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Investigating the Effects of Lithium and AKT Inhibitors on Insulin Resistance in a Rat Model

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Abstract

Insulin resistance, a fundamental aspect of metabolic diseases like type 2 diabetes and obesity, is defined by compromised insulin signaling and glucose absorption. This study sought to examine the impact of lithium, Protein Kinase B (PKB) or AKT inhibitors, and their combination on insulin resistance in a rat model generated by a high-fat diet (HFD). Fifty male albino rats were categorized into five groups: a control group, a high-fat diet (HFD) group, and three HFD treatment groups administered lithium, AKT inhibitors, or a combination thereof. Over a four-week period, we evaluated fasting insulin, blood glucose, HOMA-IR, liver function markers (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST)), triglycerides (TG), inflammatory markers (Interleukin-1 beta (IL-1β), Tumor Necrosis Factor-alpha (TNF-α)), oxidative stress parameters (Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT)), and gene expression associated with insulin signaling (insulin receptor (IR), Akt, glucose transporter type 4 (GLUT 4)). The findings indicated that both lithium and AKT inhibitors markedly enhanced metabolic, hepatic function, and inflammatory indicators. The AKT inhibitor alone was most efficacious in diminishing lipid peroxidation and inflammation, but the combined therapy offered the most comprehensive antioxidant protection. The data indicate that lithium and AKT inhibitors, either individually or in conjunction, may have therapeutic functions in the treatment of insulin resistance and metabolic diseases.

Keywords: Insulin resistance, Lithium, AKT inhibitor, High-fat diet, metabolic syndrome, Oxidative stress, Inflammation.

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1. Introduction

Insulin resistance is a disease characterized by diminished cellular responsiveness to insulin, resulting in compromised glucose absorption and elevated blood sugar levels [1]. This impairment is characteristic of metabolic diseases, including type 2 diabetes and obesity. Insulin resistance is intricately linked to disturbances in the Phosphoinositide 3-kinase/Protein Kinase B (PI3K/AKT) signaling pathway, which is essential for glucose metabolism. Insulin binding to its receptor triggers the PI3K/AKT pathway, promoting glucose transport into cells. In insulin-resistant conditions, this signaling is compromised, resulting in diminished glucose absorption and elevated blood glucose levels [2]. Insulin resistance relates to hyperglycemia and other metabolic problems, such as lipid metabolism disorders and chronic inflammation, hence increasing the risk of cardiovascular illnesses [3]. Comprehending the molecular mechanisms of insulin resistance is essential for formulating treatment strategies to restore normal insulin sensitivity and enhance metabolic health [4]. Lithium is a recognized mood stabilizer predominantly utilized in the management of bipolar disorder.

It has thoroughly investigated for its neuroprotective attributes and capacity to regulate neurotransmitter signaling; however, current studies have broadened its implications outside the field of psychiatry. The influence of lithium on multiple cellular pathways, particularly the suppression of glycogen synthase kinase-3β (GSK-3β), has created novel therapeutic opportunities in metabolic, neurological, and cardiovascular disorders [5]. Furthermore, lithium's influence on GSK-3β and many metabolic pathways establishes it as a prospective therapeutic agent for multiple disorders. In addition to metabolic health, lithium has been investigated for its possible neuroprotective properties in Alzheimer's disease and other neurodegenerative conditions. By inhibiting GSK-3β, lithium may diminish tau hyperphosphorylation and amyloid-beta buildup, two critical elements in Alzheimer's disease [6]. Moreover, lithium exhibits cardioprotective effects, namely in diminishing cardiac hypertrophy and remodeling post-myocardial infarction by modulating the AKT/mechanistic target of rapamycin (mTOR) signaling pathways [7]. AKT inhibitors are chemicals that specifically target the AKT signaling system, which is crucial for controlling cell growth, proliferation, survival, and metabolism [8-9]. The AKT pathway, or PI3K/AKT/mTOR pathway, is often dysregulated in numerous illnesses, especially cancer and metabolic disorders [10].

