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Evaluation of Several Doses of Taurine on Histological Changes and Angiogenesis in Rats

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ABSTRACT

Background: This research intended to assess the impact of various taurine dosages on angiogenesis and their effect on some hematological and biochemical traits (Hemoglobin (Hb), packed cell volum PCV, cholesterol, triglyceride ,glutathion GSH, malondialdehyde MDA, body weight of animal after and before experiments, and hepatocyte tissues. in rats males exposed to Taurine (500,1000,1500,2000) mg for months.

Material and Method: Six groups of eight rats each were created from the rats. Six groups of eight rats each were created from the rats. receiving different doses of taurine.

Result: results revealed a significant increase for (Hb, PCV, GSH, body weight before experiment) while a significant decrease to (TG, MDA, Cholesterol, body weight after experiment), and many histological changes including congestion of sinusoidal and central vein, infiltration of inflammation cell around central vein and sever vacuolation of hepatocyte.

Conclusion: High doses of taurine affect angiogenesis in hepatic tissue and stimulate hematological and biochemical responses.

Keywords: Taurine, congestion, angiogenesis, endothelial hepatocyte, biochemical, hematological, ELISA.

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INTRODUCTION

An amino acid that is not necessary for life is taurine. necessary for survival contains sulphur and it's located in every tissue in the human body [1]. Taurine concentration in platelets is six times greater than any other amino acid [2].

According to a number of clinical and experimental research, Taurine has anti-inflammatory propertiesm and A high taurine intake may aid Heart attack and stroke prevention [3,2].

After taking 1.6 g of oral taurine daily for two weeks, cardiovascular (hypertension)

significantly decreased endothelium dependent and no-endothelium vasodilation, and inhibited calcium influx [4].

Using taurine supplements for improved diabetic control in diabetic [5].
It is crucial for enhancing human vascular endothelium function [6,7].

One important homeostasis regulator is taurine and plays a variety of protective roles against oxidative stress. An example of an injury related to oxidative stress include the development of high blood pressure, muscle/nervous disorders, liver cirrhosis, heart failure, and ischemia/reperfusion. To explain the anti-oxidant properties of taurine, the current paper provides a thorough account of all the underlying mechanisms. Due to its capacity to upregulate preserving glutathione stocks, promoting membrane integrity, lowering inflammation, preventing calcium buildup, and maintaining appropriate electron transport chain function

taurine is recognized as a cytoprotective chemical. Also noted is taurine's ability to work in concert with other potential therapy techniques to treat a variety of illnesses. Through providing mechanistic insights into taurine's biological function, which support its potential clinical benefits, the new research fills the gap between the bench and the bedside., in addition to research data. Future clinical research will be necessary to support taurine's protective impact against The consequences of oxidative damage [8].

Angiogenesis is created from the endothelium of the existing vasculature. It is crucial for the development and upkeep of tissue and organs and 36 is essential in a variety of clinical circumstances, such as diabetic retinopathy [9], arthritis [10], Ischemia, and cancer growth.[11] Inflammation, bone repair [12]and immune system harm [13]. Aberrant angiogenesis is the root cause of both poor vascularization and abnormal vasculature. Cancer cells and tumors secrete proangiogenic chemicals, such as VEGF, to promote unregulated angiogenesis, which can result in metastasis [14]. In addition to these biological functions, due to the combinatorial actions of several phytochemicals, research has indicated that Taurine and its pure components may have antiangiogenic capabilities through various Cellular signaling pathways. the high doses of taurine inhibited the angiogenic process in the hepatic tissue of rats. The inhibition of angiogenesis may be partially due to inhibited growth of endothelial cells following high doses of taurine [15]. Research has indicated showed taurine

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dramatically reduces the expression of fibrosis, which could have an antifibrotic impact. and the proliferation of cultivated HSC-T6 cells [16].

An in vitro assay measuring tube formation, proliferation, and cell migration was used to determine angiogenesis activity. Cell growth was measured using an assay for the incorporation of [3 H] thymidine; nevertheless, taurine did not cause vascular inflammation or permeability [17]. According to our research, taurine may have an anti-angiogenic function because high concentrations of the amino acid prevent angiogenesis in rat liver tissue. Disrupting this route may help reduce tumors because One important element in the emergence of cancer is angiogenesis. However, new strategies are needed because traditional anti-angiogenic treatments by themselves are unable to totally remove tumors. Chemotherapy combined with anti-angiogenic techniques has demonstrated encouraging outcomes in preventing tumor development and metastasis. This study emphasizes how crucial it is to investigate taurine's function in combination medicines in order to improve the effectiveness of cancer treatment.

