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Mesenchymal Stem Cells and/or a Capsinoid Compound (Capsiate) Inhibit Inflammation and Apoptosis Caused by Ranitidine in the Liver of Rats

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Abstract: Ranitidine is considered one of the major causes of hepatotoxicity. The current study aimed to investigate the therapeutic effects of bone marrow mesenchymal stem cells alone or in combination with the sweet red pepper extract capsiate on ranitidine-induced liver disturbances. In this study, 36 male albino rats were divided into 6 groups: Control and 5 groups receiving distilled water. Ranitidine group receiving 300mg/kg/bw. Capsiate group receiving 60mg/kg/bw. Ranitidine/capsiate therapeutic group. Ranitidine/stem cells therapeutic group receiving a single intravenous injection of stem cells (2×106 cells). Ranitidine/stem cells/capsiate therapeutic group. Liver glutathione and superoxide dismutase were analyzed in tissue samples. Liver function indicators: ALT, AST, GGT, albumin, and total protein were assessed in serum. Also, inflammatory mediators: CRP, TNF-α, IL-12, and IL-10, as well as apoptotic indicators of caspase 3 and Bcl-2, were assessed in tissue samples. The results elucidated that either capsiate or mesenchymal stem cells decreased hepatotoxicity. Combining capsiate and mesenchymal stem cells revealed a better role against the effects than either treatment alone.

Keywords: inflammation; mesenchymal stem cells; sweet pepper; apoptosis; liver.

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1. Introduction

Liver toxicity may result in deviations from normal function and morphology. This may be due to the accumulation of drug metabolites or to metabolic disruption, leading to liver injury. The liver can detoxify a variety of drugs and xenobiotics. However, one of the major causes of liver diseases is the use of chemical drugs [1].

Ranitidine is a histamine receptor antagonist (H2 blocker) used as a treatment for acid-peptic disease or heartburn. H2-receptor antagonists inhibit histamine-mediated responses in gastric cells, decreasing acid secretion. Such therapy is suggested for the treatment of peptic ulcers and gastroesophageal reflux [2].

Mesenchymal stem cells (MSCs) have immune regulatory abilities and anti-fibrotic and regenerative properties. The choice of therapeutic cells may need to be tailored to the type of targeted liver disease because the required therapeutic effects may differ [3,4].

In addition, MSCs have shown great potential in regenerative medicine, where they are easily isolated and expanded, possess immunosuppressive properties, pose no risk of teratoma,

and raise no ethical concerns. Moreover, in animal models, MSCs have efficacy as a therapy for liver diseases [4].

Stem cell transplantation has been shown to restore normal liver function and improve animal survival in models of induced liver toxicity [5]. The hepatic lineage-directed differentiation showed that stem cell-derived hepatic cells expressed hepatocyte-specific markers [6].

The key features of stem cells are their capacity to divide and produce more stem cells or more differentiated precursors, which are associated with the potential to differentiate into other specific cell types [7].

Sweet red pepper is a good source of vitamins C, E, provitamin A, and carotenoids; it also contains more phenolics and flavonoids [8,9]. In addition, red pepper extracts have been suggested to have anti-inflammatory effects, lowering blood pressure, blood clotting, and blood sugar levels. Since it has analgesic and anti-inflammatory properties [8,10], moreover, the consumption of red peppers may help prevent various diseases initiated by free radical oxidation [10].

In particular, capsiate, 4-hydroxy-3-methoxybenzyl (E)-8-methyl-6-nonenoate, is the main product of (CH-19 Sweet) (*Capsicum annuum L.); it differs from capsaicin in that* it is a non-pungent cultivar of red pepper. Capsiate increases energy metabolism and possesses antioxidant and anti-inflammatory properties. However, capsiate remains much less explored [11].

It was suggested that combined treatment with MSCs and natural antioxidants may yield the best stem cell survival, thereby enhancing their therapeutic role and the combined effects of stem cells and antioxidants on the injured tissue [12].