The hyperactivation of this system correlates with unregulated cellular proliferation, rendering it a pivotal target in oncological treatment. AKT inhibitors, including

MK2206, have been engineered to obstruct this pathway, thereby diminishing tumor growth and enhancing therapeutic results [11]. Besides cancer, AKT inhibitors have demonstrated efficacy in the treatment of metabolic illnesses. The AKT pathway is crucial for insulin signaling; its dysregulation leads to insulin resistance, a characteristic of metabolic disorders. These drugs can modify glucose metabolism, enhance insulin sensitivity, and potentially mitigate insulin resistance by inhibiting AKT [12]. Recent research have investigated application of AKT inhibitors in cardiovascular disorders, wherein hyperactivity of AKT pathway leads to detrimental cardiac remodeling and hypertrophy [13-14]. Our research entailed administering lithium and AKT inhibitors, both alone and in conjunction, to rats exhibiting insulin resistance produced by a high-fat diet, in order to evaluate their impact on insulin sensitivity. This methodology may provide novel insights into the modulation of essential metabolic processes by these chemicals and present new therapeutic possibilities for addressing insulin resistance and related metabolic diseases [15].

2. Methodology

2.1. Chemicals

- ✓ Lithium Chloride (LiCl) was purchased from Sigma-Aldrich (Merck), USA.
- ✓ MK2206 was purchased from Selleck chemicals, USA, product No. S1078

2.2. Animals and experimental design

Fifty mature male albino rats were obtained from the animal facility at the Faculty of Pharmacy, Zagazig University. The rats were 8 to 10 weeks old and weighed between 180 and 200 grams on average. The rats were provided with standard care in accordance with laboratory animal standards, and study gained approval from the Zagazig University Institutional Committee for Animal Care and Use (approval number: ZU-IACUC/3/F/49/2023). Following a one-week acclimatization period, rats were randomly divided into five equal groups.

- ➢ ***Induction of Metabolic Syndrome***

The rats were subjected to a high fructose diet for a duration of 10 weeks to induce metabolic syndrome. The high fructose diet comprises 60% of total caloric intake from fructose. Fructose is integrated into a standard mouse diet by adding 60% of its weight in fructose. Following a 10-week regimen of a high-fat diet, the emergence of metabolic syndrome induced by fructose was confirmed through the observation of changes in body weight and a notable increase in fasting serum levels of glucose, insulin, and triglycerides. Rats were then utilized for a duration of 4 weeks post-therapy.

- ➢ ***Hematological specimen collection***

At conclusion of study period and following 12-hour fasting interval, rats euthanized via an intraperitoneal injection of sodium thiopental at a dosage of 60 mg/kg. Subsequently, blood samples obtained via cardiac puncture, allowed to clot, and then centrifuged at 3,000 revolutions per minute for 15 minutes. Serum samples stored at -20 °C until utilized for biochemical analysis after 4 and 8 weeks, respectively.

- ➢ ***The tissue samples collection***

The rat tissues were collected and promptly placed in a liquid nitrogen container, wrapped in aluminum foil, and stored at -80°C. This was conducted to accelerate tissue freezing and diminish the activity of endogenous RNase, facilitating preparation for real-time PCR analysis.

- ➢ ***Insulin assay***

Insulin was evaluated by ELISA (Alpco Diagnostics, Salem, Massachusetts, USA) in plasma samples. Hemolytic plasma samples were excluded.

2.3. Biochemical assays

Biochemical assays of triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood glucose were conducted using a Chemistry Analyser Olympus AU400 on non-hemolytic plasma extracted from blood samples collected post-mortem following decapitation. To compute the homeostasis model assessment for insulin resistance (HOMA-IR), divide the product of glucose (mmol/L) and insulin (μU/mL) by 22.5 [16]. Serum levels of tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) were quantified using enzyme-linked immunosorbent assay (ELISA) kits (R&D ELISA Kit, USA) following the manufacturer's guidelines.

2.4. Measurement of lipid peroxidation

Malonidealdehyde (MDA) a consequence of fatty acid peroxidation, was quantified for thiobarbituric acid (TBA) reactivity to assess lipid peroxidation. The tissue was processed for analysis with an Oxitek, ZeptoMetrix, NY, USA thiobarbituric acid-reacting compounds (TBARS) assay kit. The tissue was rinsed, weighed, and resuspended at a concentration of 50 mg/mL in isotonic saline. Subsequently, it was homogenized.