MATERIALS AND METHODS

Animal model: We used 40 male, experimental-type Sprague Dawley rats, weighing 170–220gm, that were 2.5–3 months old. They were kept in 30x40x20 cm plastic cages at Mosul University Animal House, College of Veterinary Medicine. The temperature in the room where the rats were housed ranged from 20 to 25 °C.

a 14-hour illumination cycle, and a 10-hour darkness cycle, and they were fed a regular concentrated meal provided by Animal House. the rats were fed and given fluids.

Experiment design:

Forty male are used which were distributed into: The rat was divided into five experimental groups one control group, each containing eight rats.

Control group:(No taurine administered).

Second group: Received 500 mg taurine for 30 days.

For 30 days, the third group was given 1000 mg of taurine.

The fourth group got 1500 mg of taurine for 30 days.

The fifth group was given taurine at a dose of 2000 mg for 30 days.

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Preparation of blood serum:

- .Sample collection (subcutaneous injection): A 4 ml venous blood sample was drawn using a sterile
- medical syringe.
- .Serum separation: Blood was placed in a sterile gel test tube and left for 30
- minutes before centrifugation at 3000 rpm.
- . Distribute several tubes and kept at -20°C for the ELISA (serological) investigation that follows. Storage conditions: The separated serum was aliquoted
- .Hematological testing: An additional 4 ml was collected in a plain tube
- containing EDTA for hematological examination.

Animal dissection

On day thirty, the animals were dissected after being anaesthetized with diethyl Ether. The abdomen was then incised upward, and The targeted organs were removed for

histological preparation, as per the research objectives .as it is your research objective. Rinsed with a physiological saline solution (NaCl 0.9%) , The organs were subsequently weighed and measured before being fixed in 10% formalin.& which was replaced the next day depending on the manner of [18].

ELISA, or test using enzyme-linked immunosorbents:

is a technique for assessing the measurement of peptide, protein, antibody, and hormone levels that is founded on the concept of antigen-antibody binding. An antigen-antibody bond complex is created by immobilizing the antigen on a solid surface, followed by the addition of antibodies. This is then attached to the enzyme. when employing ELISA technique. The detecting signal is created when the enzyme and substrate react. which appears as a change in color [19].

ELISA (enzyme-linked immunosorbent test) method guidelines. The Journal of Biomedicine and Transitional Research

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Table: explained the company name, type assay, number code for parameters used in research

Parameters	Company name	Type assay	Catalog number or Code
MDA	Cell BioLabs, Inc. Developing a solution for research in bio sciences	Competitive Adduct ELISA	STA-832
GSH	Science Elab	Competitive ELISA	E-E-0026
TG	The BIOTANG Lab Supply Expert	Sandwich	R6954
Cholesterol	Cell BioLabs, Inc. Developing a solution for research in bio sciences	Manual Florometric Data Sheet	AT.NU:STA-390

STATISTICAL ANALYSIS

The multiple test where the F-Value, SAS was used to evaluate the values in accordance with the randomized block design. Individual means were compared was significant (P>0.05) when utilizing Duncan's value.

RESULTS & DISCUSSION

Because it works as an antioxidant, Taurine is a semi-essential amino acid for medicine.

provides Ctoprotective properties. Osmoregulator and Ca2+ flow regulator for intracellular use. It has been shown that taurine reduces the amount of cell damage brought on by the ischemia-reperfusion phenomena. as well as help guard against a range of lung conditions brought on by different pollutants. and to increase the antibacterial activity of monocytes and neutrophils from healthy humans. Previously, Investigators discovered that Taurine may guard against apoptosis. in "tired" rat hepatocytes and human neutrophils. According to reports, those with insulin-dependent diabetes had reduced blood taurine levels... Furthermore, we have found that pharmacological taurine reduces human endothelial cell death brought on by sodium arsenite. perhaps an anti-endothelial dysfunction Ca2+ionophore.has inspired us to investigate the idea that Due to its antioxidant properties and ability to regulate

intracellular Ca2+ homeostasis, According to [20], taurine may help stop the death of endothelial cells caused by excessive hyperglycemia.