The present study aims to assess the effects of mesenchymal stem cells and/or capsiate treatments on the attenuation of ranitidine-induced hepatotoxicity in male albino rats.

2. Materials and Methods

2.1. Ethics for study and animals.

Ethical approval for animal experiments was granted by the Ethics Committee of the Faculty of Women, Ain Shams University (ASU/W/Sci-5R/23-2-31), in accordance with the ARRIVE guidelines. Male albino rats weighing an average of 130+150g were housed in a normal laboratory environment at constant room temperature (24°C) with a natural day/night light cycle; food and water were supplied ad libitum. Rats were acclimated to the laboratory environment for 1 week before the experiment began.

2.2. Administered materials.

2.2.1. Ranitidine (RA).

Zantac is a trade name for Ranitidine (RA), which was obtained from the commercial market and administered orally at a dose of 300mg/kg dissolved in distilled water daily for 6 weeks, according to [13].

2.2.2. Mesenchymal stem cells (MSCs) derived from rat bone marrow.

Bone marrow mesenchymal stem cells were labeled with PKH26 fluorescent linker dye. MSCs have been isolated and cultured in the Biochemistry and Molecular Biology Unit at the

Faculty of Medicine, Cairo University. Cells were injected as a single intravenous dose (2×10^6 cells) and left for 4 weeks [14].

Bone marrow was harvested from 6-week-old male albino rats from the tibiae and femurs with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) with 10% of fetal bovine serum (GIBCO/ BRL). Cells were incubated at 37 °C in a 5% humidified CO_2 atmosphere. At 80–90% confluence, cultures were washed with phosphate-buffered saline, and the cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) at 37°C. Cells were centrifuged, suspended, and incubated in Falcon. MSCs were found by their adherence to the plastic surface and their morphology. CD90 and CD29 were positively expressed, while CD34 was negative, as markers of MSCs by flow cytometry. Dead cells were counted using trypan blue dye, while viable cells were counted using a hemocytometer.

Flow cytometric analyses were performed on a Fluorescence Activated Cell Sorter (FACS) flow cytometer (Coulter Epics Elite, Miami, FL). The cells were incubated at room temperature. Antibody concentration was $0.1mgml^{-1}$. Cells were washed with PBS and finally diluted in PBS. The expression of the surface marker was assessed by the mean fluorescence [15].

PKH26 fluorescent linker dye was used for MSCs labeling according to the Sigma Protocol (St. Louis, MO). Briefly, cells were centrifuged and washed twice in a serum-free medium. Cells were pelleted and suspended in dye and then injected intravenously into the tail vein [16]. After 10 days, liver sections were examined with a fluorescence microscope to detect the homed-labeled cells as described in [17].

2.2.3. Sweet red pepper extract, capsiate (CA).

Capsiate was obtained from MedChemExpress USA, Cat. No.: HY-N8377 and orally administered at a dose of 60mg/kg b. wt. daily for 4 weeks [18].

2.3. Design of the study.

In the present investigation, rats were divided into 6 groups of 6 rats each. The first group served as the control, receiving distilled water; the second group represented the RA-treated group; the third group represented the CA-treated group; the fourth group represented the RA+CA therapeutic group; the fifth group represented the RA+MSCs therapeutic group; the sixth group represented the RA+CA+MSCs therapeutic group. Rats were subjected to different experimental treatments for 4 weeks.

2.4. Biochemical analysis.

At the end of the study period, all experimental animals were anesthetized and sacrificed after receiving an IV injection of sodium barbital (1.25mg/Kg) [19]. Blood samples from all groups were withdrawn by heart acupuncture. Sera was separated for assessment of liver functions, and inflammatory and apoptotic markers. Liver tissue samples were dissected for assessment of reduced glutathione (GSH), superoxide dismutase (SOD) content, and lipid peroxidation hepatic malondialdehyde (MDA).

