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## Hepatotoxic effect of popular energy drink: biochemical and histopathological evaluation

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### Abstract:

**Objective:** To assess biochemical and histopathological hepatotoxic effect of popular energy drink (POWER HORSE) **Design:** randomized controlled trial study. **Materials and Methods:** 40 adult albino rats were randomly distributed into two groups, each group formed of twenty rats, group (I), received tap water, group (II), received POWER HORSE, 3.8ml/100g orally, every day for continuous twenty one (21) days. All animals were scarified. Blood samples were gained for biochemical analysis. Histopathological examination of liver was performed through Hematoxylin and Eosin staining. **Results:** POWER HORSE energy drink treated group showed significant increase in AST, ALT and ALP levels when compared to the CONTROL group. Group received POWER HORSE energy drink showed detrimental effects of on liver histopathological examination with severe degenerative changes in liver tissue. **Conclusions:** POWER HORSE energy drink showed high degree of detrimental effects on both liver functions and histopathological examination of liver tissue revealing the hazardous effect of energy drinks on health.

**Keywords:**

Energy drink, liver function, Histopathology.

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**1. Introduction:**

Energy drinks are carbonated non-alcoholic beverages which characterized by highly sweetening taste, rapidly consumed, and often used during exercise and different sports as they are marketed for increasing physical endurance and mental alertness [1]. Recently, they could be found in stores and supermarkets so they are widely used by adolescents, athletes, and young adults. They gained their popularity through their stimulant psychoactive effects (avoid sleep, increase mental alertness, and improve activity), physical effects [2]. Energy drinks are released in the market with attractive names as POWER HORSE and RED BULL to express the speed, power and strength [3].

Energy drinks use among youth has also become increasingly socially acceptable and void of stigma, as evidenced by the growing prevalence. Energy drinks sales reached about 50 billion dollars in 2015 and they were expected to increase up to 60 billion dollars by 2021 [4].

Energy drink can of 250ml contains (80mg of caffeine, 27g of carbohydrate, 1.0g of taurine, 0.6g of glucuronolactone, 20mg of niacin, 5mg

of vitamin B6, 5mg of pantothenic acid, and 5 µg of vitamin B12 and other ingredients) [5].

Concerns about energy drinks consumption increased in many countries in the world especially after reception of 4854 calls from consumers of energy drinks on the National Poison of the U.S, Since 2004, the Food and Drug Administration (FDA) has linked 34 deaths to energy drinks consumption [6].

Since the absorbed ingredients of energy drinks are transferred via the portal venous system directly to the liver, this organ faces the highest concentration of energy drinks ingredients, consumption of energy drinks has been accompanied by the activation of lipid peroxidation in the liver and cytolysis of hepatocytes, which result in Hepatocytes apoptosis, necrosis, necro ptosis, and autophagy [7].

**2. Aim Of The Work:**

The aim of this study is to evaluate the toxicological effects of energy drink on different biochemical and pathological parameters of liver of adult Albino Rats.

### **3. Materials And Methods:**

#### **Materials:**

#### **A-substances:**

POWER HORSE energy drink is produced by Power Horse energy drinks GmbH which is a Company based in Austria. Each 100 ml of POWER HORSE energy drink contains 45 kcal, 32 mg of caffeine, 0.4 gram of taurine, 10.7 gram of carbohydrates, 0.06 gram of riboflavin (B2), 8 mg of niacin (B3), 2 mg of pantothenic acid (B5), 2 mg of pyridoxine (B6) with no fat, fiber ,or protein.

#### **B-Animals:**

Forty (40) albino rats, weighting (150-200) gram, were used in this study. They were raised and obtained from the Animal House of Faculty of Pharmacy, El-Nahda University, Beni-Suef, Egypt.

#### **Experimental Design:**

The experiment was carried out in the animal house of pharmacology and toxicology of Faculty of the Pharmacy, Beni-Suef University. This study was carried through the guidelines for the care and use of laboratory animals approved by Research Animal Committee of Beni-Suef University, Egypt (Approval number 021179). All animals were housed properly. Animals were housed five per cage. The animals were fed laboratory water ad libitum and pellet chow and were

allowed free access to diet and water. Animals distributed into 2 groups (20 rats for each group) and each rat of the Energy drink treated group received a dose of (3.8ml\100g) of body weight for 3 weeks orally [8].

The 2 groups of our study as following:

Group (I): the control group formed of 20 rats received tap water.

Group (II): the POWER HORSE energy drink treated group, formed of 20 rats received POWER HORSE energy drink.

Blood sample were obtained for liver function analysis, then all animals were scarified, rats were sacrificed via decapitation under light ether anesthesia.

