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Histopathological Study of Orlistat and Rosemary Aqueous Extract in the Brain and Liver of Obese Male Rats

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ABSTRACT

This study aimed to see how induced obesity, orlistat, and aqueous rosemary extract, affected albino rats' brains and livers. The rats were divided into 7 different groups. Each group contains 6 rats. The first group was the control. The second group was fed on a high-fat diet, and the third and fourth groups were on a high-fat diet with orlistat 100 and 200 mg/kg of B.W. The fifth group on a high-fat diet with an aqueous extract of rosemary was given 10 ml/kg of B.W. The sixth and seventh groups were given an aqueous extract of 10 ml/kg of rosemary with orlistat 100 and 200 mg/kg, respectively. The histopathological results of the brain and liver revealed pathological changes in the fattened rats, third and fourth groups represented by vacuolation of surrounding neurons and glial cells. Congestion of blood vessels and vasogenic edema in the brain while in the liver were fatty degeneration in the hepatocytes, necrosis and lymphocytic aggregation, congestion and dilatation of blood vessels, hyperplasia of epithelial cells lining bile ducts, focal infiltration of inflammatory cells and dilatation of sinusoids compared with the control group and fifth group. Whereas the histopathological changes of the sixth and seventh groups were less severe than the lesion that occurred in the second, third and fourth groups. We conclude that the aqueous extract of rosemary was efficient in alleviating tissue pathological lesions.

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INTRODUCTION

Due to the general rising prevalence of obesity as related health problems, causes in more than a third of the world's population, interest in using slimming drugs as a quick and effective treatment for obesity has grown, despite thoughts that differ in terms of effectiveness, mechanism of action, and side effects. According to one study of **Thaler *et al.*, (2012),** HFD diet causes neuronal damage in the hypothalamus, leading to reactive glia including glial and astrocyte populations; it appears that these reactions are selective.

The transient nature of this hypothalamic response suggests that neuroprotective responses are modulated and limit or reverse injury during its early stages, but that gliosis is re-established with continued HFD exposure, and that obesity is thus linked to neuronal injury in the body weight-control brain region **(Padwal and Majumdar, 2007).**

Orlistat is an anti-obesity gastric and pancreatic lipase inhibitor that has been approved by the Food and Drug Administration (FDA) **(Heal *et al.*, 2012).** Orlistat has a cholesterol-lowering effect, yet there is limited information on its adverse effects and problems. This drug and its long-term consequences **(Song *et al.* 2018).** Furthermore, interest in medicinal plants has grown as a safer alternative to slimming drugs, as it has been demonstrated that they play a significant role in preventing a variety of diseases. As they contain components with antioxidant properties due to the presence of a diverse range of active compounds, rosemary is one of these plants. It is one of the most important medicinal herbs; it is utilized in foods, nutritional supplements, and cosmetics as flavor additives **(Misra et *al.*, 2009).**

Because it contains antioxidants and antibacterials and is an antidiuretic, it has a variety of biological functions. It's also anti-inflammatory, and it's been shown to help with a variety of medical issues,

including obesity, weight reduction, and hyperglycemia **(Himed-Idir*et al*., 2021).**

The focus of the research is to see how induced obesity affected the histological structure of several organs (brain and liver) in male rats and how orlistat and rosemary aqueous extract influenced it.

MATERIALS AND METHODS

Experimental animal:

Forty-two male Albino rats (aged 6-8 weeks, weighing between 170-200 grams) were used for this investigation. The animals were housed in tight plastic cages in the College of Veterinary Medicine/Mosul University's animal house of college, considering the cleanliness conditions. In addition to the conventional rearing conditions of 12 hours of light and 12 hours of darkness, and at laboratory temperature (20±22) °C, cleaning was performed and gave the animals water and the appropriate feeding during the trial time.

Chemicals:

Orlistat was utilized in this study in the form of 120 mg capsules from the Jordanian Al-Hikma Company, bought from local pharmacies. It had been dissolved in distilled water.

