IASJ المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/j

Interstop [Dioraleze] Drug-induced hepatotoxicity and Liver Injury In Rats

Asraa Abed Thiyab

Department of Biology , College of Education for Pure Sciences , Tikrit University, Tikrit , Iraq

https://doi.org/10.25130/tjps.v25i4.264

ARTICLE INFO.

Article history:

-Received: 5 / 9 / 2019

-Accepted: 3 / 10 / 2019

-Available online: / / 2020

Keywords: Interstop, Dioraleze, Anti-Diarrhea.

Corresponding Author:

Name: Asraa Abed Thiyab

E-mail: esraa.abed@tu.edu.iq

Tel:

ABSTRACT

Interstop (Also known as Dioraleze) was the focus of this research paper to elaborate and find out the effect of Interstop administration on Hepatocytes in Rats.

Rats from both sexes were randomly distributed into three main groups as follows:

Group 1 (Control): Orally given distilled water daily for three weeks.

Group 2 (Treatment): Orally given 1mg/kg body weight Interstop daily for three weeks.

Group 3 (Overdose): orally given 2.5mg/kg body weight Interstop daily for three weeks.

At the end of the experiment, rats were dissected and the liver was obtained under aseptic technique for laboratory study .

Drug-induced liver disease is usually a result of metabolites that could affect the cell and tissue biochemistry directly or result in an immune response which was found in this study as the occurrence of several Vascular Sacs directly attached to the liver in all rats present in group 2 and 3. The sac ranged in size from 0.5 cm-1 cm compared to control that lacked the presence of any vascular sac.

Liver in both treatment groups showed several abnormalities after treatment compared to Group 1 which includes Karyolysis, pyknosis and Karyohexsis. Atrophy and necrosis in addition to degeneration of hepatocytes was seen.

In conclusion, Interstop has shown remarkable damaging effects on the Liver, especially when used without medical awareness and at higher doses than recommended, hence, leading to Hepatocyte abnormalities increasing organs malfunction and performance deficiency after long time of treatment.

Introduction

Interstop (Dioraleze) is an over-the-counter capsules for oral administration for the treatment of anti-diarrhea Stored below 25°C in a dry place. This drug works by lowering gastro-intestinal motility by effecting both circular and longitudinal muscles of the intestine and peripheral intestine μ-receptors, hence reducing the number of discharge in patients following ileostomy and for the treatment of inflammatory bowl disease. This drug clearly works as a symptom healer rather than finding a final solution for diarrhea itself. When Interstop slows down the digestion process, the gut and small intestine has more time to absorb nutrients and fluid from food [1].

Interstop is present as 2 mg capsules. Dosage of drug depends on the condition, age and weight of patient. Interstop is broken down by the liver that could eventually over time cause liver malfunction. Various studies on distribution in rats showed a high affinity for the gut wall with a preference for binding to receptors of the longitudinal muscle layer. The plasma protein binding of Interstop is 95% which binds mainly to albumin[2].

Interstop is predominantly metabolized and extracted by the liver then conjugated and excreted via the bile. It has a half-life range of 9-14 hours.

Interstop has a rapid metabolism /diffusion through the Central Nervous System making people to believe it is free of abuse but when hepatic function

necessary for the drug's metabolism is defective (e.g. in cases of severe hepatic disturbance), as this might result in a relative overdose leading to CNS toxicity. Nevertheless, Interstop abuse has significantly accelerated the past few years with many life threatening conditions such as cardiac arrhythmia and cardiac arrest as a result, hence more research is needed on a histological/histo-pathological level [2,3].

This study emphasizes on adverse toxic effects of Interstop on the hepatocytes and liver tissue.

Materials and Methods

Table (1): Materials and Equipment used.