#### **Biochemical Analyses**

##### **Liver function tests:**

a. Estimation of serum aspartate transaminase (AST) activity: The serum AST activity was estimated photometrically using AST test kits supplied by DIAMOND Diagnostics, Egypt according to the method described by [9].

b. Estimation of serum alanine transaminase (ALT) activity: The serum ALT activity was estimated photometrically using ALT test kits supplied by DIAMOND Diagnostics, Egypt according to the method described by [9].

c. Estimation of serum alkaline phosphatase: The blood serum alkaline phosphatase was estimated photometrically by using alkaline phosphatase test kits supplied by BioSystems reagents & instruments according to the method described by [10]

#### Histopathological study:

Autopsy samples were taken from the rats in the previously mentioned experimental groups. Then liver samples were fixed in 10% formal saline solution for 24 hours. The obtained sections were fixed on glass slides, cleared from paraffin and stained with H & E stain for histopathological examination under the light microscope [11].

#### Statistical analysis:

Data was analyzed using SPSS version 25 for windows 10.

### 4. Results:

This randomized controlled trial study was conducted to 40 adult albino rats to assess toxicological effects of one popular energy drink of POWER HORSE on the biochemical and histopathological parameters of the liver tissues of rats.

#### 1-Estimation of liver Function Tests

##### a- Estimation of Alanine Aminotransferase (ALT):

The levels of Alanine Aminotransferase (ALT) that measured in serum are shown in **Table (1)**

**Table (1) Comparison between the studied groups regarding the Alanine Aminotransferase (ALT) level:**

ALT(U/L)	CONTROL group No=20	POWER HORSE group No=20	P-value
Mean±SD	41.28±4.94	62.78±4.25	<0.001*

*\*P-value is significant*

**Table (1)** showed that there was a significant difference between groups regarding the ALT level in POWER HORSE group showed significant increase in ALT level , when compared to the CONTROL group. The higher level was in the POWER HORSE group than CONTROL group. The

mean ALT level was  $41.28 \pm 4.94$  and  $62.78 \pm 4.25$ , U/L for CONTROL group and POWER HORSE respectively.

### b-Estimation of Aspartate Transaminase (AST)

The levels of Aspartate Transaminase (AST) that measured in serum are shown in **Table (2)**.

**Table (2) Comparison between the studied groups regarding the Aspartate Transaminase (AST) level:**

AST(U/L)	CONTROL group No=20	POWER HORSE group No=20	P-value
Mean±SD	135.17±5.14	163.47±5.67	<0.001*

*\*P-value is significant*

**Table (2)** showed that there was a significant difference between CONTROL & POWER HORSE groups. POWER HORSE group showed significant increase in AST levels when compared to the CONTROL group.. The mean AST level was  $135.17 \pm 5.14$  and  $163.47 \pm 5.67$ U/L for CONTROL and POWER HORSE group respectively.

### c- Estimation of Alkaline Phosphatase(ALP):

The levels of Alkaline Phosphatase (ALP) that measured in serum are shown in table (3)

**Table (3) Comparison between the studied groups regarding the Alkaline Phosphatase (ALP) level:**

ALP(U/L)	CONTROL group No=20	POWER HORSE group No=20	P-value
Mean±SD	256.8±9.75	321.44±34.20	<0.001*

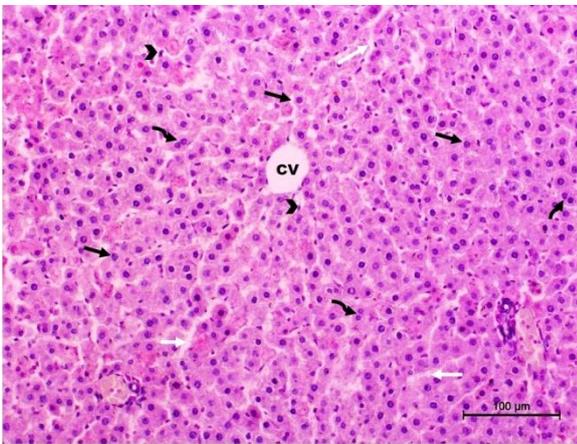
*\*P-value is significant*

**Table (3)** showed that there was a significant difference between groups regarding the ALP level in POWER HORSE group showed significant increase in ALT level , when compared to the CONTROL group. The higher level was in the POWER HORSE group than CONTROL group. The mean ALT level was  $256.8 \pm 9.75$  and  $321.44 \pm 34.20$  U/L for CONTROL group and POWER HORSE respectively.