Rat feed:

A conventional rat diet with a percentage defined by the National Research Council (NRC) (National Research Council, 1994) was utilized in this experiment. The diet's components were: (feed wheat 24 %, feed maize 45%, soybean meal 20%, animal protein 10%, lime 0.5 percent, table salt 0.5 percent). A high-fat diet was used during the experiment period by adding 35 percent fat (30 percent beef fat, 5 percent sunflower oil) to the standard diet (Galaly*et al*., 2014; Kim *et al*., 2004).

The plant used in the current study:

Only the plant's leaves were used, and they were dried on big filter sheets in the shade at room temperature for 15 days, with occasional stirring to prevent rotting. The dried leaves were then pulverized with an electric grinder and stored at room temperature in clean, dry bags until usage.

Preparation of the aqueous extract of rosemary leaves:

The extraction process was followed by (Halloui*et al*., 2000), who prepared the extract daily by combining 8 g of leaf powder with 100 ml of heated distilled water, boiling it for two minutes, and then allowing it to cool for an hour. The solution is then filtered through Whatman No.1 filter paper and yielding 60 mL within 24 hours of preparation. it is administered daily via oral dosage with a Gavage oral dosing syringe at a rate of 10 mL/kg body weight.

The rats utilized in this study were 42 white male Albino rats with weights ranging from 170-200 grams and ages ranging from 6 to 8 weeks. The trial lasted 20 weeks and was split into two sections:

Experimental Design:

The first stage (fattening stage):

The rats were divided into seven groups, each of which had six rats. The starting weights of the rats were recorded once the preparatory period finished, and then the stage of induced obesity began using high-fat food (Kim et al., 2004), which lasted from week 0 to week 12. Except for the control group, which was provided regular food throughout the experiment, the rats were fed on this diet.

The second stage (treatment stage):

The rats were fed a high-fat diet only with a daily dose of distilled water from week 12 to week 20, and the totals were divided as follows:

- The first group was a control group dosed daily with distilled water.
- The second group was fed a high-fat diet only with a daily dose of distilled water.
- The third group was fed a high-fat diet while being given Orlistat at a dose of 100 mg/kg.
- The fourth group, in which the rats were fed a high-fat diet while also being given the Orlistat at a dose of 200 mg/kg.
- The fifth group consisted of rats fed a high-fat diet ~~and~~ given 10 ml/kg of body weight of aqueous rosemary extract.
- The sixth group consisted of rats fed a high-fat diet and given aqueous rosemary extract at a concentration of 10 ml/kg of body weight for the duration of the study.
- The seventh group, the rats, were given Orlistat at a 100 mg/kg dosage after being fed a high-fat meal with aqueous rosemary extract (10 ml/kg).
- The eighth group, in which rats were fed a high-fat diet with aqueous rosemary extract (10 ml/kg) and subsequently Orlistat medication at a 200 mg/kg concentration. The rats were dosed daily with it using an oral dosing system.

Histological Sample Collection and preparation:

The animals were euthanized with ether. For histological testing, the target organs (brain and liver) were removed and rinsed thoroughly in physiological saline 0.9 percent NaCl. Brain and liver samples were taken from each animal and were collected and fixed for 48 hours in a 10% neutral buffed formalin solution. According to the approach, histological slides for each section were conducted as outlined by Suvarna et al. 2019. A light microscope was used to view and photograph the tissue sections.

RESULTS

Histopathological changes in the brain:

The cerebral cortex of the control rat group had normal histological architecture represented by neurons, glial cells, and blood vessels (Fig.1). The fattened rats' cerebral cortex revealed vacuolation surrounding neurons, glial cell proliferation, congestion and dilatation of blood vessels, and vasogenic edema (Fig. 2). The cerebral cortex sections of both Orlistat 100 and 200 mg/kg treated rats group revealed the same histopathological changes as the fattened rats group, in addition to perineuronal and periaxonal edema, severe congestion and dilatation of blood vessels in the meninges of the Orlistat 200 mg/kg treated rats group (Figs. 3, 4 and 5).

