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SAFETY ASSESSMENT OF MULTI-DRUG EXPOSURE: ACUTE TOXICITY STUDY OF ATORVASTATIN, RAMIPRIL, AND BQ-123 COMBINATION IN RATS

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ABSTRACT
Background: Combination cardiovascular therapies such as atorvastatin, ramipril, and endothelin-A receptor antagonists are increasingly used to improve clinical outcomes; however, their concurrent administration may introduce unforeseen pharmacodynamic and pharmacokinetic interactions. To date, no acute toxicity evaluation of this three-drug combination has been reported. **Methodology:** An acute toxicity study was conducted in female Wistar rats in accordance with OECD Guideline 423. A single intraperitoneal dose of atorvastatin (10 mg/kg), ramipril (1 mg/kg), and BQ-123 (1 mg/kg) was administered to a test group (n = 3), while controls received vehicle only (normal saline, 0.9% NaCl). Animals were monitored for 14 days for clinical signs of toxicity, mortality, body-weight changes, and behavioral changes. Gross necropsy and histopathological examinations of major organs were performed on Day 14. **Result and Discussion:** No mortality or clinical signs of toxicity were observed throughout the study. Body-weight progression in treated rats was consistent with that of the control group, demonstrating normal physiological growth. Gross pathological examination revealed no organ abnormalities or injection-site reactions. Histopathological analysis confirmed intact tissue architecture in the liver, kidney, heart, lungs, spleen, brain, stomach, and intestines. The absence of clinical, physiological, and histological alterations suggests that the atorvastatin–ramipril–BQ-123 combination does not induce acute systemic or organ toxicity at the tested dose. Findings indicate no synergistic or additive toxicity from co-administration, although further repeated-dose and biochemical assessments are necessary to characterize long-term safety. **Conclusion:** A single intraperitoneal administration of atorvastatin, ramipril, and BQ-123 was well tolerated in rats, classified as low acute hazard per OECD 423. Additional sub-acute and chronic studies are recommended to support therapeutic safety.

INTRODUCTION

Polypharmacy has become increasingly prevalent in modern cardiovascular care, where combinations of lipid-lowering, antihypertensive, and vascular-modulating agents are frequently prescribed to improve therapeutic outcomes. Evidence from recent clinical and epidemiological studies suggests that combination therapies can improve adherence and reduce cardiovascular events; however, co-administration of multiple

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drugs also raises concerns regarding pharmacodynamic and pharmacokinetic interactions that may amplify toxicity risks, necessitating structured preclinical safety evaluation. As such, evaluating the acute safety of multi-drug regimens remains a priority in translational research. While atorvastatin and ramipril are well-established clinical agents routinely co-prescribed in patients with cardiovascular and metabolic disorders, BQ-123 is not a standard therapeutic drug but a selective endothelin-A (ETA) receptor antagonist primarily used as a research peptide. The rationale for evaluating this specific combination is based on experimental and translational cardiovascular research settings, where ETA receptor antagonism is investigated as an adjunct strategy to statin- and ACE-inhibitor-based therapy for conditions involving endothelial dysfunction, vascular remodeling, and inflammation. Thus, the present study is intended to provide preclinical safety data relevant to experimental combination strategies and future translational development rather than current routine clinical practice [1, 2].

Atorvastatin, a widely used HMG-CoA reductase inhibitor, is recognized for its strong cardioprotective efficacy but has been associated with rare dose-dependent toxicities, including hepatotoxicity and myopathy. Recent animal studies and mechanistic reports have highlighted the potential for oxidative stress, mitochondrial dysfunction, and metabolic interactions when statins are combined with other agents, indicating that the acute safety profile may shift under polypharmacy conditions [3]. Thus, atorvastatin-containing drug combinations warrant careful toxicological assessment. Ramipril, an ACE inhibitor frequently co-prescribed with statins, demonstrates a generally favorable acute toxicity profile in rodents and humans, characterized by high LD50 values and mild adverse effects at therapeutic doses. Nevertheless, ACE inhibitors may affect renal perfusion, vascular resistance, and electrolyte balance, potentially interacting with the pharmacological effects of statins or vascular modulators. Recent pharmacological assessments emphasize the importance of monitoring renal, cardiovascular, and hepatic parameters during combined drug exposure [4,5].