2.4.1. Liver function enzymes.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum were assessed spectrophotometrically at 340nm [20]. Commercial kits were purchased from

Spin React Company. Gamma-glutamyl transferase (γ-GT) was measured according to the method described by Gendler & Kaplan (1984) [21] using a commercial kit purchased from Spin React Company. Serum albumin level was estimated calorimetrically according to the method of Doumas *et al*. [22]. Also, total protein in serum was determined calorimetrically, according to Henry [22].

2.4.2. Antioxidants and oxidative stress.

Hepatic reduced glutathione (GSH) content in tissue was determined colorimetrically according to the manufacturer's instructions provided by Bio-diagnostic, Egypt [23]. Hepatic superoxide dismutase (SOD) content was determined according to the method of Nishikimi [24]. Malondialdehyde (MDA) in tissue was determined according to Botsoglou’s method [25].

2.4.3. Immune response indicators.

Pro-inflammatory factors, C-reactive protein (CRP) and Tumor necrosis factor (TNF-α), in the liver lysate were measured using ELISA kits purchased from Alpha Diagnostics and determined according to the manufacturer’s instructions. Interleukin-12 (IL-12) level in the liver was determined using an ELISA kit according to the method of Trinchieri *et al*. [26].

Anti-inflammatory factors: Interleukin-10 (IL-10) levels in the liver were measured using an ELISA kit [26].

2.4.4. Apoptotic indicators.

Caspase-3 content in liver tissue was determined by the ELISA technique according to Harrington *et al*. [27] using a rat Caspase-3 kit purchased from Glory Science Co., Ltd, USA, according to the manufacturer’s instructions.

Liver B-cell leukemia/lymphoma 2 (Bcl-2) was estimated by an ELISA kit for rat Bcl-2 purchased from Glory Science Co., Ltd, USA, according to Bauer & Bryant’s assay method [28].

2.5. Statistical analysis.

In the present study, all results were expressed as Mean ± S.E. of the mean. Data were analyzed using one-way ANOVA in SPSS, version 19, followed by least significant difference (LSD) to compare group differences. The difference was considered significant when the p-value was < 0.05.

3. Results

3.1. Characterization and identification of MSCs are important.

MSCs in culture were visualized morphologically as fibroblast and spindle shapes and by their adhesiveness (Figure 1A–C). Also, MSCs were identified by surface markers; CD90 and CD29 were positive, while CD34 was negative, as determined by flow cytometry (Figure 2).

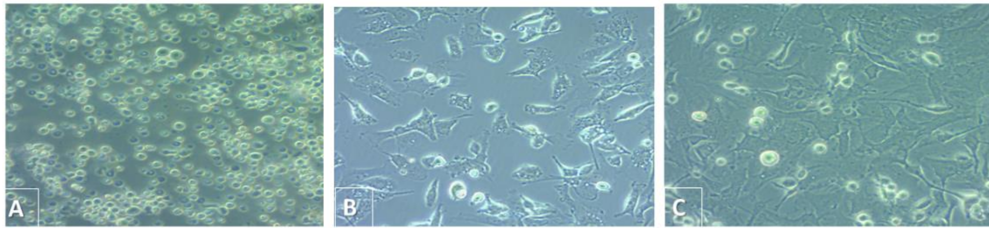

Figure 1. Rat bone marrow mesenchymal stem cells in culture. (A) MSCs at 3 d appeared rounded in shape; (B) at 7 days, MSCs appeared as fibroblast and spindle-shaped cells. (C) MSCs at 10 d reached 80–90% confluence; they were identified by their fusiform morphology.