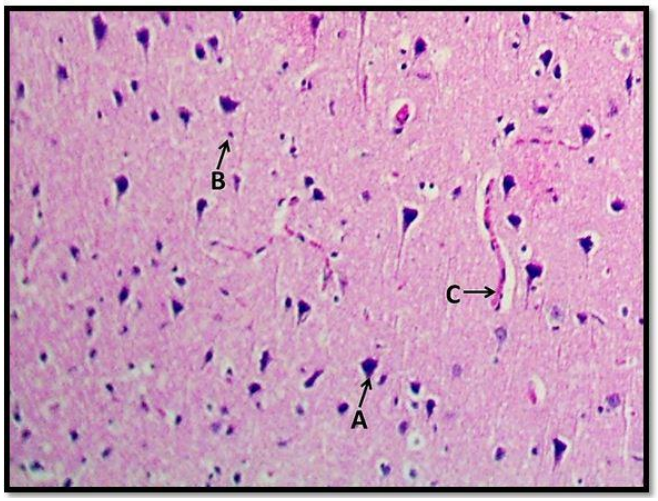

Fig. 1: A photomicrograph of the brain of the control group shows the normal architecture of the cortex of the cerebrum represented by neurons (A), glial cells (B), and blood vessels (C). H&E stain, 400X.

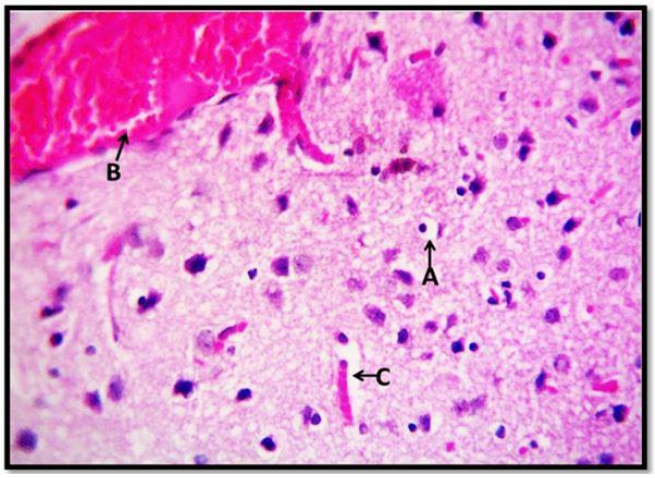

Fig. 2: A photomicrograph of the brain of fattened rats group shows vacuolation of glial cells (A), congestion and dilatation of blood vessels (B), and vasogenic edema (C). H&E stain, 400X.

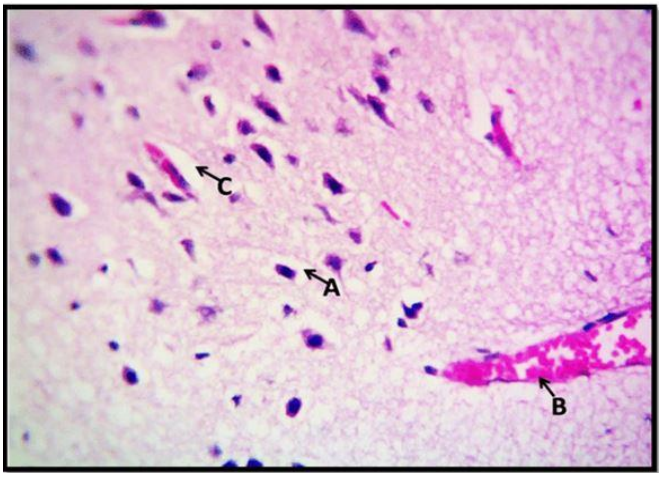

Fig. 3: A photomicrograph of the brain of Orlistat 100 mg/kg treated group shows vacuolation of neurons (A), congestion and dilatation of blood vessel (B) and vasogenic edema (C). H&E stain, 400X.

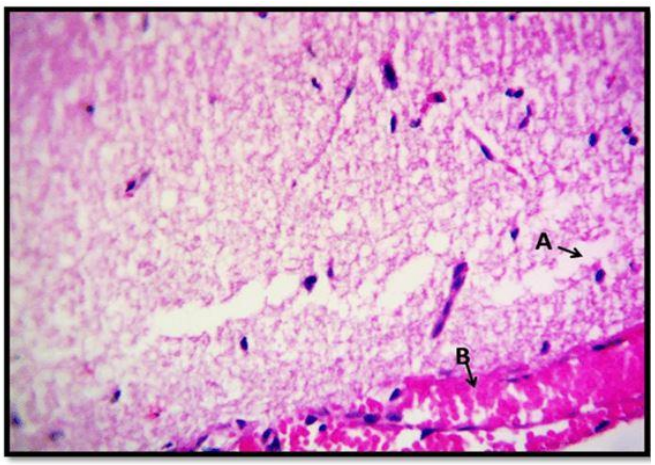

Fig. 4: A photomicrograph of the brain of Orlistat 200 mg/kg treated group shows vacuolation of myelin sheaths (A), severe congestion, and dilatation of blood vessels in the meninges (B). H&E stain, 400X.