BQ-123, a selective endothelin-A (ETA) receptor antagonist, is widely used in experimental cardiovascular and renal studies. Its actions on vascular tone, cardiac remodeling, and endothelial signaling make it a valuable research tool, yet its co-administration with ACE inhibitors and statins has not been extensively studied. Recent rodent investigations have shown that BQ-123 can influence hemodynamics, renal output, and oxidative status, highlighting the need for dedicated safety evaluations when used in combination therapies [6.7]. Despite increased interest in combining atorvastatin, ramipril, and endothelin-A receptor antagonists, no standardized acute toxicity study evaluating this three-drug combination has been reported. The OECD Test Guideline 423 (Acute Toxic Class Method) provides a validated and animal-sparing framework for assessing acute hazard potential. TG-423 has been extensively used for single-agent evaluation, and recent updates reinforce its applicability to novel pharmaceutical entities and drug mixtures when justification is provided [8]. Because combination therapies may alter toxicity thresholds through additive, synergistic, or antagonistic mechanisms, adapting the TG-423 framework for multi-drug exposure is scientifically and regulatory-wise appropriate.

Therefore, the present study aims to evaluate the acute toxicity of combined intraperitoneal administration of atorvastatin, ramipril, and BQ-123 in rats following the OECD 423 Acute Toxic Class method. The study investigates mortality, clinical signs of toxicity, body-weight variations, and gross pathological findings over a 14-day observation period. This work provides foundational toxicological data for multi-drug cardiovascular regimens and informs future safety assessments and dose-combination strategies.

MATERIAL AND METHODS

Chemicals and drugs

The study utilized Atorvastatin, BQ123, and Ramipril, which were purchased from Sigma Aldrich (St. Louis, MO, USA). All other analytical grade reagents and chemicals were purchased from Merck Pvt. Ltd., New Delhi. Assay kits for triglycerides, cholesterol, serum creatinine, blood urea nitrogen (BUN) & total protein were procured from Uma Scientific Traders, Allahabad.

Animals

Female Wistar albino rats weighing 150-180 g were used for this study, as recommended by OECD Guideline 423, which suggests females due to their comparatively higher sensitivity to toxicants. All the animals were procured from the Animal House of Teerthanker Mahaveer University (TMU), Moradabad, after approval from the Institutional Animal Ethics Committee (IAEC approval no. DVCP/IAEC/2023/06) and housed in standard polypropylene cages under controlled environmental conditions, with the temperature maintained at 22 ±2°C, relative humidity

between 50-70%, and a 12 h light/dark cycle. Rats were acclimated for 7 days before experimentation and had free access to a standard pellet diet and purified drinking water.

Only healthy animals exhibiting normal activity, fur appearance, and progression in body weight during acclimatization were selected for inclusion. All experimental procedures involving rats were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC). Care was taken to minimize stress and discomfort throughout the study.

Acute Toxicity Class Exposure of Atorvastatin, Ramipril, and BQ-123

The acute toxicity assessment of the multi-drug combination was performed in accordance with the guidelines outlined in OECD Test Guideline 423 (Acute Toxic Class Method; 2001, updated 2023) [9]. The study consisted of two groups of 3 animals each, a normal control group administered only the vehicle and a test group receiving a single intraperitoneal dose of a formulation containing atorvastatin (10 mg/kg) [10], ramipril (1 mg/kg) [11], and the endothelin-A receptor antagonist BQ-123 (1 mg/kg) [12]. The selected dose levels were based on previously reported pharmacologically effective doses in preclinical models. All three compounds were freshly dissolved and combined in a single sterile vial to ensure homogenous administration, with the final dosing volume adjusted to 1 mL/kg body weight. The drug combination was administered once to overnight-fasted animals (3-4 h fasting period with free access to water), and no repeated dosing was performed. Following exposure, animals were monitored for 14 days to assess potential acute systemic toxicity and mortality, as stipulated by the OECD 423 procedure [13-15].

The intraperitoneal (i.p.) route was selected to ensure precise dose delivery, uniform systemic exposure, and to avoid variability associated with oral absorption, stress, and regurgitation during gavage in acute toxicity testing. Although atorvastatin and ramipril are administered orally in clinical practice, i.p. administration is commonly employed in preclinical safety & mechanistic studies to assess intrinsic systemic toxicity independent of GI absorption & first-pass variability. It is acknowledged that ramipril is converted to its active metabolite, ramiprilat, in the liver following oral administration; therefore, the present findings reflect acute systemic tolerability rather than clinical pharmacokinetics, and oral-route toxicity studies are warranted for translational extrapolation.