Materials	Instruments
Alcohol (100%-30%)	Beakers, Glasses, Slides
Chloroform	Rotary-microtome
Paraffin Wax	Oven 57°C
Distrene Plasticizer Xylene (D.P.X)	Worm Water Bath
H & E Stains	Filter Paper
Xylene	Light Microscope
Interstop pure powder	Blocks and Hot Plate

Experimental Animals

Animals were obtained from the University of Tikrit Animals House. Experiment was designed to fit 20 Swiss-Rats for 3 weeks.

Rats were housed in special cages (4 animal per cage) and given pellet rodent diet in addition to water, well ventilated housing was provided in a stress free environment (elimination of excess feces, crowdedness and environmental change).

Groups:

Group 1 (Control): 4 Rats treated with 1ml distilled water for 3 weeks.

Group 2 (Therapeutic Dose): 8 Rats treated with 1 mg/kg of Interstop for 3 weeks.

Group 3 (Overdose Dose): 8 Rats treated with 2.5mg/kg of Interstop for 3 weeks. [8]

Experiment Design

Rats were divided randomly into three groups, all were given the drug after dissolving it in 1ml distilled water and given orally. Rats were given the drug twice a day.

After the end of experimental period of animals, Rats were killed using chloroform in a closed glass box with no ventilation. Liver was obtained after aseptic dissecting technique and processed according to Bancroft& Stevens 1987 as follows:

1- Fixation- [10% neutral buffered formalin] for 24 hours at room temperature.

2- Dehydration- tissues obtained were passed through progressively graded concentrations of alcohol baths [30%, 50%, 70%, 80%, 90%, 100%].

3- Clearing- xylene was used by keeping the tissues for about 20 minutes twice allowing transparency to the tissues.

4- Infiltration and Embedding- two changes of molten paraffin wax [57-58° C] for 30 minutes, to ensure the complete removal of xylene. Special Aluminum containers were used for embedding the tissues and labeling the blocks for future recognition.

5- Tissue sectioning- rotary microtome with disposable sharp blades were used. Tissues were cut into 6 micrometer before transporting the thin ribbons into 44 ° C water bath then put on the slides.

6- Tissue Attachment - Mayer's glycerol-albumin mixture was used to smear the slides before attachment of the sections.

7- De-waxing and Hydration- two changes of xylene used to remove excess wax followed by hydration by inserting the slides in descending concentration of alcohol baths [100%, 90%, 80%, 70%, 50%, 30%].

8- Staining- with Heamatoxylin and Eosin [H & E].

9- Mounting- with D.P.X and examined under Light Microscope while taking the pictures by Multiple Power Microscope with computer screen.

Results

Gross Pathology

Color of Liver was changed with rough edges compared to Control Group. Small to medium large sacs (0.5-1 cm) was observed attached to the Liver in both group 2&3 compared to Control Group.

Histopathology

Group1 : Control

The sections of liver hepatocytes which forms the main composition of the liver in addition to epithelial cells. Liver central vein and bile canal is shown in (Figure 1)

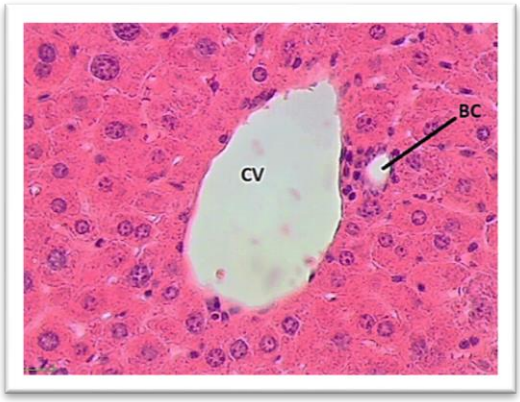

Fig. 1: Photomicrograph of Liver showing Central Vein (CV) and Bile Canaleculi (BC). (H & E, 40X)

Group2 : Therapeutic Dose

Figure 2 shows a clear section of the liver with normal radial alignment in the Hepatocytes while sinusoids does not show any abnormalities. However, Tissues lining the Central Vein appear to be slightly enlarged. Infiltrated lymphocytes appear scattered in this section.