2.5. Measurement of total glutathione peroxidase (GPx) activity

In accordance with the guidelines provided with the complete GPx test kit (OXItek, ZeptoMetrix), kidney tissue was homogenized in six liters of cold GPx assay buffer relative to the tissue weight. The solution was subsequently centrifuged at 8000 rpm for 15 minutes at 4°C. A kinetic ultraviolet-visible spectrophotometer (Ultraspec 3100, Amersham Biosciences, and Cambridge, UK) was employed to evaluate the level of GPx activity in the tissue. The reduction in absorbance at 340 nm was employed to assess the oxidation of NADPH to NADP+.

2.6. Superoxide dismutase activity

The Kono method was employed to assess the activity of superoxide dismutase. The testing system comprised 96 mmol/L of nitro blue tetrazolium (NBT), 50 mmol/L of sodium carbonate, and 0.1 mmol/L of EDTA. In the cuvette, 2 mL of the aforementioned combination, 0.05 mL of hydroxylamine, and 0.05 mL of the post-nuclear fraction of the kidney homogenate were combined, and the auto-oxidation of hydroxylamine was monitored by measuring the absorbance at 560 nm. Enzyme units are defined as the quantity of enzyme that completely suppresses the reaction rate by 100%. The findings are expressed as units per milligram of protein [17].

2.7. Catalase activity

The Luck method was employed to quantify the catalase concentration. The degradation of hydrogen peroxide (H2O2)

was quantified at 240 nm with an Erba Chem 5 Plus semi-automated analyzer. The assay combination consisted of 3 mL of H2O2 phosphate buffer (1.25 × 10−2 mol H2O2) and 0.05 mL of 10% kidney homogenate supernatant. Enzyme activity was evaluated using millimolar extinction coefficient of H2O2 (0.07). The reported values are in μmol H2O2 decomposed every minute per milligram of protein [17].

2.8. qRT-PCR

Total RNA was extracted from the tissue in a single step using the Trizol Kit (15596-026, Beijing Solabo Technology Co., Ltd., China). Subsequently, cDNA was reverse transcribed from extracted RNA according to the instructions of the Takara Reverse Transcription Kit (RR037A, Takara, Japan) and then diluted to a concentration of 50 ng/μL. A 25 μL reaction system was employed to amplify 5 μL of the cDNA product utilizing the subsequent primers: 0.5 μL of upstream and downstream primers, 13 μL of 2× quantitative SYBR Green RT-PCR master mix, 5 μL of reverse transcripts, and 6 μL of DNase-free water. The procedure for the reaction was as follows: Forty-five cycles in total—95°C for 5 minutes, 95°C for 20 seconds, 60°C for 1 minute, and 72°C for 30 seconds. Gene expression was analyzed utilizing the 2-ΔΔCt method. The experiment was performed in three distinct replicates, with β-actin as the internal control.

2.9. Statistical analysis

SPSS 22.0 (SPSS Inc., Chicago, USA) was employed for statistical analysis. The measured values were presented as mean ± standard deviation. If $P < 0.05$, a substantial difference exists.

3. Results and discussion

3.1. Results

Table (2) demonstrates the effects of lithium, AKT inhibitor, and their combination on multiple metabolic (fasting insulin, fasting blood glucose, HOMA-IR, TG, liver enzymes ALT and AST) and inflammatory indicators (IL-1β, TNF-α) in rats subjected to a high-fat diet. The HFD group demonstrated a marked elevation in fasting insulin levels relative to the control group, indicating insulin resistance. Both lithium and AKT inhibitor therapies reduced insulin levels towards control values, with the combination therapy exhibiting the highest insulin levels among the treatment groups, albeit still much lower than those in the HFD group. The HFD group exhibited a significantly higher fasting blood glucose level relative to the controls, indicating of hyperglycemia. All treatment groups decreased blood glucose levels, with the lithium group demonstrating the most significant reduction, while the combination therapy also yielded considerable reductions relative to the HFD group. The HFD group exhibited a significant elevation in HOMA-IR, signifying substantial insulin resistance. Lithium and AKT inhibitor therapies markedly decreased HOMA-IR levels, with the combination group exhibiting slightly elevated levels compared to the individual treatments, although remaining much lower than the HFD group.