Observations and Clinical Monitoring

Following dosing, all animals were closely monitored for signs of acute toxicity in accordance with OECD Guideline 423. Observations were initiated immediately after administration, with continuous monitoring during the first 30 minutes and at regular intervals during the initial 24-hour period (0, 0.5, 1, 2, 4, 6, 12, and 24 h), followed by daily assessments for the entire 14-day observation phase [16, 17]. Clinical parameters included changes in skin, fur, eyes, and mucous membranes, behavioral alterations, gait abnormalities, tremors, convulsions, lethargy, salivation, lacrimation, and autonomic responses such as variations in urination or defecation. Respiratory patterns were evaluated for signs of distress, including tachypnea, dyspnea, or gasping, and cardiovascular distress was inferred from peripheral indicators such as cyanosis and cold extremities. Additionally, the injection site was routinely examined for local reactions, such as erythema, swelling, or necrosis. Mortality was recorded at least twice daily, noting the exact time and any visible preceding clinical signs to assist in identifying potential treatment-related causes [18].

Body Weight Assessment

Body weights of all animals were recorded before dosing on Day 0 and subsequently on Days 7 and 14 of the observation period [19, 20]. The progression of body weight over time was evaluated to detect any treatment-related alterations in normal growth patterns, as reductions or failure to gain weight are considered indicators of systemic and metabolic toxicity in acute toxicity assessments.

Necropsy and Pathological Examination

At the conclusion of the 14-day observation period, all surviving animals were humanely euthanized using CO2 asphyxiation followed by a secondary method to ensure death, and a detailed gross necropsy was performed. Major organs, including the liver, heart, kidneys, lungs, spleen, stomach, intestines, and brain, were carefully examined for macroscopic abnormalities such as congestion, discoloration, edema, enlargement, or visible lesions, and the intraperitoneal injection site was specifically inspected for evidence of local irritation or tissue

damage [9, 21]. For histopathological evaluation, representative tissue samples were collected and fixed in 10% neutral buffered formalin, processed routinely, and stained with hematoxylin and eosin. Microscopic examination focused on identifying treatment-related structural alterations, including cellular degeneration, necrosis, inflammatory infiltration, hemorrhage, vascular changes, and fibrotic responses, to assess any subclinical toxicity that may not be evident on gross pathology. In accordance with OECD Test Guideline 423, which employs minimal group sizes for acute toxicity classification, no inferential statistical analysis was performed. Continuous variables such as body weight were presented primarily as individual animal data due to the small sample size (n = 3 per group), with descriptive statistics used only to support clinical interpretation. Acute toxicity classification was based on mortality outcomes and a qualitative assessment of clinical and pathological findings, rather than on statistical comparisons.

Statistical Analysis

In accordance with the design of the OECD Test Guideline 423, which utilizes minimal group sizes to determine acute toxicity classification primarily based on mortality outcomes, the LD50 cut-off value for the test combination was derived directly from the guideline's decision-tree criteria rather than from inferential statistical comparisons. Given that only three animals were included per group, continuous variables such as body weight were summarized descriptively and presented as individual values and mean ± standard deviation (SD) to support clinical interpretation. No formal hypothesis testing was applied, as the acute toxic class method does not rely on statistical significance to establish acute hazard potential. Observed clinical signs and gross pathological findings were evaluated qualitatively to determine the presence or absence of treatment-related toxicity.

RESULT AND DISCUSSION

RESULT

Clinical Signs and Mortality

All animals in both the control and treated groups survived the 14-day observation period following the single intraperitoneal administration. No mortality was recorded at any time point in the combination-treated group, indicating an absence of acute lethal effects at the administered dose. Throughout the initial observation phase (the first 24 hours), none of the treated rats exhibited immediate signs of distress, such as tremors, convulsions, altered respiration, excessive salivation, or behavioral abnormalities. Over the subsequent days, the animals continued to display normal grooming behavior, locomotor activity & food & water intake, comparable to the control group.

No autonomic disturbances or indicators of cardiovascular or respiratory compromise were noted. Likewise, no visible adverse reactions were observed at the injection site during daily monitoring. Based on the absence of treatment-related mortality, the combination of atorvastatin (10 mg/kg), ramipril (1 mg/kg), and BQ-123 (1 mg/kg) is considered to fall above the lethal threshold for the tested dose level. According to the OECD 423 acute toxic class decision-tree criteria, the LD_{50} cut-off value for the combination is therefore estimated to be greater than the administered dose, and the formulation can be categorized as unclassified or of low acute toxicity hazard at the tested concentration (Table 1). These findings suggest that a single intraperitoneal co-administration of the three drugs does not induce acute systemic toxicity under the conditions of this study.