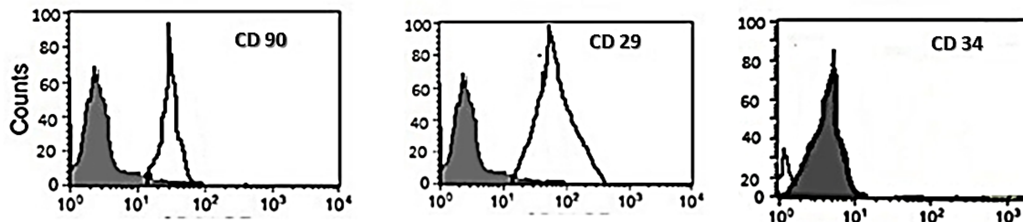

Figure 2. Characterization analyses of rat bone marrow–mesenchymal stem cells by flow cytometry show positive expression of CD90 and CD29, and negative expression of CD34.

3.2. Detection of MSCs in the tissue.

PKH26-labeled stem cells were detected in the liver after 10 days of MSCs transplantation; more homing of labeled cells was seen in hepatic sections treated with mesenchymal stem cells and capsiate together (Figure 3) (A, B).

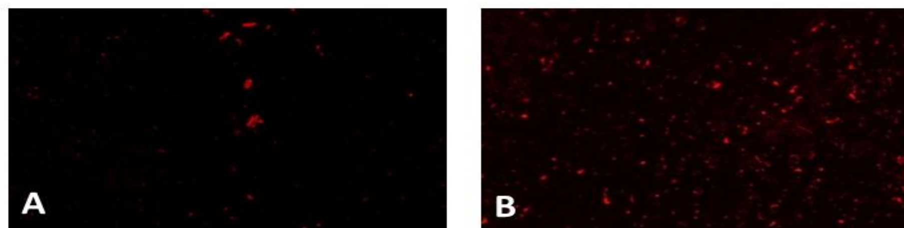

Figure 3. PKH26-labeled stem cells in liver showing: (B) a redder fluorescence after treatment with both MSCs and capsiate and (A) after MSCs alone.

3.3. Liver enzymes.

The mean values of ALT, AST, and GGT of the control and experimental groups are shown in Table 1. Control rats showed constant levels during the study. On the other hand, in the RA group, ALT, AST, and GGT levels were significantly elevated compared with the control group. This elevation recorded mean values of (55+0.44), (100+0.58), and (12±0.05) at the end of the experiment. A considerable improvement was observed in RA+CA, RA+MSCs, and RA+CA+MSCs therapeutic groups. This improvement was reflected in mean values of (40+0.52, 35+0.11, and 30+0.22), (60+0.35, 55+0.62, and 45+0.22), and (8.2±0.04, 7.8±0.02, and 7.2±0.03) for ALT, AST, and GGT, respectively, at the end of the experimental period.

Table 1. The therapeutic role of capsiate or /and MSCs on liver enzymes in the serum of ranitidine-treated rats.

	Parameters		
Groups	ALT(u/l)	AST(u/l)	GGT(u/l)
Control	25±0.28	35±0.45	6±0.02
RA	55±0.44 a**	100±0.58 a**	12±0.05 a**
CA	26±0.32 b**	38±0.27 b**	6.5±0.01 b**

	Parameters		
RA+CA	40±0.52 a* b c	60±0.35 a* b* c*	8.2±0.04 a* b* c*
RA+MSCs	35±0.11 a b*c*	55±0.62 a b*c	7.8±0.02 a b*c
RA+CA+MSCs	30±0.22 b**	45±0.22 b**	7.2±0.03 a b**c

a: Significant change concerning control group; **b:** Significant change concerning ranitidine-treated group (RA); **c:** Significant change concerning capciate-treated group (CA). *= $p<0.05$; **= $p<0.01$.