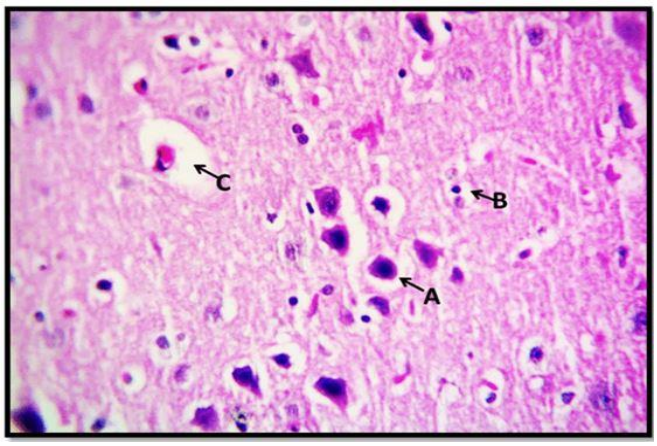

Fig. 5: A photomicrograph of the brain of the Orlistat 200 mg/kg treated group shows perineuronal edema (A), gliosis (B), and periaxonal edema (C). H&E stain, 400X.

The brain of the aqueous extract of rosemary plant treated histological group sections showed normal architecture of cortex of the cerebrum represented by neurons and glial cells with mild periaxonal edema (Fig. 6). While the histopathological changes were less severe in the Orlistat 100 mg/kg with aqueous extract of rosemary plant treated group, which were congestion and dilatation of blood vessel and vasogenic edema (Fig. 7), and also in the orlistat 200 mg/kg with aqueous extract of rosemary plant treated group, which revealed mild perineuronal edema and intact glial cells with mild vasogenic edema (Fig. 8).

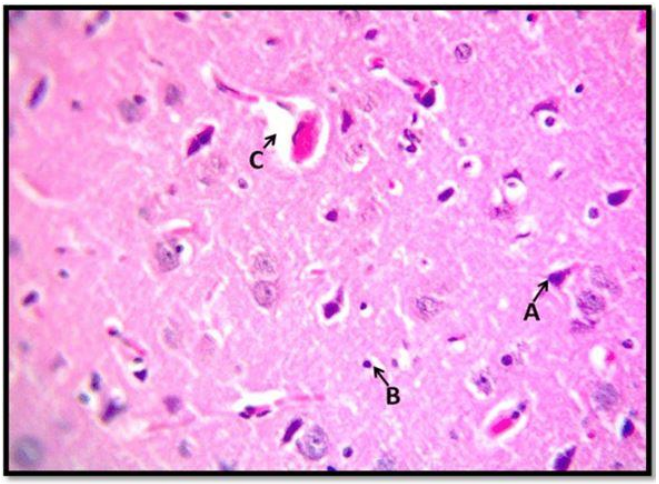

Fig. 6: A photomicrograph of the brain of aqueous extract of rosemary plant treated group shows the normal architecture of cortex of the cerebrum representing by neurons (A), glial cells (B) with mild periaxonal edema(C). H&E stain, 400X.