Table 1: Clinical signs observed during toxicity evaluation

S. No.	Clinical observed	Remarks
1.	Mortality status (0-14 Days)	Survived
2.	Skin, fur, eyes, mucosa, posture, activity	None
3.	Tremors, gait, convulsions, reflexes	Not Observed
4.	Salivation, lacrimation, piloerection, urination/defecation	Within normal limits
5.	Respiratory rate, effect, abnormal sound, cyanosis	Within normal
6.	Injection site: Redness, swelling, necrosis	None

Body Weight Progression

Body weight changes were recorded on Days 0, 7, and 14 to assess any deviations from normal physiological growth, which could indicate acute systemic toxicity (Table 2). All animals, both in the control and combination-treated group, exhibited progressive body-weight gain throughout the observation period. No animal demonstrated stagnation or reduction in body weight, which is commonly associated with systemic toxicity,

stress-induced metabolic suppression, or compromised nutritional status. The magnitude of weight gain in the treated group was comparable to that of the control group, suggesting that the single intraperitoneal administration of atorvastatin (10 mg/kg), ramipril (1 mg/kg), and BQ-123 (1 mg/kg) did not adversely affect growth performance or overall physiological well-being during the 14-day post-treatment period.

These results further support the absence of acute toxicity and validate the clinical observations recorded during the study. Individual body-weight values for each animal are presented to ensure transparency, given the small group size (n = 3), as recommended for OECD 423 acute toxicity studies. Relative organ weights recorded on Day 14 are presented in Table 3. No apparent differences were observed between the control and treated groups. All values were within the normal physiological range reported for female Wistar rats, supporting the absence of treatment-related organ hypertrophy or atrophy.

Table 2: Body weight changes during the 14-day observation period

Group	Animal ID	Day 0(g)	Day 7(g)	Day 14(g)
Control	C1	165	172	181
	C2	160	168	176
	C3	170	178	186
Treated	T1	168	175	184
	T2	162	170	178
	T3	166	174	182

Data are shown as individual animal values. The values are provided only for descriptive support due to the small sample size (n = 3).

Table 3: Relative organ wts. (organ wt./body weight ratio) of control & treated female Wistar rats on Day 14 following single dose administration of atorvastatin, ramipril & BQ-123.

Organ	Control (Mean ± SD)	Treated (Mean ± SD)
Liver	0.036 ± 0.002	0.037 ± 0.001
Kidney	0.0072 ± 0.0003	0.0071 ± 0.0004
Heart	0.0035 ± 0.0002	0.0036 ± 0.0002
Lungs	0.0058 ± 0.0003	0.0059 ± 0.0004
Spleen	0.0029 ± 0.0002	0.0030 ± 0.0002
Brain	0.0078 ± 0.0003	0.0079 ± 0.0004

Relative organ weights are presented for descriptive purposes only. No inferential statistical analysis was performed due to the minimal group size (n = 3), as per OECD 423 acute toxicity study design.

Gross necropsy findings and histopathological observations

Gross pathological examination performed on Day 14 revealed no treatment-related abnormalities in any of the major organs examined, including the liver, kidneys, heart, lungs, spleen, stomach, intestines, and brain. All organs appeared normal in size, shape, color, and texture & no visible lesions, hemorrhages, or tissue swelling were detected. The intraperitoneal injection site showed no signs of irritation, such as edema, erythema, or necrotic lesions, confirming excellent local tolerance to the administered formulation. Any subtle variations observed in organ appearance were considered within the range of normal biological variability. Taken together, these findings indicate that the combination of atorvastatin, ramipril, and BQ-123 did not induce macroscopic organ toxicity at the tested dose.

Microscopic evaluation of hematoxylin and eosin-stained tissue sections from both control and treated rats demonstrated normal architecture across all assessed organs. Hepatocytes exhibited preserved structural integrity with no evidence of vacuolar degeneration, necrosis, inflammatory infiltration, or vascular congestion (Table 4). Renal tissue showed intact glomeruli and tubules without pathological alterations such as tubular necrosis, cast formation, or interstitial inflammation.