Effect of several doses on biochemical and hematological parameters

Table (1) showed that significant elevation of glutathione levels P>0.05 (0.21±3.23 in 1500,2000,2500 mg/ml)groups; whereas significant decrease of malondialdehyde (7.31±269.61) in the same groups when compared to control .while the body weight of animal 3.54±243.17 in gram) before the experiment demonstrated a much greater rise than the control (0.87±199.92).

Table 1 shows how different taurine dosages affect the body weight, glutathione, and malondialdehyde in male albino rats.

2500,2000,1500 mg/ml Taurine	1000mgL/ml Taurine	500mgL/ml Taurine	control	Groups/standards
0.21±3.23 D	0.11±3.0 B	0.10± 3.6 A	0.10±2.5 c	Glutathione Mm/g of weight tissue
7.31±269.61 D	1.42±301.0 C	0.73±31.73 B	0.51±353.76 a	Malondi aldehude Mm/g of weight tissue
3.54±243.17 C	2.44±232.15 D	2.10±230.11 A	3.11±23.89 b	Body weight before experiment
0.87±199.92 C	1.10±209.64 B	3.12±211.19 B	2.10±236.50 a	Body weight after experiment

Different alphabetical indicate on presence of P>0.05 indicates a significant difference.

Table (II) showed that significant elevation of Hemoglobin (Hb) concentration, packed cell volume p>0.05 in relation to the control, while, cholesterol concentration, Triglyceride concentration significant decreasing p>0.05 in contrast to the control.

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Table 2 shows how different taurine dosages affect a few hematological and biochemical characteristics in male albino rats.

2500,2000,1000mg/ml Taurine	1000mg/ml taurine	500 mg/ml Taurine	control	Groups/standards
0.11±14.82 A	0.15±14.35 B	0.23±13.64 C	0.11±13.17 d	Hb concentration Gm/100ml
2.38±151.83 D	1.41±37.45 B	1.65±37.25 B	1.32±33.58 b	Packed cell volum
2.38±151.83 D	0.79±161.82 C	3.63±173.98 B	2.91±182.11 a	Cholesterol concentration mg/100ml
2.28±145.52 C	1.62±150.72 B	3.11±155.94 B	4.16±202.33 a	Tri glyceride concentration mg/100 ml

Different alphabetical indicate on presence of significant different *P>0.05.

[21]correlates with this study in table 2, As the taurine supplementation has an effective role on biochemical parameters of immunoglobulins, anti-oxidants and hormones in angora rabbits, whereas, there were decreased level of cholesterol, LDL, MDA in serum which enhances anti-oxidants role wool production then decreased lipid metabolism

And table (1,2) in this study, correlated with [22] whereas the treatment with taurine decreased lipid peroxidation and increased GSH this indicate that taurine role as anti-oxidant, in addition increased significant for (triglyceride, ALT, AST). Table 1 Table 1 according with [23] where using taurine against stenohepatitis during cafeteria deity, result that MDA interleukin, necrosis factor a decreased in other hand increased in GSH .

Effect of several doses on hepatocyte tissue and angiogenesis:

They were prepared on the way of the [18]. liver examination showed that the Taurine (500mg): Rat liver photomicrograph of taurine group 500 mg reveals sinusoidal congestion. (A) thickining of some hepatocyte(B). while, (A) presence of some hepatocyte contain di nucleus(B). (as in fig:3,4).
Taurine (1000mg): A photomicrograph of taurine group 1mg rat liver demonstrates sinusoidal congestion (A)hepatocyte congestion(B)infiltration of inflammation cell around central vein(C), while; the presence of some hepatocyte congestion(B)Infiltration of inflammation cell around central vein(C) Thickening of hepatocyte nucleus (blue) (as in fig :5,6), compared to healthy controls which showed A microscopic examination of rat liver tissue from the untreated control group shows typical liver tissue architecture, which includes Hepatocytes (B), sinusoids (C), and central veins (A). 100X H&E stain and 400X magnification (as in fig:1&2).