Lithium therapy seems to produce the most significant enhancement in HOMA-IR. Both ALT and AST levels, indicators of hepatic injury, were markedly elevated in the HFD group relative to the control group, signifying hepatic distress. All therapies decreased ALT and AST levels, with combination therapy exhibiting the lowest AST levels and lithium demonstrating the lowest ALT levels, signifying superior liver protection. The HFD group exhibited a significant elevation in triglycerides, an indicator of dyslipidemia. All treatment cohorts exhibited decreases in triglyceride levels, with the combination therapy (HFD-Li+AKT Inhibitor) yielding the most significant enhancement, followed closely by the lithium and AKT inhibitor groups. The HFD group exhibited increased IL-1β levels, indicating inflammation. The AKT inhibitor demonstrated the most efficacy in diminishing IL-1β levels, succeeded by lithium. The combined therapy exhibited a marginal elevation in IL-1β relative to the individual therapies, however remained lower than the HFD group. Levels of TNF-α were markedly elevated in the HFD group, serving as an additional signal of inflammation.

Both lithium and AKT inhibitor therapies decreased TNF-α levels, with the AKT inhibitor exhibiting the most significant reduction. The combination therapy group demonstrated somewhat increased TNF-α levels relative to individual therapies, however remained lower than the HFD group. The combination therapy (HFD-Li+AKT Inhibitor) was the most efficacious in diminishing triglycerides and AST levels, signifying substantial advantages in enhancing lipid metabolism and mitigating hepatic damage. Nonetheless, regarding insulin sensitivity (HOMA-IR) and inflammation (IL-1β and TNF-α), the separate treatments, especially the AKT inhibitor, demonstrated the most equitable enhancement without significantly increasing inflammatory indicators compared to the combination therapy. Consequently, the HFD-AKT Inhibitor group seems to provide the most favorable overall mix of advantages, yielding substantial enhancements in metabolic health, hepatic function, and inflammation. This table (3) illustrates the effects of lithium, AKT inhibitor, and their combination on critical oxidative stress biomarkers—MDA, SOD, GPx, CAT—in rats generated by a high-fat diet. The comparison of these indicators across the control group, HFD group, and treatment groups elucidates the efficacy of the treatments in mitigating oxidative stress. MDA serves as a marker for lipid peroxidation and oxidative injury.

The HFD group had a significant elevation in MDA levels relative to the control group, signifying increased oxidative stress. Treatment with lithium, the AKT inhibitor, and their combination markedly decreased MDA levels, with the AKT inhibitor group exhibiting the lowest MDA levels. SOD is a crucial antioxidant enzyme that alleviates the detrimental impacts of free radicals. The HFD group had a significant reduction in SOD activity relative to the control, signifying compromised antioxidant defense. All treatment groups demonstrated elevated SOD levels, with combination therapy (HFD-Li+AKT Inhibitor) displaying highest SOD activity. This suggests that combination therapy is most efficacious in augmenting antioxidant defense system. GPx is essential for safeguarding cells against oxidative damage. The HFD group had reduced GPx activity relative to the control group. Lithium, AKT inhibitor, and their combination all elevated GPx levels, with the combination therapy resulting in the most significant increase. This indicates that the combo therapy yields most significant restoration of GPx activity. Catalase (CAT) is an essential enzyme that mitigates oxidative stress by decomposing hydrogen peroxide. In the HFD group, CAT activity reduced relative to control group.

All treatments resulted in elevated CAT activity, with the combination therapy exhibiting the greatest levels.