3.4. Serum albumin and total protein (g/dl) levels.

The mean values of serum albumin and total protein levels are given in Table 2. The control rats were designated constantly during the study period. In the control rats, a significant decrease of 35.67% in total protein level was reported in rats treated with RA for 6 weeks. Furthermore, partial improvement took place in the groups treated with RA+CA, RA+MSCs, and RA+CA+MSCs; their mean values were 5.35±0.02, 5.65±0.03, and 6.01±0.04 (g/dl), respectively, after 30 days of treatment. Moreover, a partial change occurred in the RA+CA therapeutic group. The percentage decline in albumin level reached -8.71% after 30 days of treatment. Also, changes in albumin levels were recorded in the RA+MSCs and RA+CA+MSCs therapeutic groups, with percent changes of 2.82% and 0.71%, respectively, after 30 days of treatment.

Table 2. The therapeutic role of capsiate and MSCs on serum albumin and total protein in ranitidine-treated rats.

	Parameters	
Groups	**Albumin (g/dl)**	**Total protein (g/dl)**
Control	4.25±0.04	6.14±0.01
RA	2.85±0.02 a**	3.95±0.02 a**
CA	4.55±0.02 b**	6.23±0.01b**
RA+CA	3.88±0.03 a* b* c*	5.35±0.02 a b* c
RA+MSCs	4.13±0.03 b*c	5.65±0.03 b*
RA+CA+MSCs	4.22±0.02 b**	6.01±0.04 b*

a: Significant change with respect to the control group; **b:** Significant change with respect to the ranitidine-treated group (RA); **c:** Significant change with respect to the capciate-treated group (CA). *= $p<0.05$; **= $p<0.01$.

3.5. Liver GSH, SOD, and MDA content.

No remarkable changes were reported after CA treatment throughout the experimental duration, as shown in Table 3. But, in RA rats, a significant depletion ($p<0.001$) in the content of tissue GSH was recorded and reached (2.05±0.01) as compared to the control group (3.24 ±0.01), with a percentage of change (-36.73%). Moreover, GSH content was partially increased to 2.73±0.01 nmol/g compared with the RA group. Also, therapeutic with MSCs induced a partial increase in GSH level, recording 2.82±0.01nmol/g with a percent change (-12.96%) compared to the control group.

On the other hand, some improvement in GSH level in the therapeutic group by CA+MSCs reached 3.01±0.02nmol/g with a percentage change (-7.11%) compared to the control group.

Concerning the control rats, insignificant changes were obtained in the CA rats. On the other hand, the results showed that the level of SOD was significantly decreased (43.50±0.23) in RA group compared to the control (75.45 ±0.42); the percentage of change was (-42%). Moreover, SOD content was partially increased to 64.35±0.21 nmol/g compared with the RA group. Also, therapeutic with MSCs induced a partial increase in SOD level, recording 67.45±0.32nmol/g with a percent change (-10.60%) in comparison with the control group.

Some improvement in SOD level in the CA+MSCs group reached 69.38±0.12nmol/g with a percentage of change (-8.05%) as compared to the control group.

Regarding the control group, no changes were observed after CA administration, except in Table 3. Whereas a significant elevation (p<0.001) was realized in tissue MDA content in RA rats as compared with the control group, with a percentage increase of 86.35% from the control.

A considerable improvement in MDA content was observed in treated rats with RA+CA compared to the RA group. This improvement was recorded (5.50±0.01), with the percentage of change of 29.41% from control rats.

Moreover, great improvement was also seen in therapeutic groups (GV) in MDA content as compared to the control group (GI); the percentage of change reached 20.47%. Meanwhile, MDA content in the therapeutic group by MSCs+ CA (GVI) revealed a significant improvement and recorded change (8.24%) from the control group.

Table 3. The therapeutic role of capsiate and MSCs on hepatic oxidative and antioxidant status in ranitidine-treated rats.

