
6	MCHC (g/l)	318.66±6.74	326.16±2.41*	319.83±2.21*
7	WBC (10^3/UL)	5.81±0.2	5.73±0.17*	4.68±0.45*

Values are expressed as mean ± S.E.; *: Significant at ($p<0.05$)

Table. 2 Serobiochemical changes in rats given various levels of (MDMB-4EN-PINACA) orally for 2 weeks

No	Parameters	Control	MDMB-4en-PINACA (5mg)	MDMB-4en-PINACA (50 mg)
1	AST (U/L)	149.16±1.24	164±1.46*	158.16±1.01
2	ALT1 (U/L)	68.33±1.83	75±1.59*	71.16±0.94*
3	ALP1(U/L)	195.16±1.57	210.3±1.6*	245.6±9.6*
4	Creatine kinase (U/L)	324.33±3.32	899.5±1.97*	355.6±1.78*
5	Total protein (g/L)	67.49±1.7	72.3±0.93*	68.5±1.29*
6	Albumin (g/dl)	5.81±0.14	6.78±0.33*	5.9±0.23*
7	Cholesterol (mmoI/l)	1.44±0.08	1.19±0.03*	1.4±0.1*
8	Triglyceride	0.27±0.01	0.4±0.002*	0.62±0.03*
9	(HDL) (mmol/l)	1.16±0.01	1.12±0.04*	0.83±0.02*
10	Total bilirubin (umol/L)	2.46±0.14	3.03±0.18*	2.68±0.13*
11	Direct bilirubin (umol/L)	0.55±0.03	0.34±0.01	0.82±0.02*
12	Urea (mmol/l)	7.7±0.26	7.11±0.13*	7.81±0.19*
13	Creatinine (umol/L)	30.51±0.67	40.16±0.43*	36.5±0.61*

Values are expressed as mean ± S.E.; *: Significant at ($p<0.05$)

Discussion

Average body weight growth was all different across the groups throughout the trial since all animals got the same meal. There has been no evidence of the effects of MDMB-4en-PINACA taken orally on the development of Wistar rats at this time in the literature available. Most of the studies reporting toxic effects of SCs are case studies of ER patients with recent consumption of SC substances. The most-reported system affections are CNS, gastrointestinal, and cardiopulmonary. Acute renal toxicity and acute hepatotoxicity were also reported at a lower rate as a complication of acute toxicity. Case studies give attention to the clinical symptoms and leading causes of death without paying attention to specific organ pathology. Organized animal studies evaluating the histopathological toxic effects on different body organs are scarce[9]. As a result, the current investigation was designed to look into the histopathological and biochemical profiles of MDMB-4en-subacute PINACA's effects in the liver, kidneys, and heart of control and treated animal groups.

The current investigation found that group 2 liver tissue underwent histological evaluation 2(5mg/kg of MDMP-4en-PINACA) showed small aggregation of inflammatory cells besides to some cytoplasmic degeneration, while liver tissue of group 3 (50mg/kg of MDMP-4en-PINACA) exhibited gathering of inflammatory cells and cytoplasmic degeneration accomplished by karyolysis which means complete digestion of nucleus. The liver's role in metabolism and excretion may provide an explanation for these outcomes of MDMB-4en-PINACA[5]. On reviewing the available literature there was no published experimental study revealing the histopathological effects of SC on body organs. However, many studies were found exploring the toxicological effects and the leading cause of death in human cases of acute intoxication. Some of them reported postmortem histological analysis of different body organs. hepatic affection is not a common presentation of SC intoxication, there are confirmed cases of fulminant hepatic failure and liver affection after smoking SC. However, there was no histopathological assessment in these cases.

[10]reported a case of acute hepatic failure after consumption of SC substance. The case developed hyperbilirubinemia, an increase in INR, shooting liver enzymes, and coma. However, the case improved with supportive treatment. Similar findings were described by[11] who reported three cases with hepatic affection from SC use and the cases had been improved with supportive treatment. Histopathological changes can be explained by oxidative stress in tissues causing cell apoptosis[12].

The increase in ALT and AST may result from liver disease, a disruption in the production of these enzymes, a shift in the likelihood of liver membrane potential, and other factors [13]. Other studies [14] revealed a correlation between liver cell damage and an elevation in transaminases and overall protein level.

The present study revealed that histopathological examination of kidney tissue after 14 days of treatment, group 2 (5mg/kg of MDMP-4en-PINACA) showed relative healthy renal tissue with abundant glomeruli and proximal and distal convoluted tubules while kidney tissue of group 3 (50mg/kg of MDMP-4en-PINACA). Aside from the appearance of tubular casts, tubular degeneration increased. Glomeruli atrophy was accompanied by an increase in Bowman's space. The current study's rise in urea concentration is a symptom of decreased renal function. Clinical assessment of renal function is based on Urea and Creatinine measurements [15]. High levels of creatinine are seen in the liver, cardiac and skeletal muscle, kidneys, where glomerular filtration accounts for most of its elimination [16]. According to these results, MDMP-4en-PINACA significantly altered the liver and kidneys' general health. These changes might have been the result of MDMP-4en poisoning. The results of the current investigation show that the liver and kidneys are more susceptible to the negative effects of the drug used, MDMP-4en-PINACA.

Hematological characteristics acted as biomarkers while detecting organ damage- cellular or tissue level, in animal reproductive, and in the identification of infections, parasitism, and other illnesses. The RBC, HCT, HGB, MCV, MCH, and MCHC counts are routinely employed in clinical practice to evaluate the erythrogram [17]. The ability to investigate the link between erythrocyte size

and HGB content in its interior is provided by the RBC, MCV, MCH, and MCHC databases, which is important for determining the differing levels of anemia [18]. MCV is used to categorize anemia among normocytic, microcytic, and macrocytic erythrocytes and to determine the degree of anisocytosis [19]. The decrease in MCV and rise in MCHC in this research imply microcytic hyperchromic anemia.

The evaluation of biochemical parameters provides crucial information on the diagnostic state, dietary balance, metabolism, functioning of organs and tissues, as well as proof of concealed diseases, allowing treatment and prognosis monitoring [20].

Changes in the ALT, AST, Albumin, and Total protein levels can be symptoms of certain disorders. Rapid ALT increase implies a liver lesion [21], When combined with a rise in AST concentration, it implies severe hepatocyte injury[22].

Conclusion

The liver and kidneys are the organs most vulnerable to the negative effects of synthetic cannabinoids, therefore it is reasonable to conclude that MDMP-4en-PINACA may induce microcytic hyperchromic anemia. MDMP-4en-PINACA.

Ethics Approval: Ethical approval obtained from the Experimental Animals Ethics Committee of Naif Arab University for Security Science, following the international standards for the handling of experimental animals. (Nauss-Rec-22-03)

Conflict of Interest: Nill

Consent to Participate: Nill

Consent for Publication: Not Applicable

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