This suggests that combined treatment is most efficacious in enhancing CAT activity and, consequently, overall antioxidant response. The HFD group continuously exhibited markedly increased oxidative stress (elevated MDA levels) and diminished antioxidant enzyme activity (SOD, GPx, and CAT) relative to the control, indicating detrimental impact of a high-fat diet. All treatment groups exhibited enhancements in oxidative stress markers, with combination therapy yielding highest levels of antioxidant enzymes (SOD, GPx, and CAT). The combined therapy (HFD-Li+AKT Inhibitor) yielded most significant enhancement in antioxidant defenses, evidenced by highest levels of SOD, GPx, and CAT. Consequently, the combination therapy (HFD-Li+AKT Inhibitor) seems to be the most effective in augmenting antioxidant capacity, but the AKT inhibitor alone is superior in diminishing lipid peroxidation. Table (4) indicates that the control group had a standard diet, whereas other groups were administered a high-fat diet (HFD) with or without supplementary treatments: lithium, AKT inhibitor, or a combination of both. Marked elevations in gene expression ($p < 0.05$) were noted in the groups administered lithium, the AKT inhibitor, or their combination relative to the HFD group. The data indicate that both lithium and the AKT inhibitors separately and synergistically restore or augment expression of these genes repressed by the high-fat diet (HFD).

3.2. Discussion

The research illustrated the impacts of lithium, AKT inhibitor, and their combination on metabolic and oxidative stress indicators in rats induced by a high-fat diet. In comparison to the HFD group, all therapies diminished fasting insulin, blood glucose, HOMA-IR, ALT, AST, TG, IL-1β, and TNF-α, signifying enhancements in metabolic health, hepatic function, and inflammation. The AKT inhibitor optimally reduced inflammation and lipid peroxidation, but the combo therapy delivered most robust antioxidant assistance [18]. The HFD group demonstrated markedly increased fasting insulin and blood glucose levels, signifying insulin resistance, a characteristic of metabolic syndrome. Both lithium and AKT inhibitor therapies significantly decreased fasting insulin and glucose levels, with lithium cohort exhibiting most pronounced enhancements in glucose regulation. The Delangre et al. [19] corroborate these findings, demonstrating lithium alleviates diabetogenic consequences of metabolic abnormalities by improving insulin sensitivity. Studies by the Campbell et al. [20] and Jung et al. [21] indicate that lithium enhances glucose absorption via the AKT signaling pathway, hence mitigating hyperglycemia and insulin resistance.

This study demonstrated lithium resulted in most significant decrease in HOMA-IR, aligning with findings indicating lithium improves insulin signaling through activation of glycogen synthase kinase-3 (GSK-3) and AKT pathway [5]. AKT inhibitors markedly lowered HOMA-IR levels, corroborating findings of Lee et al. [22]which indicated that inhibition of AKT activation enhances insulin sensitivity and mitigates hyperglycemia in mouse models. Increased ALT and AST levels in the HFD group indicate liver damage resulting from fat buildup, potentially leading to non-alcoholic fatty liver disease (NAFLD). Both lithium and AKT inhibitor therapies decreased ALT and AST levels, with combination therapy exhibiting most significant drop in AST. Lithium has demonstrated hepatoprotective properties by mitigating hepatic steatosis and inflammation via modulation of insulin signaling and lipid metabolism [5-23].

AKT inhibitors have shown ability to reduce hepatic fat accumulation and enhance liver function by down regulating lipogenic pathways [20-24]. Results of this investigation corroborate existing findings, emphasizing potential of both lithium and AKT inhibitors in safeguarding liver from diet-induced stress [25]. The HFD group demonstrated markedly increased triglyceride levels, an indicator of dyslipidemia. Treatment with lithium, AKT inhibitor, and their combination significantly lowered triglyceride levels, with combination therapy yielding most pronounced reduction. Studies by Tye et al. [26] indicate, lithium mitigates fat buildup by enhancing lipid metabolism through modulation of insulin signaling pathways, especially in adipose tissue and skeletal muscle. AKT inhibitors have been demonstrated to diminish lipid synthesis by obstructing downstream targets of the PI3K/AKT pathway, which facilitate fat accumulation [7]. The HFD group exhibited increased concentrations of IL-1β and TNF-α, both indicators of inflammation often linked to metabolic syndrome and obesity.