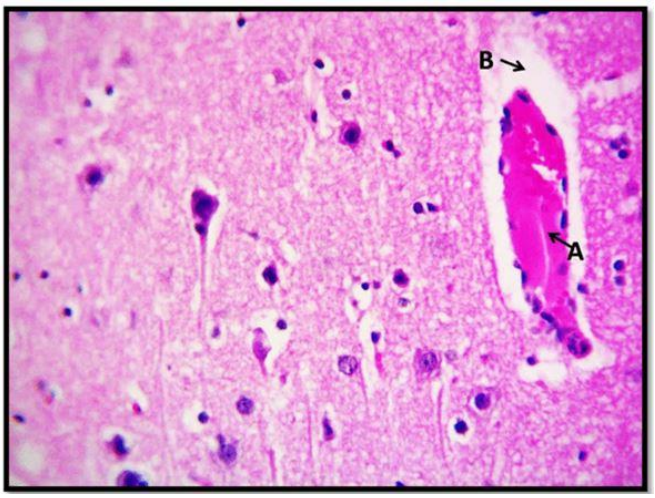

Fig. 7: A photomicrograph of the brain of Orlistat 100 mg/kg with aqueous extract of rosemary plant treated group shows congestion (A) and vasogenic edema (B). H&E stain, 400X.

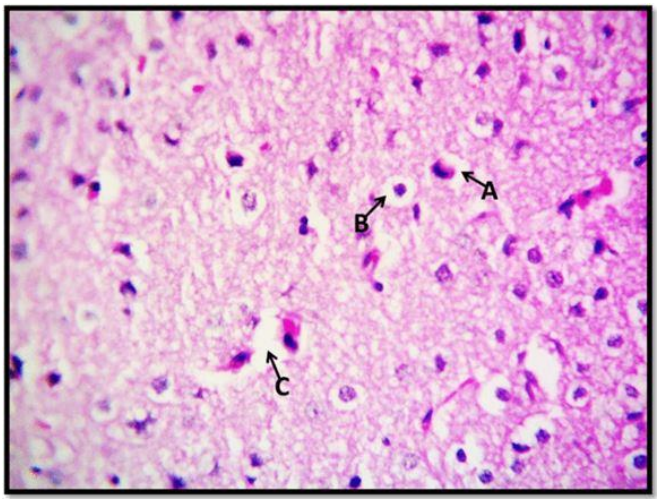

Fig. 8: A photomicrograph of the brain of Orlistat 200 mg/kg with aqueous extract of rosemary plant treated group shows mild vacuolation of neurons (A), intact glial cells (B), and vasogenic edema (C). H&E stain, 400X.

Histopathological changes in the liver:

The control group's histological sections of the rat's liver showed normal architecture of the central vein surrounded by organized hepatocytes as ~~a~~ cord, sinusoids, and Kuepfer cells (Fig.9).

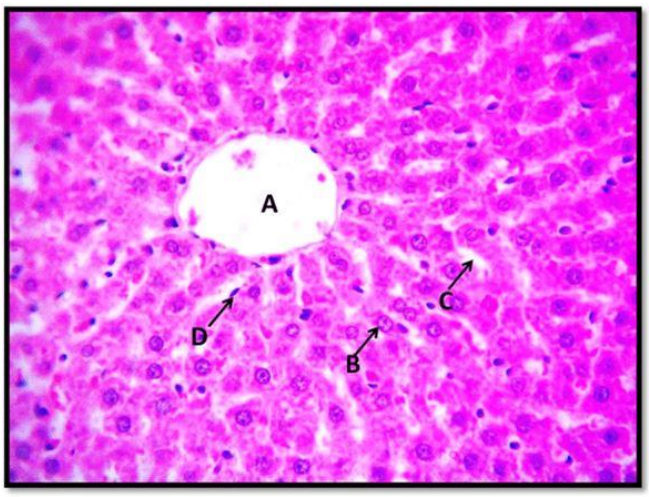

Fig. 9: A photomicrograph of the liver of the control group shows the normal architecture of the central vein (A) surrounded by organized hepatocytes as a cord (B), sinusoids (C), and Kuepfer cells (D). H&E stain, 400X.

The histopathological changes of the liver of fattened rats group were severe vacuolar degeneration in the hepatocytes with necrosis of others and lymphocytic aggregation, congestion, and dilatation of the portal vein, hyperplasia of epithelial cells lining bile ducts, focal infiltration of inflammatory cells and dilatation of sinusoids (Figs. 10 and 11).