	Parameters		
Groups	**GSH (nmol/g tissue)**	**SOD (u/ g tissue)**	**MDA (nmol/g tissue)**
Control	3.24±0.01	75.45±0.42	4.25±0.01
RA	2.05±0.01a**	43.50±0.23 a**	7.92±0.02 a**
CA	3.11±0.02 b**	74.64±0.35 b**	4.15±0.01b**
RA+CA	2.73±0.01a* b* c*	64.35±0.21 a* b* c*	5.50±0.01 a* b* c*
RA+MSCs	2.82±0.01 a b*c	67.45±0.32 a* b*c*	5.12±0.02 a b*c
RA+CA+MSCs	3.01±0.02 b**	69.38±0.12 b**	4.6±0.01 b**

a: Significant change with respect to control group; **b:** Significant change with respect to ranitidine-treated group (RA); **c:** Significant change with respect to capciate-treated group (CA). *= p<0.05; **= p<0.01.

3.6. Liver CRP, TNF-α, IL-12 and IL-10 levels.

The values of CRP, TNF-α, and IL-12 in liver tissues of control and experimental groups are presented in Table 4. In the results, RA rats exhibited a significant elevation (p<0.001) of CRP and TNF-α and IL-12 levels, reaching (8.35±0.23 and 140.25±1.64), with a percent of change (279.55% and 222.04%) compared to the control rats (43.55±3.62 and 2.20±0.12). In contrast, no remarkable changes were reported in CA rats compared with Control rats.

A significant improvement in TNF-α and CRP was observed in treated rats with RA+CA compared with the RA group. This improvement was recorded (68.21±4.25 and 4.52±0.21), with percentage of change (56.62% and 105.45%), respectively, from control rats.

Moreover, great improvement was also seen in therapeutic groups in TNF-α and CRP as compared to the control group; the percentage of change reached (27.72% and 61.36%). Whereas TNF-α and CRP in the therapeutic group treated with MSCs+CA revealed a significant improvement, recorded change (15.34% and 41.36%) from the Control group.

Upon IL-12 detection, insignificant changes were observed in the CA rats throughout the experimental period. On the other hand, in RA rats, a significant elevation (p<0.01) in liver IL-12 was observed compared with the control group, with a 25.05% increase. At the same time, treatment with RA+ CA resulted in a greater reduction in IL-12 levels than in RA rats. The percentages of change were (-3.69%) from the RA group. Moreover, some improvement was observed in other groups compared to RA rats. This improvement was greater (-5.83% and -14.16%) than in the RA group.

About the anti-inflammatory marker, IL-10, the values of IL-10 in the control and experimental groups are presented in Table 4. The data showed that IL-10 content was significantly decreased (p<0.001) in RA rats, recording 27.20±4.62 pg/g compared to control rats (122.12±5.60 pg/g). At the same time, no significant difference (p>0.05) was found between CA rats and the Control group.

Moreover, IL-10 content was partially increased to 56.56±2.50 pg/g compared with the RA group. Also, MSC therapy induced a partial increase in IL-10, with levels of 74.43±3.67 pg/g, compared to the RA group. On the other hand, a significant improvement in IL-10 in the therapeutic group by CA+MSCs reached 84.75±2.65pg/g with a percent of change (-30.60%) compared to the control group.

Table 4. The therapeutic role of capsiate or/and MSCs on liver inflammatory and anti-inflammatory factors in ranitidine-treated rats.

	Parameters			
Groups	**CRP(u/g)**	**TNF-α (pg/g)**	IL-12 **(pg/g)**	**IL-10 (pg/g**
Control	2.20±0.12	43.55±3.62	98.23±0.22	122.12±5.60
RA	8.35±0.23 a**	140.25±1.64 a**	122.84±0.45 a*	27.20±4.62 a**
CA	2.40±0.35 b**	44.25±3.02 b**	97.52±0.31 b*	120.25±5.32 b**
RA+CA	4.52±0.21 a* b* c*	68.21±4.25 a* b* c*	118.31±0.14 a*b*c*	56.56±2.50 a**b* c**
RA+MSCs	3.55±0.32 a b*c	55.62±1.30 a b*c	115.68±0.62 a b c	74.43±3.67 a* b**c*
RA+CA+MSCs-	3.11±0.12 a b*c	50.23±2.54 a b*c	105.45±0.33 b*	84.75±2.65 a b** c

a: Significant change with respect to control group; **b:** Significant change with respect to ranitidine-treated group (RA); **c:** Significant change with respect to capciate-treated group (CA). *= *p*<0.05; **= *p*<0.01.