The AKT inhibitor medication was most efficacious in decreasing IL-1β and TNF-α levels, succeeded by lithium. Decrease in inflammation aligns with results of Sharma et al. [17-27] and Oyabambi et al. [28] established that AKT inhibitors limit production of inflammatory cytokines in obesity-related diseases. AKT inhibitors, through modulation of downstream pathways including mTOR and GSK-3, diminish inflammation and oxidative stress, enhancing metabolic profile noted in this study [20].Anti-inflammatory properties of lithium extensively described in literature, especially regarding its neuroprotective and metabolic advantages [29]. Lithium diminishes cytokine production, especially TNF-α, by regulating GSK-3 activity, consequently attenuating inflammatory responses in liver and adipose tissues [28].Oxidative stress marker MDA markedly increased in HFD group, whereas activity of antioxidant enzymes (SOD, GPx, CAT) diminished, signifying higher oxidative stress. Both lithium and AKT inhibitors markedly decreased MDA levels, with the AKT inhibitor exhibiting most substantial effect.

The findings corroborated by research including Agarwal et al. [24] and Jung et al. [21-30] which indicated that AKT inhibition diminishes oxidative damage by augmenting production of antioxidant enzymes. Lithium significantly contributed to reduction of oxidative stress, as seen by elevated levels of SOD, GPx, and CAT in this study. Literature extensively documents lithium's capacity to augment antioxidant defenses, with prior research demonstrating that lithium enhances activity of essential antioxidant enzymes via modulating oxidative stress pathways [5-31]. Combined medication produced highest levels of antioxidant enzymes, indicating a synergistic impact of lithium and AKT inhibitors in alleviating oxidative stress. Expression of genes associated with insulin signaling, such as insulin receptor (IR), Akt, and GLUT 4, markedly down regulated in the HFD group. Both lithium and theAKT inhibitors reinstated expression of these genes, with combination therapy demonstrating most significant enhancement.