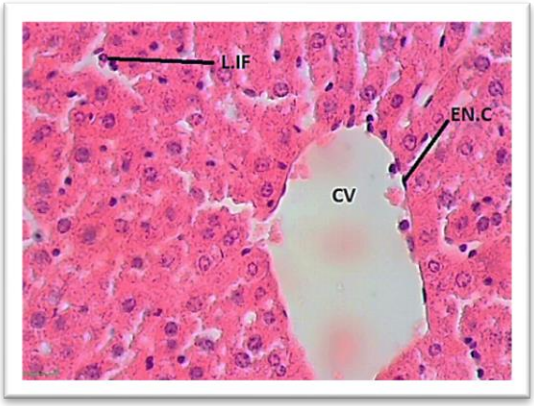

Fig. 2: Photomicrograph of Liver showing Central Vein (CV), Lymphocyte Infiltration (L.IF) and Endothelium cells (EN.C). (H & E, 40X)

Figure 3 shows a severe congestion hemolysis that appears in the central vein and in all of hepatocytes.

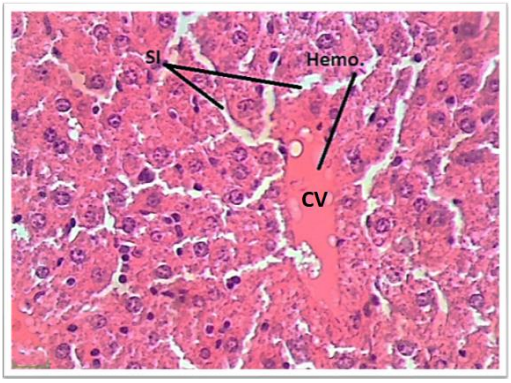

Fig. 3: Photomicrograph of Liver showing sinusoid (SI) and Hemolysis (Hemo.). (H & E, 40X)

Group 3 : Overdose Dose

Figure 4 shows hepatocyte arrangement in cords around the central sinusoid (CV), karyorrhexis, absence of clear sinusoids within Hepatocytes, with the presence of severe congestion in between Hepatocytes and the central vein. Necrosis of the nuclei is also seen at various stages. Karyopyknosis of the nuclei is seen due to cell degeneration and necrosis.

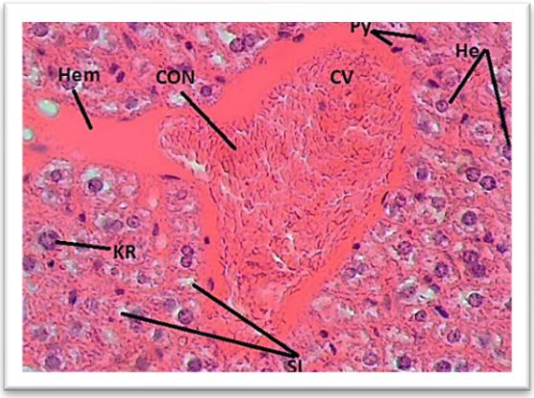

Fig. 4: Photomicrograph of Liver showing Central Vein (CV), Hemolysis (Hem), Congestion (CON), Karyorrhexis (KR), Sinusoid (SI), Hepatocytes (He) and Pyknosis Cells (Py). (H&M, 40X)

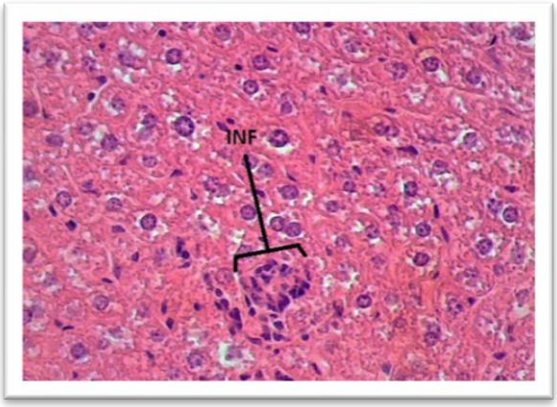

Fig. 5: Photomicrograph of Liver showing Infiltration of Lymphocytes (INF). (H & E, 40X)

Mild Hemolysis in the central vein is observed in Figure 6 with Severe Congestion.