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While, the taurine (1500 mg): A photomicrograph of the taurine group 1500 mg rat liver indicates sinusoidal obstruction. (A)hepatocyte congestion(B) Infiltration of inflammation cell around central vein(C) thickening of hepatocyte nucleus (blue) (as in fig:7)

Taurine(2000 mg): photomicrograph of rat liver of taurine 2000 mg :shows sinusoidal congestion (A) and severe vacuolation of hepatocytes (B), cells necrosis around central vein (C). H&E stain, (as in fig:8,9), this means that taurine reduced the process of angiogenesis in the hepatic tissue of rats. Especially in high doses.

Although Taurine has anticancer properties, cyanide can negative side and induce toxic impacts especially when taken orally [24]According to the findings of the current investigation, rats given relatively large doses of taurine 1000 and 1500,2000 mg per kilogram body weight displayed a moderate to high intensity compared to those in the control group which displayed a normal architecture & antigenic process in hepatic tissue .So, the high doses of taurine 2000 mg inhibited the angiogenic process in the hepatic tissue of rats,. The inhibition of angiogenesis may be partially due to inhibited growth of endothelial cells following high doses of taurine . Our findings are in agreement with [15],hepatic segments and Tau organizations. The TAA group's liver sections revealed Disorganized portal regions, central veins, and hepatic cords and there is substantial portal bridging, isolated necrosis, fibrous growth of the portal regions, and infiltration of leukocytic cells. in addition extending from the primary portal. Hepatic fibrosis was the TAA group's liver sections revealed Disorganized portal regions, central veins, and hepatic cords and there is substantial portal bridging, isolated necrosis, fibrous growth of the portal regions, and infiltration of leukocytic cells. in addition extending from the primary portal. The liver sections of the late TAA + Tau and Con TAA + Tau groups showed signs of hepatic fibrosis.

Microscope photos of A significant amount of collagen was deposited in the TAA-induced liver fibrosis as seen in sirius red stained liver sections, although taurine administration Significantly reduced this amount (long arrows indicate fibrous tissue).

Additionally, it was proposed that taurine possesses anti-angiogenic properties, which may help explain why it helps reduce cancer. According to research, taurine significantly lowers fibrosis expression, which may have antifibrotic effects. and the growth of HSC-T6 cells during cultivation. [16]. According to earlier studies (Hassan et al., 2003), high dosage of these histological changes Compared to animals treated with taurine plus CCL4, those treated with CCL4 alone showed more pronounced and widespread changes. In contrast to taurine-protected rats,

animals treated with CCL4 showed significantly lower rates of total protein content and SDH reactivity. Rats treated with CCL4 exhibited noticeably greater levels of lipid and Alk.ph. activity in contrast to rats protected by taurine. Taurine improved

liver function and prevented hepatocellular damage in this investigation. higher lipid content and Alk.ph. activity compared to taurine-protected rats .

These results imply that taking taurine supplements enhances Further research on the impact of altered endothelial function on macrovascular endothelial function in dyslipidemic situations is required. [25].The results of the study align with previous research on this amino acid's

cytoprotective action in a number of endothelium injury cases that have been investigated in vivo as well as in vitro [26,27].

These findings suggest that taking taurine supplements enhances Further research on the impact of altered endothelial function on macrovascular endothelial function in dyslipidemic situations is required. [25]. Previous reports of this amino acid's cytoprotective action in other endothelium injury instances that have been examined both in vitro and in vivo are consistent with the findings of this investigation [26, 27].

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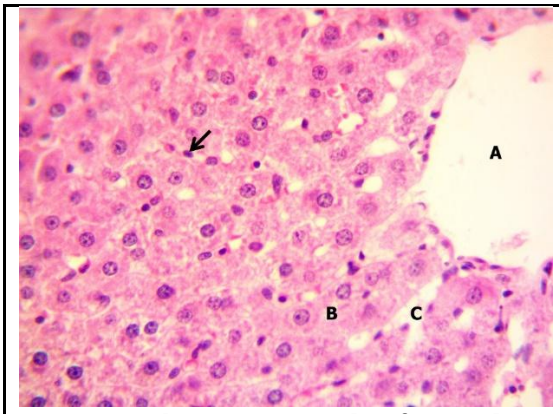


Fig.2: A photomicrograph of a rat liver from a control positive group (without treatment) demonstrates the usual architecture of the liver tissue, which is made up of sinusoids (C), the major vein (A), and cupffer cells (D). H&E stain 100X.