3.7. Tissue caspase-3 and Bcl-2 contents.

Data recorded for the levels of liver caspase-3 and Bcl-2 were presented in Table 5. In the results, RA rats showed a significant elevation (*p*<0.001) of caspase-3 level reaching (5.12±0.11), with a percent of change (126.55%) in relation to the control rats (2.26±0.15). On the other hand, Bcl-2 content was significantly decreased in RA rats, recording 2.95±0.37 ng/g compared to control rats (3.82±0.45 ng/g). A significant improvement in caspase-3 levels was observed in treated rats with RA+CA compared with the RA group. This improvement was recorded (3.71±0.26), with a percentage of change (64.16%) from control rats.
Moreover, greater improvement was observed in the therapeutic groups at the caspase-3 level compared to the control group; the percentage change reached 15.04%. Meanwhile, the caspase-3 level in the therapeutic group by MSCs+ CA revealed a significant improvement and recorded change (12.83%) from the control group.

Furthermore, Bcl-2 content was partially increased to 3.11±0.26 ng/g compared with the RA group. Also, therapeutic with MSCs induced a partial increase in Bcl-2 level, recording 3.32±0.41ng/g with a percent change (-13.09%) compared to the control group.

On the other hand, some improvement in Bcl-2 level in the therapeutic group by CA+MSCs reached 3.55±0.24ng/g/g with a percentage change (-7.07%) as compared to the control group.

Table 5. The therapeutic role of capsiate or/and MSCs on tissue pro-apoptotic and anti-apoptotic factors in ranitidine-treated rats.

	Parameters	
Groups	**Caspase-3 (ng/g)**	**Bcl-2(ng/g)**
Control	2.26±0.15	3.82±0.45
RA	5.12±0.11a**	2.95±0.37 a*
CA	2.28±0.23 b**	3.80±0.30 b*
RA+CA	3.71±0.26 a* b* c*	3.11±0.26 a b c
RA+MSCs	2.60±0.25 b**	3.32±0.41 a b c
RA+CA+MSCs	2.55±0.13 b**	3.55±0.24b*

a: Significant change with respect to control group; **b:** Significant change with respect to ranitidine-treated group (RA); **c:** Significant change with respect to capciate-treated group (CA). *= p<0.05; **= p<0.01

4. Discussion

Liver function has been found to be disrupted by ranitidine's toxic effects [30]. This may be reflected in routine liver function tests, which are detected by disturbances in serum liver enzymes ALT, AST, and GGT, and in the protein profile. These results confirm damage to liver cells and disruption of their membrane permeability, leading to the leakage of enzymes into the circulation and a decrease in protein synthesis [31]. Also, the decrease in albumin and total protein may be due to reduced food intake and decreased protein absorption [32]. On the other hand, data showed improvements in liver enzymes and protein profiles after treatment with capsiate and/or MSCs. [33,34].

The damage to liver cells by ranitidine may be related to the fact that ranitidine induced oxidative stress in the liver, as it increased hepatic tissue content of MDA while decreasing GSH and SOD, which, in turn, postulated an inflammatory response [35].

The protective effect of capsiate on lipid peroxidation in the liver may be related to its phenolic component, which acts as a free radical scavenger [36]. In the same manner, the antioxidant activity of capsiate could be attributed to its phytochemical contents, which increase the activity of antioxidant enzymes. Increased GSH levels induced by capsiate may explain its incorporation into the cell membrane, altering membrane fluidity and permeability and inducing other metabolic pathways that lead to elevated GSH biosynthesis [37].

The ameliorative role of MSCs may be enhanced through their regenerative properties, which are among the powerful skills of stem cells; MSCs can differentiate into functional liver cells and then produce several growth factors and cytokines to improve liver injury and enhance antioxidant enzymes [38-40].