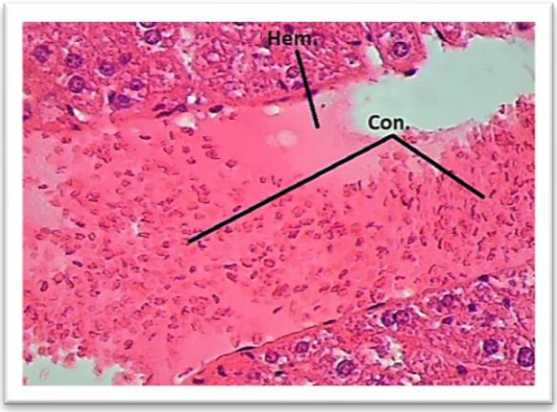

Fig. 6: Photomicrograph of Liver showing Mild Hemolysis (Hem.), Congestion (Cong.). (H & E, 40X)

Cord Radial Hepatocyte Alignment appear normal in Figure 7 with small narrow Sinusoids and unclear cells lining the Bile Duct in addition to Lymphocyte Infiltration.

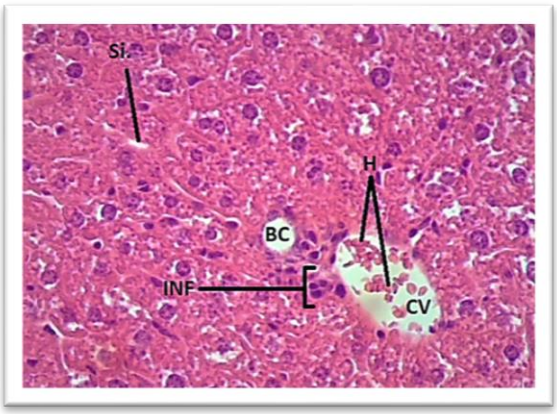

Fig.7: Photomicrograph of Liver showing Sinusoid (S), Bile Canaleculi (Bc), Hemorrhage (H), infiltration of Lymphocytes (IF). (H & E, 40X)

Discussion

Dioraleze is a yellowish powder that is mainly soluble in ethanol, methanol and chloroform while it is slightly soluble in water. The drug reduces the loss

of water and salts from the intestines in addition to slow their activity, hence, resulting in a stiff stool [1].

Modern research regarding Dioraleze (Interstop) states that Interstop acts as an anti-diarrheal drug due to its ability to bind to the opiate receptor located on the wall of the gut. Furthermore, it inhibits the release of acetylcholine and prostaglandins which reduces propulsive peristalsis[3].

Several research show the effects of this drug mainly on the Central Nervous System (CNC) commonly causing mild symptoms while in rare cases could cause loss of consciousness' muscular hypertonia and respiratory depression. Immune System Disorders such as hypersensitivity reaction and Anaphylactic shock. Eye Disorders such as Miosis could also form in rare cases. Gastrointestinal Disorders can also be found with mild side effects such as fatigue, Constipation, Nausea or could be more severe causing ileus [including paralytic ileus], toxic megacolon, abdominal distension and abdominal pain. Other researches indicate the formation of Renal and Urinary disorders such as Urinary retention. [5]. These research could explain the adverse effects found as a result of therapeutic and toxic dose given to rats.