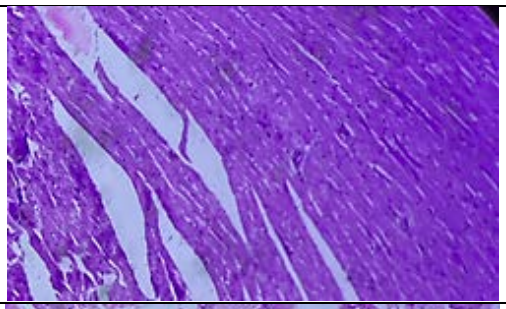
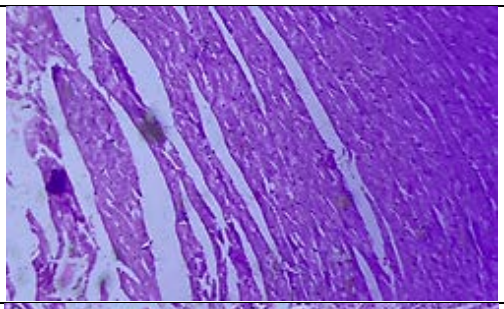
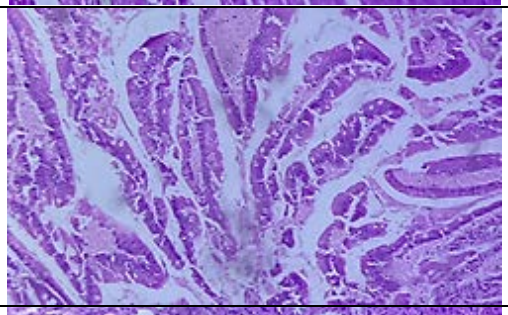
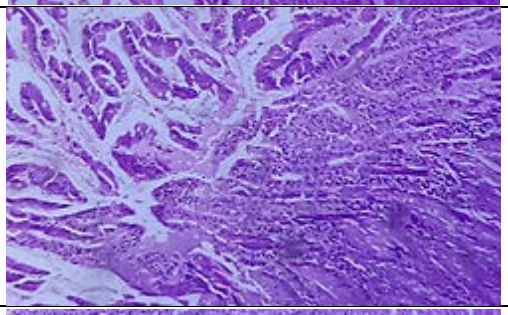
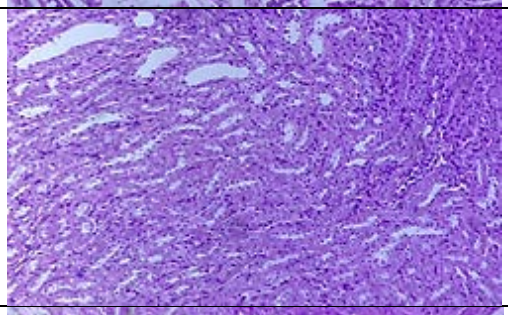
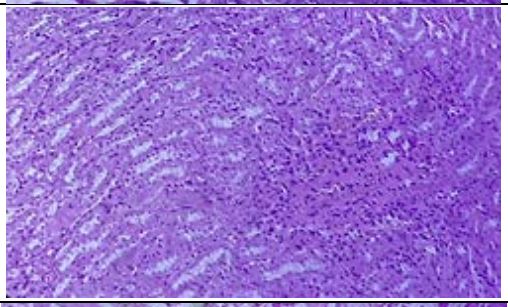
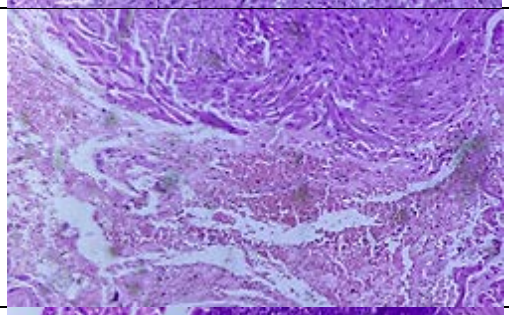
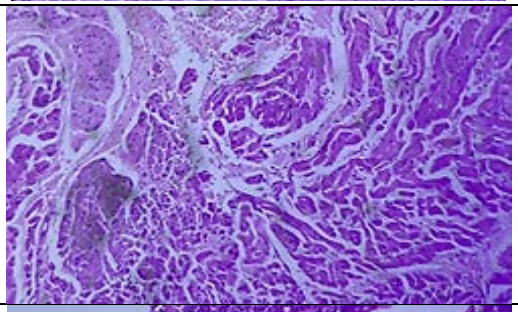
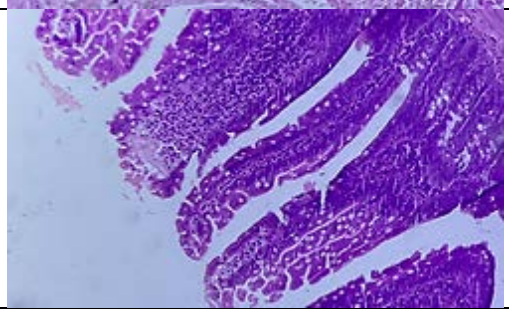
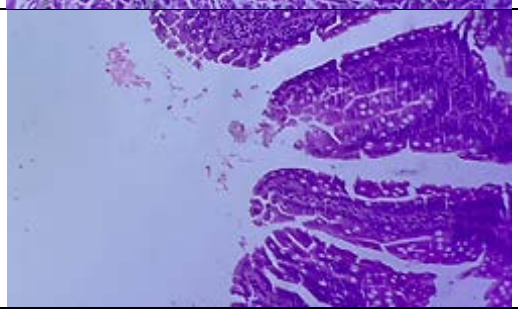
Cardiac and pulmonary tissues revealed normal cellular morphology, with no edema or inflammatory changes. Splenic and cerebral tissues demonstrated normal histological features. No microscopic changes suggestive of treatment-induced toxicity were detected in any animal. These results support the conclusion that no acute cellular or tissue damage occurred following the single intraperitoneal administration of the drug combination.