CRP plays important roles in inflammatory responses, including the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, and cytokine production. In the current results, the ranitidine-treated groups showed a significant elevation in inflammatory markers, including CRP and TNF-α. Ranitidine activated Kupffer and stellate cells in the liver, thus increasing the production of CRP and TNF-α [35]. TNF-α levels are increased at the site of inflammation and, together with IL-1β and IL-6, can induce systemic inflammatory responses that may fuel liver injury [41].

In T cells, the pro-inflammatory IL-12 triggers polarization of Th0 cells to pro-inflammatory Th1, while suppressing the induction of regulatory T cells (Treg), which secrete anti-inflammatory mediators. More to the point, IL-12 enhances IFN-γ secretion and cytotoxic activity of CD8 T and natural killer cells [42]. This means that ranitidine induces inflammation via the adaptive immune response. The suppression of Treg by IL-12 may be associated with decreased anti-inflammatory cytokine IL-10 in response to ranitidine. However, free radicals

are considered a key mediator of inflammation, so modulation of these inflammatory markers reflects the perfect scavenging role of capacitate for free radicals and inhibits oxidative inflammation crosslinking [43].

The positive outcomes have led to trials to study the role of MSCs and their products in several clinical applications [44]. The most vital role of MSCs is to respond to the inflammatory environment and to modulate the immune response via paracrine signaling, switching macrophages from M1 to M2 [45,46]. Similarly, MSCs can communicate with immune cells, including B cells, T cells, dendritic cells, natural killer cells, neutrophils, and macrophages [47]. The interaction mechanisms are particularly based on intercellular contact, acting in collaboration with the secretion of soluble immune particles, including cytokines and growth factors, to stimulate MSC-regulated immunosuppression [48].

The oxygen-inflammation environment triggers mitochondrial-initiated responses involved in the intrinsic apoptosis pathway. Once the outer mitochondrial membrane is permeabilized, pro-apoptotic proteins, such as cytochrome c, are released from the intermembrane space into the cytosol; this triggers caspase activation. On the other hand, extrinsic apoptosis is triggered by ligand binding to death receptors [49]. The transmembrane death signal induces a cascade that leads to the activation of caspase 8. Both intrinsic and extrinsic routes converge on common factors that lead to caspase-3. Once caspase-3 is activated, several substrates are cleaved, leading to apoptotic cell death [50].

In contrast, Bcl-2 proteins are essential for regulating mitochondrial-initiated apoptosis; they act by binding apoptotic activators such as Bax and Bak and inhibiting apoptotic pathways [51]. Based on the above discussion, the ameliorative role of capciate and MSCs against apoptosis, as confirmed by increased caspase-3 and decreased Bcl-2, may be challenged by their role in antagonizing the crosslinking influence of oxidation-inflammation. Moreover, several studies reported that MSCs exert anti-apoptotic effects via secreted factors that trigger the formation of Bcl-2 protein [52,53].

5. Conclusions

From the earlier results and discussion, it can be concluded that MSCs and capsiate, alone or together, may play an effective therapeutic role against ranitidine-induced toxicity in rat livers. It looks best to enhance the role of MSCs with a natural antioxidant such as capsiate. The authors suggest more studies on the effects of MSCs with capsiate on liver toxicity.

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Author contributions

Conceptualization. N.E.S., E.S.E., and N.Z.H.; methodology, A.A.S.; data analysis and initial writing, N.Z.H., E.A.S., and A.A.S.; the graphical abstract and finishing writing, N.Z.H., and all authors, final revision and confirmation of publication.

Institutional Review Board Statement

Ethical approval for animal experiments was confirmed by the Ethics Committee of the Faculty of Women, Ain Shams University (ASU/W/Sci-5R/23-2-31).

Informed Consent Statement

Not applicable.

Data availability

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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