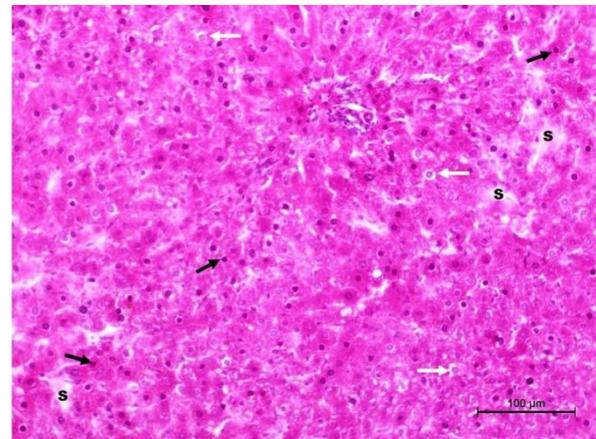
## **2-Histopathological examination :**

Histopathological examination of liver sections of control group showed in **figure (1)** that revealed normal hepatic architecture of well-preserved hepatic lobules that formed of cords of hepatocytes which radiate from central veins, blood sinusoids with Van kuffer cells.

Histopathological examination of POWER HORSE treated group in **figure (2)** showed severe degenerative changes with coagulative necrosis, moderate leukocytic infiltration and nuclear necrosis.



**Figure (1): A photomicrograph of a section in the liver of adult male albino rat of CONTROL group showing:** Normal hepatic architecture with the central vein (CV) and radiating cords of normal hepatocytes (**Black arrow**) with central rounded vesicular nuclei and prominent nucleoli. Hepatic cords are separated by blood sinusoids (**white arrow**) lined with endothelium and Von-Kupffer cells (**arrowhead**). Binucleated cells (**curved arrows**) can be seen. (H&E X 200).



**Figure (2): A photomicrograph of a section in the liver of adult male albino rat of POWER HORSE group showing:** Some hepatocytes have dark eosinophilic cytoplasm with pyknotic nuclei (**Black arrow**). Others have cytoplasmic vacuolations (**white arrow**). Some blood sinusoids are dilated (S). (H&E X 200).

## **5. Discussion:**

Our study showed significant increase in biochemically tested liver function (ALT,AST,ALP) in the group treated with POWER HORSE energy drink when compared to the control group , these results are concurrent with [12] of significant high levels of liver enzymes( ALT, AST, ALP) in groups received POWER HORSE in dose of (3.1ml/100g) and dose of (6.3ml/100g) for 21 days and this alteration in liver enzymes are most probably caused by caffeine, also [13] showed significant increase in liver enzymes( ALT, AST, ALP) indicating liver damage by toxic agents with significant decrease in superoxide dismutase, catalase, glutathione dismutase enzymes indicating increasing oxidative stress in groups received different doses ranging from (0.4ml : 2.2 ml/100g) of an energy drink for 12 weeks. These findings are consistent with result of [14] of liver enzymes in groups treated by energy drinks alone or in combination of alcohol.

Also, there are reported cases of acute toxic liver damage resulted from abuse of energy drinks consumption as in [15] that reported a 22 years old woman suffered from acute hepatitis with high levels of ALT, AST and normal ALP levels, there was history of consuming 10 cans of energy drinks daily in the previous 2 weeks, no history of alcohol ingestion and no positive results for toxicological analysis and the most assumed

probable cause was high levels of niacin ingested in energy drinks for its known adverse effect of hepatitis.

Concerning our histopathological findings in the liver showed severe degenerative changes of nuclear necrosis, coagulative necrosis and moderate leukocytic infiltration in the group received POWER HORSE energy drink.

Our findings of histopathological alterations in liver tissue of nuclear necrosis and degenerative changes are corresponding to findings of [7] that revealed euchromatic or hyperchromatic nuclei of hepatocytes with increased von-Kupffer and lymphocytic cells with small areas of necrosis and these changes are caused by activation of apoptosis of hepatocytes which detected by increasing levels of caspase-3(plays a central role in the execution-phase of cell apoptosis) in liver homogenate of groups treated with energy drinks, the increase in apoptosis were attributed to the caffeine, taurine, and D-glucuronolactone ingredients of energy drinks. Our study showed significant histopathological changes of liver in contrast to [16] which demonstrated no pathological changes in liver of the group that received POWER HORSE energy drink in a dose of (1.2ml/100g) daily for 4 weeks.

## **6. Conclusion And Recommendations:**

POWER HORSE energy drink showed toxic effects on hepatocytes accompanied by elevation of liver function tests which come

along with histopathological changes in liver tissue which showed severe degenerative changes of nuclear necrosis, coagulative necrosis and moderate leukocytic infiltration. This showing potential hazardous effect of energy drinks on health so there should be awareness campaigns and informative workshops for health hazards of energy drinks on public health.

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