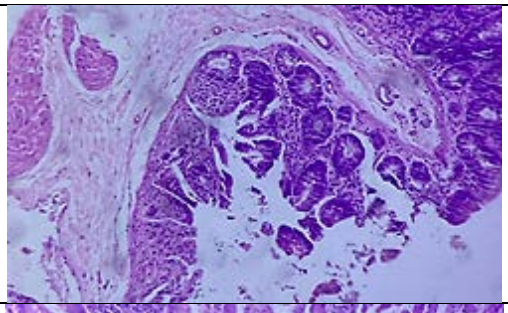
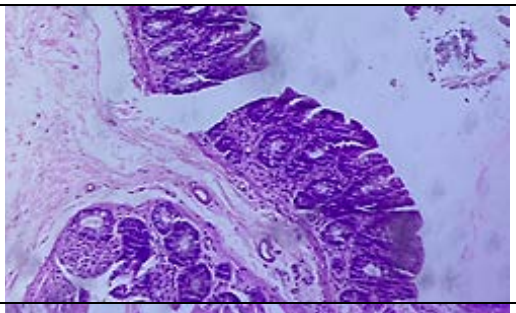
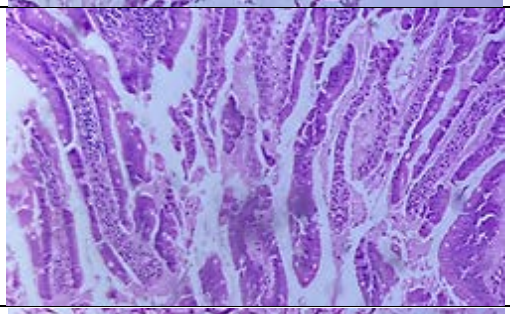
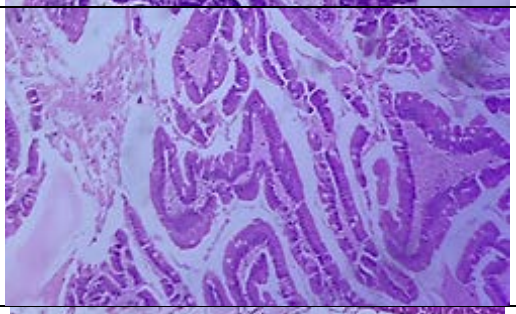
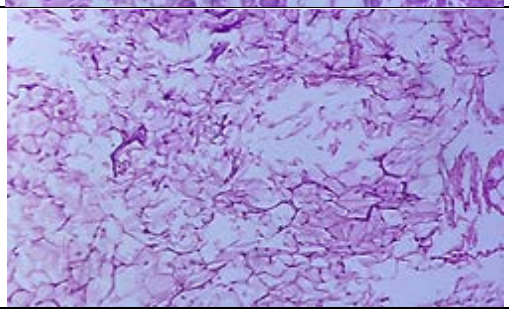
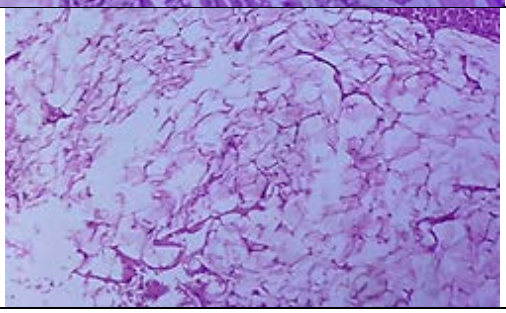
Acute Toxicity Classification