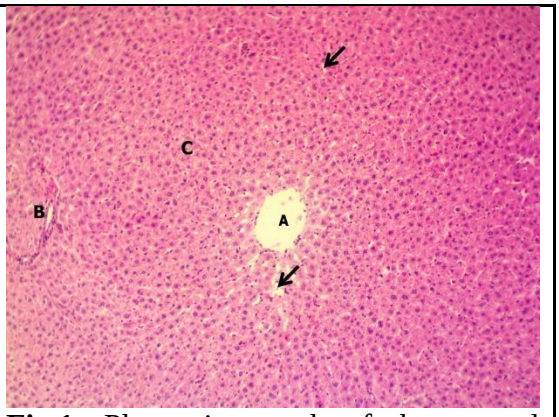


Fig.1: Photomicrograph of the control positive group's rat liver (without treatment) demonstrates the organ's typical architecture, which is represented by the organ's central vein (A), sinusoids (C) and hepatocytes (B). H&E stain 100X.

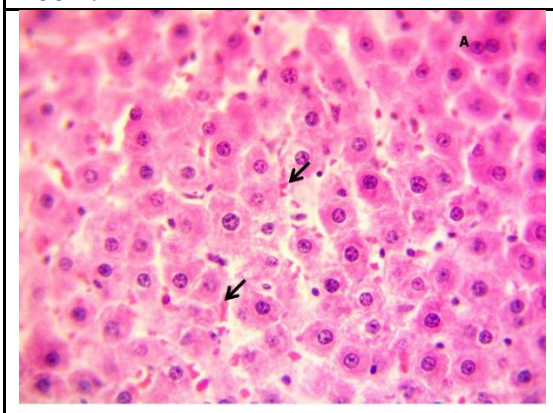
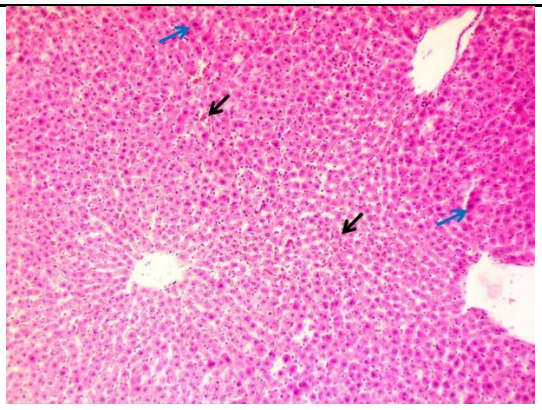


Fig.4: pictures of 500 mg taurine-treated rat liver reveals sinusoidal congestion (A) and the presence of certain hepatocytes with dinuclei (B). 400X The H&E stain.



0Fig.3: photomicrograph of rat liver containing 500 mg of taurine how certain hepatocytes are thickening hepatocyte due to sinusoidal congestion (A). 100X E& H stain.

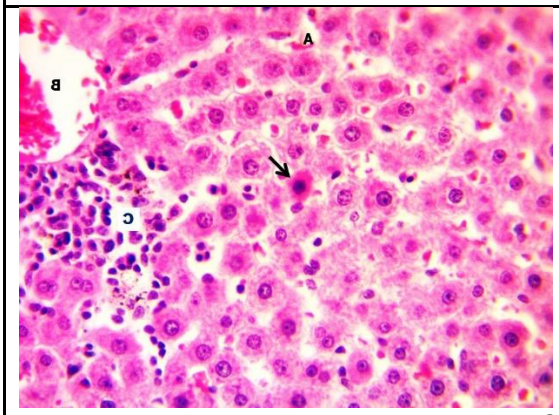


Fig.6: Rat liver photomicrograph of taurine group 1000 mg reveals sinusoidal congestion (A)hepatocyte congestion (B)infiltration of inflammation cell around central vein