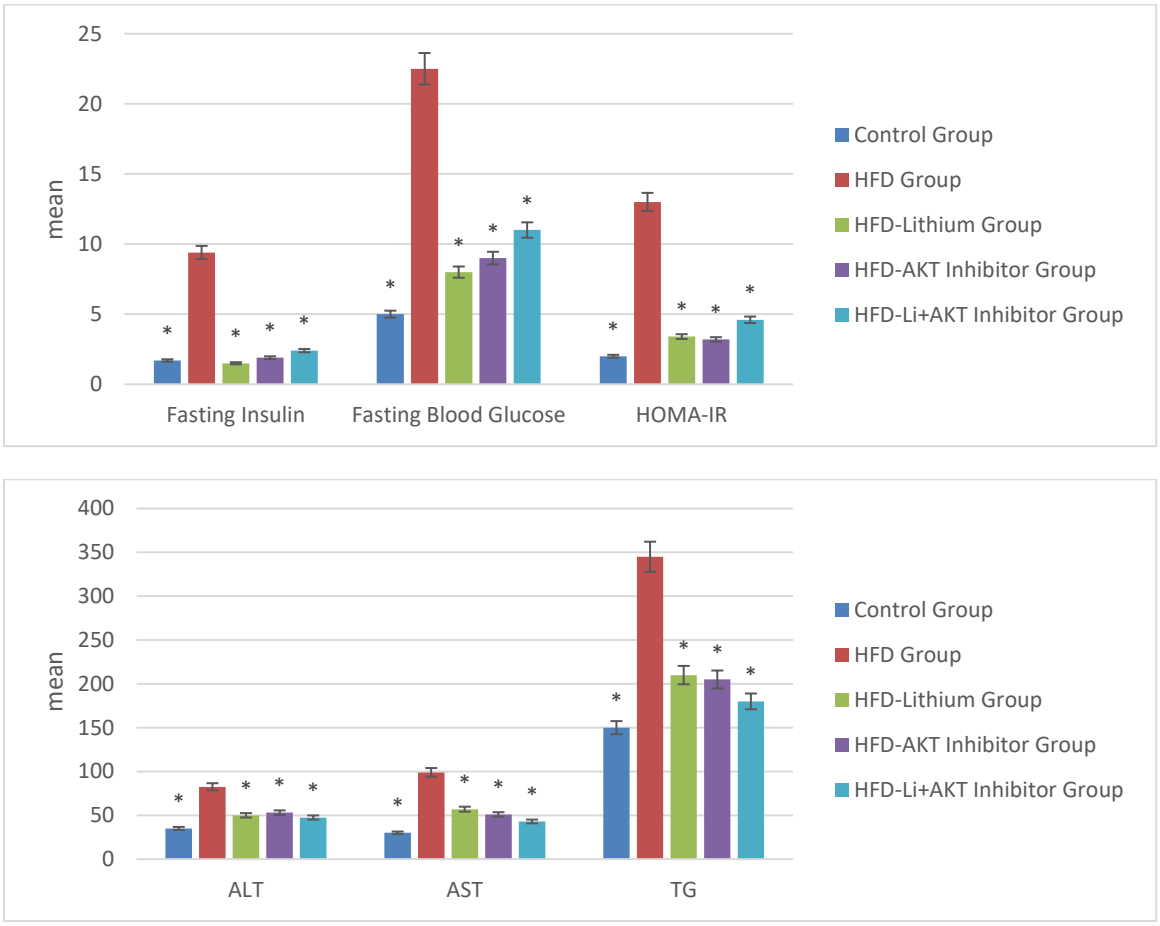

Figure 1. Effect of Lithium, AKT Inhibitor, on chemical biomarker levels in HFD- induced rats. Data are expressed as means ± SEM. * $p < 0.05$= indicate significant difference with HFD group.

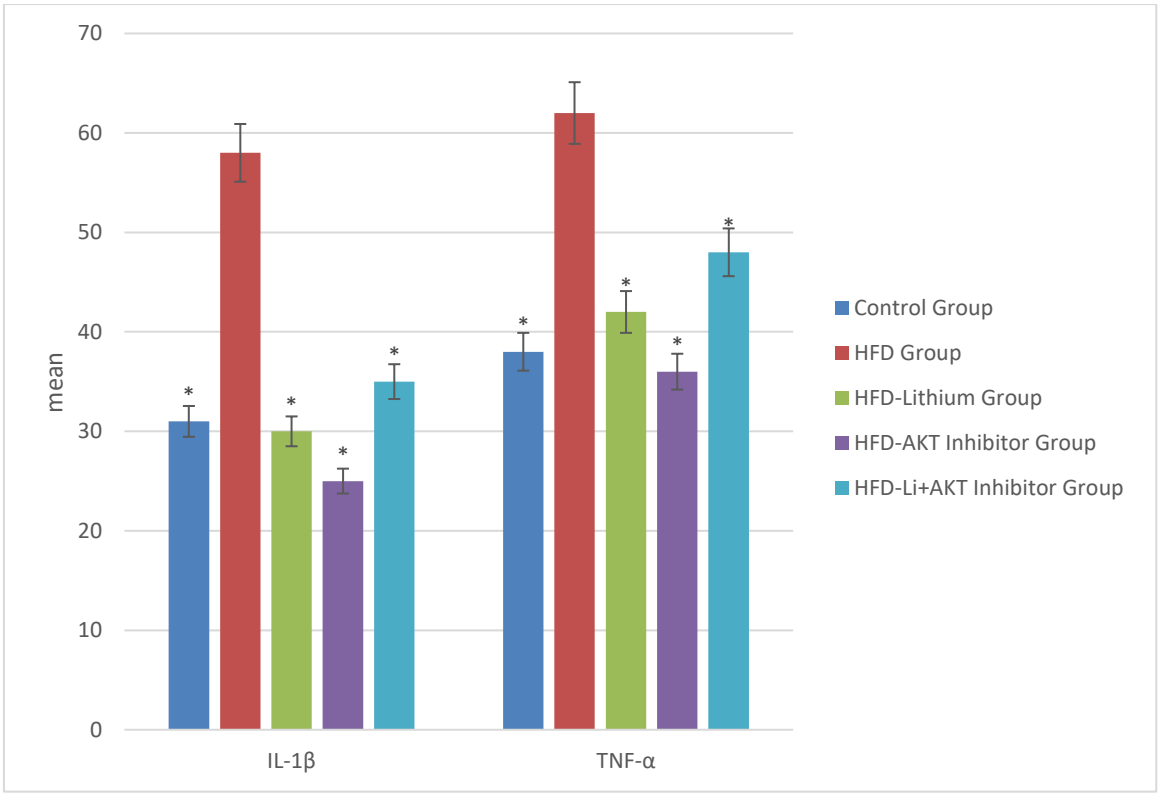

Figure 2. Effect of Lithium, AKT Inhibitor, on inflammatory biomarker levels in HFD- induced rats. Data are expressed as means ± SEM. * $p < 0.05$= indicate significant difference with HFD group.