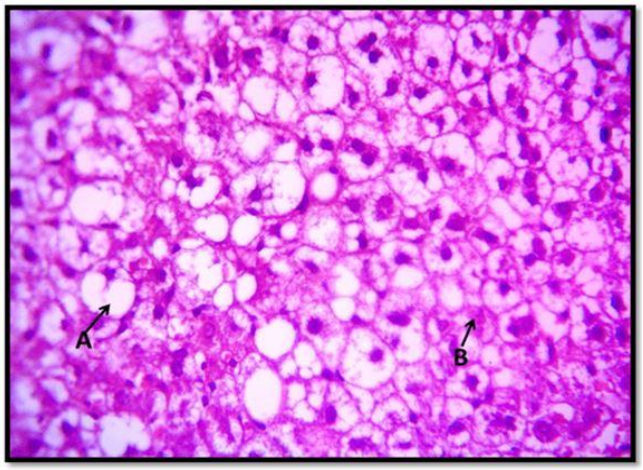

Fig.10: A photomicrograph of liver of fattened rats group shows fatty degeneration in the hepatocytes (A) with necrosis of others (B). H&E stain, 400X.

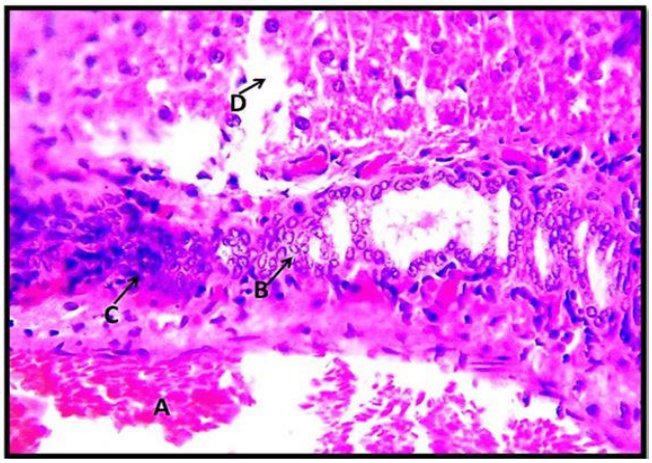

Fig.11: A photomicrograph of liver of fattened group shows congestion and dilatation of portal vein (A), hyperplasia of epithelial cells lining bile ducts (B), focal infiltration of inflammatory cells (C) and dilatation of sinusoids (D). H&E stain, 400X.

Whereas the lesions of the liver of the Orlistat 100 mg/kg treated group revealed infiltration of inflammatory cells mainly lymphocytes, necrosis of hepatocytes, and dilatation of sinusoids (Fig.12), in addition to these lesions, there was congestion and enlargement of Kuepfer cells and disappearance of chromatin material in the nuclei of a few hepatocytes in the liver of the Orlistat 200 mg/kg treated group (Fig. 13).

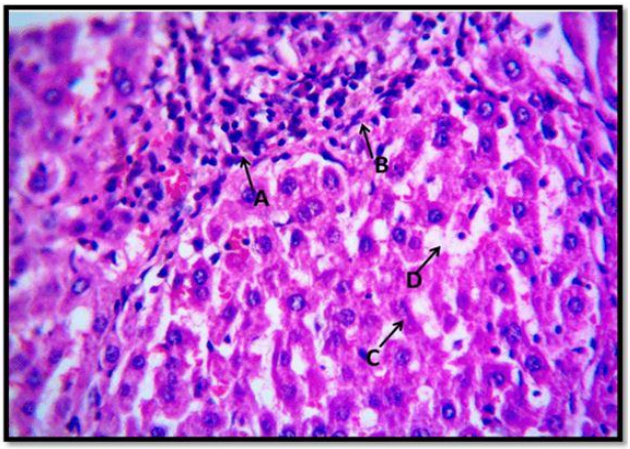

Fig.12: A photomicrograph of liver of Orlistat 100 mg/kg treated group shows infiltration of inflammatory cells (A) mainly lymphocytes (B), necrosis of hepatocytes (C) and dilatation of sinusoids (D). H&E stain, 400X.