Very few studies was found on the effect of Interstop on hepatocyte and liver tissue but overall observations found in this study was the heavy breathing causing a heavy ventilation for Rats treated in the Overdose Group and the death of 1 rat after two weeks of oral dosage concluding it's lethal effect when given a higher dose than recommended, hence, resulting in an endo-toxic substance that led to necrosis of cells in the tissues and organs effecting its overall activity and function. This was in agreement with [Julia, 2015] that recorded a young patient complaining from abnormal abdominal pain, acute respiratory failure, sinus rhythm and intra-ventricular conduction defect for weeks. After close toxicology tests it was found the presence of high amount of Interstop substance in the blood and was reported as Drug Abuse[6].

Another study also was in agreement regarding the effects of this drug on muscles [Maccherer *et al*., 1977] that concluded the inhibition of the contractions of electrically driven longitudinal muscle from guinea-pig ileum and naloxone antagonized these effects.

This study was in agreement with a recent research on Rats after giving Rats anti-diarrhea drug of the same component as used in this study. According to [Apotex. 2012]. The secretion/absorption in Rats was reduced which effected the intestinal activity greatly as a result of ion permeability of the mucous layer. This is due to the occurrence of endotoxins and hormones which led to diarrhea concluding the effect of this drug on a toxic level when using an overdose [7].

The toxicity of Interstop at higher doses was also recorded in several studies such as the (CNITV) research records of 13 cases on dog poisoning by Interstop; with severe cases that led to coma and respiratory distress and the death of a 6 week old poppy after a high dose of Interstop. [4,7].

Conclusion

Interstop [Dioraleze] is an over-the-counter capsule that is given without any care on its negative effects when given at a higher dose than recommended or when taken for longer periods. It effects several organs in addition to the Liver with long term and lethal side effects. Further study on its Toxicity is recommended due to the lack of histopathological research found so far.

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تسمم وتضرر خلايا الكبد نتيجة استعمال دواء الانترستوب (Dioreleze) في الجرذان

اسراء عبد ذياب جزاع

قسم علوم الحياة ، كلية التربية للعلوم الصرفة ، جامعة تكريت ، تكريت ، العراق

الملخص

تم تصميم هذا البحث لتوضيح واكتشاف تأثير عقار الانترستوب [او ما يعرف بـ ديولوريز] علي خلايا الكبد في الجرذان.

تم اختيار الجرذان عشوائيا من كلا الجنسين وتصنيفهم عشوائيا إلي ثلاثة مجاميع رئيسية:

المجموعة 1 : [مجموعة السيطرة] تم تجريع الحيوان الماء في الفم مرة واحدة يوميا لمدة 3 اسابيع

المجموعة 2: [الجرعة العلاجية] تم تجريع الحيوان عن طريق الفم بـ 1 مغاكيلو لمدة 3 اسابيع

المجموعة 3: [الجرعة العلاجية الزائدة] تم تجريع الحيوان عن طريق الفم بـ 2.5 مغاكيلو لمدة 3 اسابيع

في نهاية الفترة المخصصة للتجربة تم تشريح الجرذان وحفظ الكبد تحت ظروف معقمة لدراستها نسيجيا

عادةً ما يتم الحصول علي تغيير نسيجي للأعضاء نتيجة الاستمرار بتجريع دواء كان له تأثير مباشر علي عملية الايض مما ينتج عنه استجابة مناعية مثلما تم الحصول عليه في هذه الدراسة حيث اظهرت نتائج البحث خصول العديد من التغييرات في نسيج الكبد ظاهرياً وتحت المجهر. تم العثور علي اكياس خارج الجدار المحيط بالكبد في أغلب جرذان المجموعة 2 و 3 بالإضافة إلي موت الخلايا, تحلل وتنخر في خلايا الكبد, ارتشاح في الخلايا اللمفاوية واحتقان دموي.

نستنتج أن لدواء الأتوستوب المستخدم (بدون رقابة) تأثير كبير علي خلايا الكبد وتظهر هذه التجربة التأثير السلبي الذي يصاحب تناول عقار الانترستوب بكثرة بالأخص فوق الجرعات المحددة لكل مريض.