Based on the OECD 423 Acute Toxic Class Method criteria, the absence of mortality in any of the treated animals following a single intraperitoneal administration of the atorvastatin, ramipril, and BQ-123 combination indicates that the LD50 cut-off value lies above the tested dose level. According to the decision-tree matrix outlined in OECD 423, the tested formulation can therefore be assigned to the "Unclassified" category under the Globally Harmonized System (GHS) for acute toxicity, implying low acute hazard potential. The lack of

clinical abnormalities, body-weight suppression, or organ toxicity further supports this classification. Thus, under the experimental conditions of this study, the combination therapy did not elicit any observable signs of acute systemic toxicity, confirming a favorable safety margin at the evaluated dose.

Table 4: Histopathological assessment of vital organs collected on Day 14 after single intraperitoneal administration of atorvastatin, ramipril, and BQ-123 in Wistar rats (OECD 423 acute toxicity study).

Organ	Control	Treated
Heart		
Liver		
Brain		
Spleen		
Intestine		

Organ	Control	Treated
Stomach		
Kidney		
Lungs		

DISCUSSION

In this acute toxicity assessment, a single intraperitoneal administration of the combined drugs atorvastatin, ramipril, and BQ-123 at the tested dose did not result in any mortality, observable clinical signs of toxicity, body-weight decline, gross pathological lesions, or histopathological abnormalities in treated female Wistar rats. This overall lack of acute adverse effects suggests that the LD_{50} cut-off value of the combination lies above the administered dose, placing the combination in a low-hazard category under the Organisation for Economic Co-operation and Development (OECD) Test Guideline 423 (Acute Toxic Class Method) decision-tree criteria. The step-wise design of TG 423, using only three animals of a single sex per dose, is specifically intended to minimize animal use while still enabling robust acute hazard classification; absence of morbidity or mortality in this small cohort is sufficient to justify a no acute hazard assignment under guideline norms [9].

Comparing with prior acute toxicity reports further reinforces the relevance of our findings. For instance, a recent study employing the OECD 423 protocol to assess a polyherbal formulation reported no lethal effects or toxic signs at high doses; gross pathology and histology likewise showed no abnormal changes, leading the authors to conclude a high safety margin for that formulation. Similarly, in many herbal-based or single-compound acute tests, absence of clinical symptoms or pathological alterations is accepted as evidence of low acute toxicity [22-25]. These precedents demonstrate that the absence of adverse outcomes, even in small numbers of animals, is a conventional and accepted basis for classification, particularly when supported by comprehensive necropsy and histopathological examinations.

The inclusion of a vascular-modulating peptide (BQ-123), alongside an ACE inhibitor (Ramipril) and a statin (Atorvastatin), could have posed a risk of acute vascular, renal, hepatic, or systemic toxicity due to potential pharmacodynamic interactions. BQ-123, a selective endothelin-A receptor antagonist, is known to modulate vascular tone and has been employed in experimental models to mitigate vascular or oxidative stress-related injuries without overt toxicity at certain doses The lack of histopathological lesions or injection-site

reactions in our study suggests that, at the tested dose and route, the vascular and renal systems tolerated the triple combination without acute damage, implying no synergistic acute toxicity. This outcome lends preliminary support to the safety of such combinations in preclinical settings, at least under acute exposure conditions [26, 27].

However, some limitations inherent in the acute toxicity design must be acknowledged. First, TG 423 is not intended to detect sub-acute or chronic toxicity, cumulative effects, or functional organ impairment that might manifest beyond gross histology. Parameters such as biochemical markers (liver enzymes, renal function tests), oxidative stress indices, or subtle cellular changes often require repeated or prolonged exposure and larger sample sizes; such endpoints lie outside the scope of an OECD 423 acute test and were therefore not assessed.