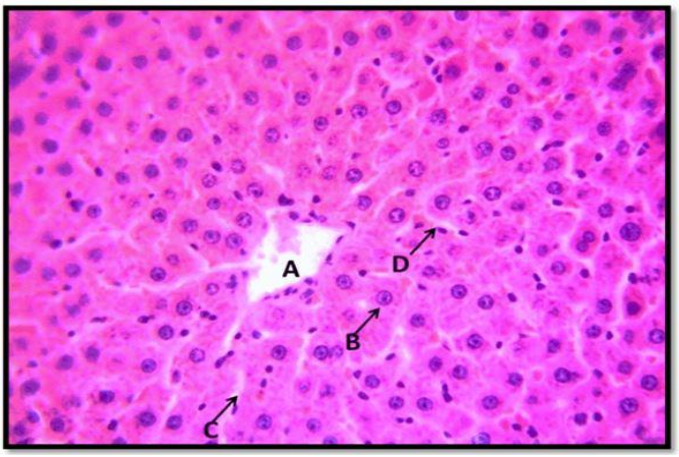

Fig.13: A photomicrograph of liver of Orlistat 200 mg/kg treated group shows congestion of central vein (A), necrosis of hepatocytes (B), dilatation of sinusoids (C) with congestion (D) and infiltration of lymphocytes with enlargement of Kuepfer cells (E). H&E stain, 400X.

The histological sections of the liver of aqueous extract of the rosemary plant treated group showed normal architecture of hepatic tissue (Fig.14). While both Orlistat 100 and 200 mg/kg with aqueous extract of rosemary plant treated groups revealed less severe lesions represented by mild congestion of central vein and mild dilatation with congestion of sinusoids with intact hepatocytes organized as cords and Kupffer cells (Fig. 15); in addition to mild cloudy degeneration of hepatocytes, mild focal infiltration of inflammatory cells and mild dilatation with congestion of sinusoids in the Orlistat 200 mg/kg with aqueous extract of rosemary treated group (Fig.16).

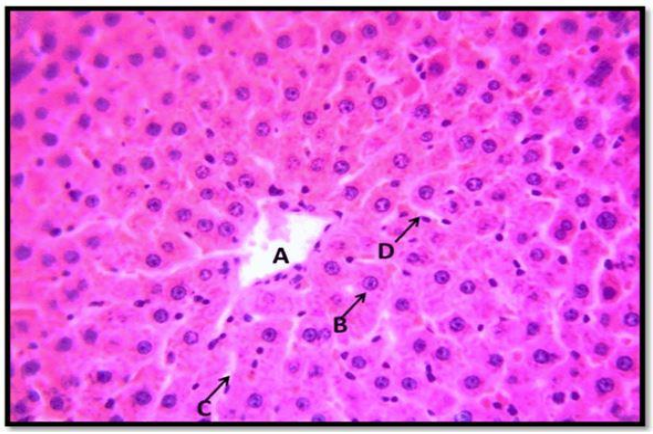

Fig.14: A photomicrograph of liver of aqueous extract of rosemary plant treated group shows normal architecture of central vein (A) surrounding by organized hepatocytes as a cord (B), sinusoids (C) and Kuepfer cells (D). H&E stain, 400X.

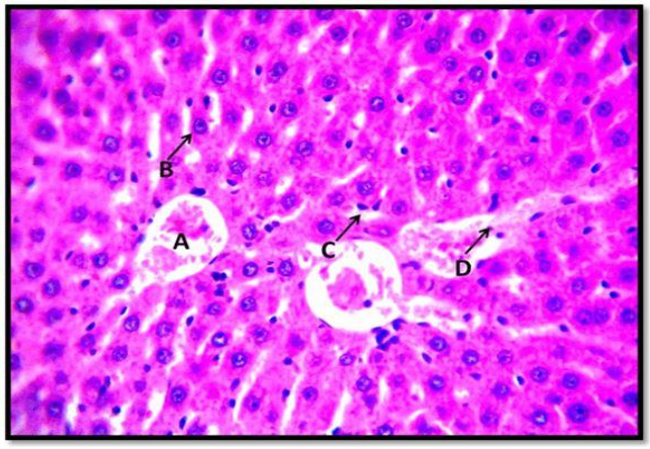

Fig.15: A photomicrograph of liver of Orlistat 100 mg/kg with aqueous extract of rosemary plant treated group shows mild congestion of central vein (A) intact the hepatocyte organized as cords (B), Kuepfer cells (C) and mild dilatation with congestion of sinusoids (D). H&E stain, 400X.