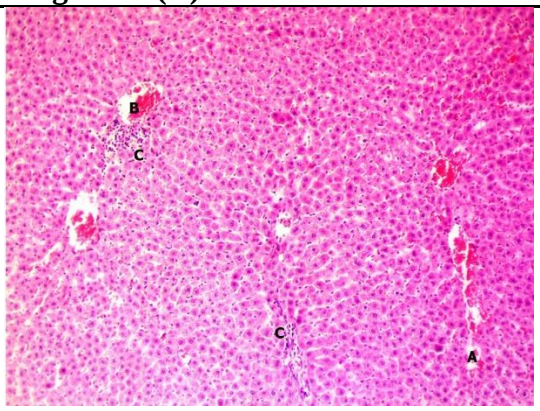


Fig.5: Rat liver microscopy image of taurine group 1000mg reveals sinusoidal congestion (A)hepatocyte congestion (B)infiltration of inflammation cell around central vein

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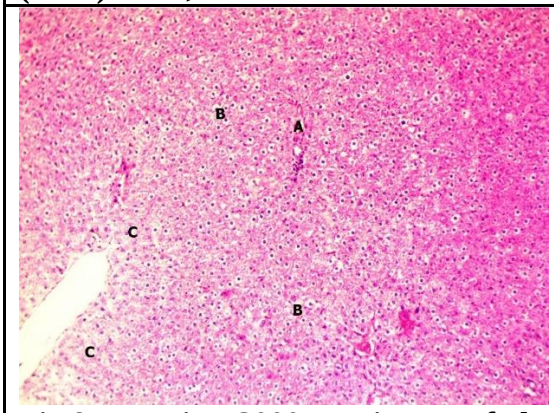
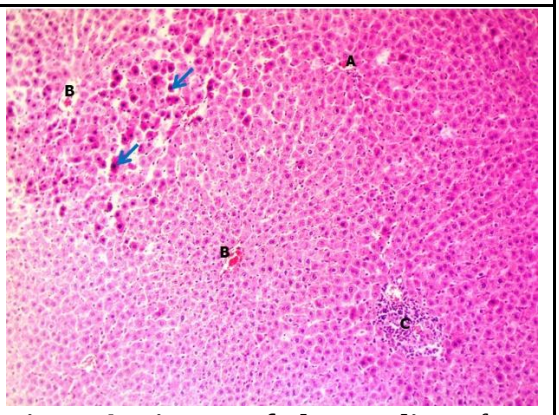
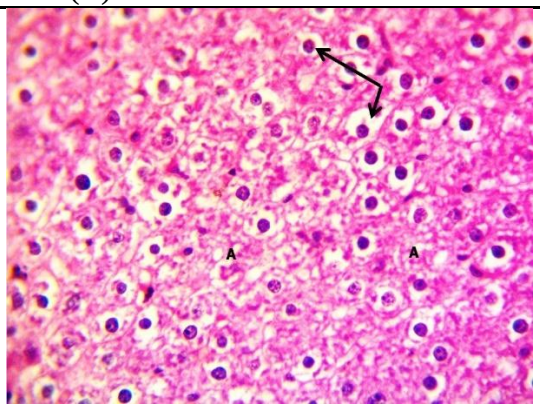
(C) thickening of hepatocyte nucleus (blue) 400X,H&E stain	(C)100X,H&E stain
 **Fig.8: Taurine 2000 mg image of the rat liver under a microscope reveals sinusoidal congestion (A), significant vacuolation of the hepatocytes (B), and necrosis of the cells surrounding the principal vein (C). 100X H&E stain.**	 **Fig.7: A picture of the rat liver** from the taurine group after 1500mg of taurine reveals sinusoidal congestion (A), hepatocyte congestion (B), infiltration of inflammatory cells surrounding the hepatocyte nucleus (blue) thickening at 400X using H&E stain, and the central vein (C).
	 **Fig.9:** A photomicrograph of the rat liver containing 2000 mg of taurine reveals significant hepatocyte vacuolation (A) and necrosis of the cells surrounding the principal vein (C). 400X H&E stain.

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CONCLUSION

Our findings indicate that high doses of taurine inhibit angiogenesis in rat liver tissue, suggesting its potential role as an anti-angiogenic agent. Since angiogenesis is a fundamental process in cancer progression, disrupting this pathway may contribute to tumor suppression. However, because conventional anti-angiogenic therapies alone do not completely eradicate tumors, novel approaches are required. Combining anti-angiogenic strategies with chemotherapy has shown promising results in halting tumor growth and metastasis. This study underscores the importance of exploring taurine role in such combination therapies to enhance cancer treatment efficacy.

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Conflict of interests.

There are non-conflicts of interest.

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