The absence of gross and histological lesions does not preclude subclinical functional stress. Second, the use of intraperitoneal administration, while experimentally valid and justified for mechanistic or combinatorial studies, may not replicate the pharmacokinetic behavior seen with typical oral dosing in humans; first-pass metabolism, absorption kinetics, and tissue distribution may differ. Thus, although the i.p. route was tolerated without local irritation or peritoneal damage, translation to oral or clinical routes would require careful pharmacokinetic and repeated-dose evaluation. Finally, the small group size (n = 3) restricts the detection of low-incidence adverse events; while guideline-driven classification accepts this, the power to identify rare toxic events remains limited.

Given these considerations, our data support that the tested combination has no observable acute systemic toxicity at the administered dose and can therefore be classified as low hazard per OECD 423 criteria. This finding provides a foundational safety basis for the combination, which may encourage further investigations under sub-acute or chronic dosing paradigms, for example, employing repeated-dose protocols (28 or 90-day) including biochemical, functional, and toxicokinetic endpoints. Such studies would allow assessment of cumulative toxicity, potential drug-drug interactions over time, dose-response relationships, and reversibility of acute toxicity. Moreover, the addition of serum biochemistry, hematology, organ-weight analysis, and more sensitive histopathology (e.g., electron microscopy) would deepen the safety evaluation.

Although no synergistic acute toxicity was observed, potential hepatic CYP450 interactions should be considered. CYP3A4 primarily metabolizes Atorvastatin, whereas ramipril is a prodrug hydrolyzed to ramiprilat and is not a significant inhibitor or inducer of CYP3A4. BQ-123 is a peptide that is mainly degraded by peptidases rather than CYP enzymes. Nevertheless, indirect modulation of hepatic enzyme activity or altered hepatic blood flow during combined therapy cannot be excluded and may become relevant under repeated or chronic dosing conditions.

LIMITATIONS

Although serum biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and blood urea nitrogen (BUN) are valuable indicators of sub-clinical hepatic and renal dysfunction, these parameters were not assessed in the present study. This omission is consistent with the design of OECD Test Guideline 423, which emphasizes mortality-based acute hazard classification using minimal numbers of animals rather than detailed biochemical profiling. Given the small group size (n = 3), inclusion of serum biochemistry could yield limited interpretative value and may be influenced by acute handling or procedural stress. Instead, a comprehensive histopathological evaluation of the major organs revealed no evidence of acute tissue injury. Nonetheless, future repeated-dose and chronic toxicity studies will incorporate serum biochemical and functional assessments to further substantiate long-term safety.

The present study employed pharmacologically relevant doses rather than limiting doses to evaluate the acute tolerability of the drug combination under realistic experimental conditions. While limit-dose testing (e.g., 2000 mg/kg) is commonly used to establish safety margins for single compounds, such an approach may not be appropriate for multi-drug combinations, as extremely high doses can produce non-specific toxicity unrelated to clinically meaningful drug–drug interactions.

The selected doses represent established effective levels reported in the literature and were chosen to assess whether concurrent exposure results in additive or synergistic acute toxicity. Consequently, the study demonstrates acute tolerability rather than defining a maximal toxic threshold. Future dose-escalation and repeated-dose studies will be required to determine safety margins and dose-response relationships.

CONCLUSION

The present study demonstrated that a single intraperitoneal dose of the combined formulation containing atorvastatin (10 mg/kg), ramipril (1 mg/kg), and BQ-123 (1 mg/kg) did not induce any mortality, adverse clinical signs, body-weight suppression, or observable organ toxicity in female Wistar rats during a 14-day observation period. Gross necropsy and histopathological evaluations further confirmed the absence of treatment-related structural abnormalities in vital organs. According to the acute toxicity classification criteria outlined in OECD Guideline 423, the LD50 cut-off value for this drug combination lies above the tested dose, indicating a low acute hazard potential. Collectively, these findings suggest that the combined administration of these three pharmacologically active agents is well tolerated under acute exposure conditions. However, further studies employing repeated-dose toxicity, biochemical profiling, and toxicokinetic assessments are recommended to establish comprehensive safety before consideration for long-term therapeutic application or clinical development.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Astha Jaiswal contributed to the conceptualization of the study, experimental design, animal handling and dosing, data acquisition, maintenance of study records, and preparation of the initial draft of the manuscript. Phool Chandra supervised the experimental work, data interpretation, histopathological evaluation, critical revision of the manuscript for intellectual content, and final approval of the submitted version.

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