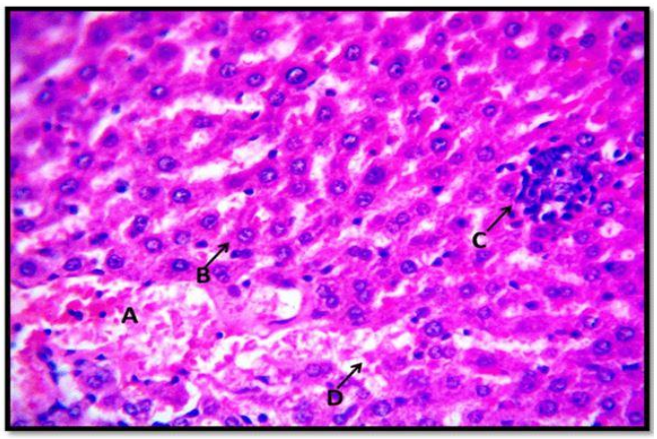

Fig.16: A photomicrograph of liver of Orlistat 200 mg/kg with aqueous extract of rosemary plant treated group shows mild congestion of central vein (A), slight cloudy degeneration of hepatocytes (B), mild focal infiltration of inflammatory cells (C) and mild dilatation with congestion of sinusoids (D). H&E stain, 400X.

DISCUSSION

According to the current study's findings, many histological abnormalities in the brain were found in a group of fattened rats. Pathological lesions in the cerebral cortex were observed during microscopic examination of brain tissue in a group of rats given orlistat.

The involvement of tissue inflammation mediated by immune cells as an important mechanism where obesity is associated with insulin resistance in various organs could be the cause of brain damage. Orlistat has also been shown to cause histological alterations in the hypothalamus, such as changes in the levels of numerous neuropeptides or the primary monoamine neurotransmitters of the central nervous system (CNS) **(Calderón *et al.*, 2011).**

According to the microscopic inspection of brain tissue, the histological structure of the cerebral cortex was improved in a group of rats treated with an aqueous extract of rosemary leaves, which accords with a study conducted by **Lahouel and his colleagues (2020).** This explanation is that rosemary suppresses mitogen activation and phosphorylation of protein kinase (MAPK). Nuclear factor-kappa (NF-kB) B inhibitor Mitogen-activated Protein Kinas, Pro-inflammatory enzymes, and other substances such as nitric oxide (NO) and interleukin-1 beta (IL-1) are also inactivated **(Benincá *et al.*, 2011) .**

The capacity of rosemary leaf aqueous extract to repair tissue damage in tissues was also demonstrated, because of its high abundance of phenolic chemicals, the brain is a good place to start **(Cui *et al.*, 2018).** According to a microscopic inspection of brain tissue, the histological structure of the cerebral cortex was improved in a group of rats treated with an aqueous extract of rosemary leaves. On the other hand, the orlistat-treated rats had pathological abnormalities in their liver tissue. The pathogenicity observed in liver tissue following orlistat use is related to the suppression of the carboxylesterase-2 gene (CES-2), the liver's principal detoxifying enzyme, which accelerates drug breakdown and detoxifies the liver by activating metabolic processes. The enzyme promotes the oxidation of fatty acids and triglycerides by minimizing the buildup of harmful chemicals in the liver **(Nwobodo, 2015).**

As for the group treated with the aqueous extract of rosemary leaves, our current study showed that the aqueous extract has beneficial effects in repairing tissue damage and agrees with **Hassanen (2015)** results. Antioxidant enzymes and defense systems scavenge free radicals to remove them and provide anti-inflammatories **(Hegazy *et al.*, 2018).**

Our recent investigation found that the aqueous extract of rosemary leaves had therapeutic benefits in

mending tissue damage, which is inconsistent with **Hassanen's findings (2015).** Antioxidant enzymes and defense mechanisms scavenge free radicals and anti-inflammatories to eliminate them **(Hegazy *et al.*, 2018).**

CONCLUSION

The results of this investigation showed that rats that were given orlistat plus an aqueous extract of rosemary leave improved histological brain and liver structures. The dose concentration determines the extent of orlistat's damage and its potential to cause histological alterations in the organs studied.

Declaration of Conflicting Interests:

The authors revealed that there is no potential conflicts of interest.

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