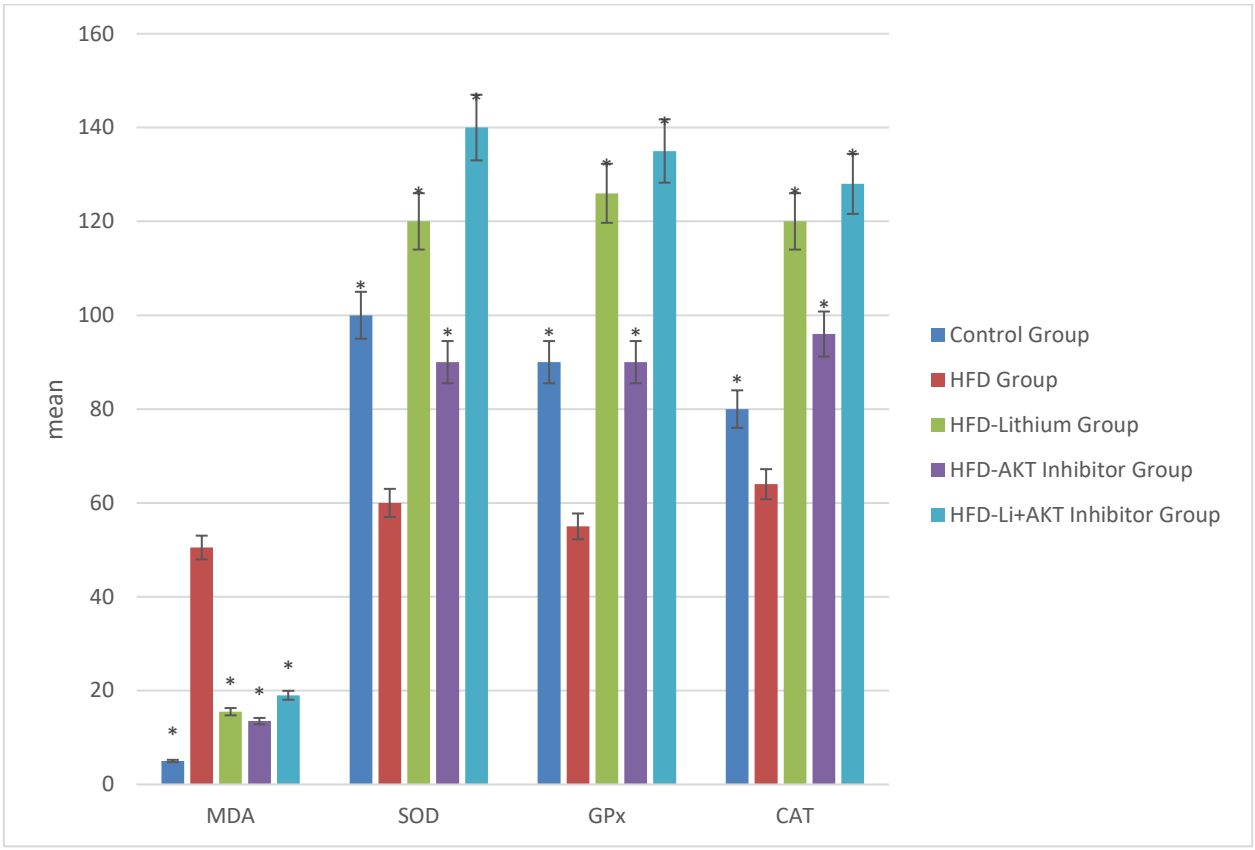

Figure 3. Effect of Lithium, AKT Inhibitor, on oxidative stress biomarker levels in HFD- induced rats. Data are expressed as means ± SEM. * $p < 0.05$= indicate significant difference with HFD group.

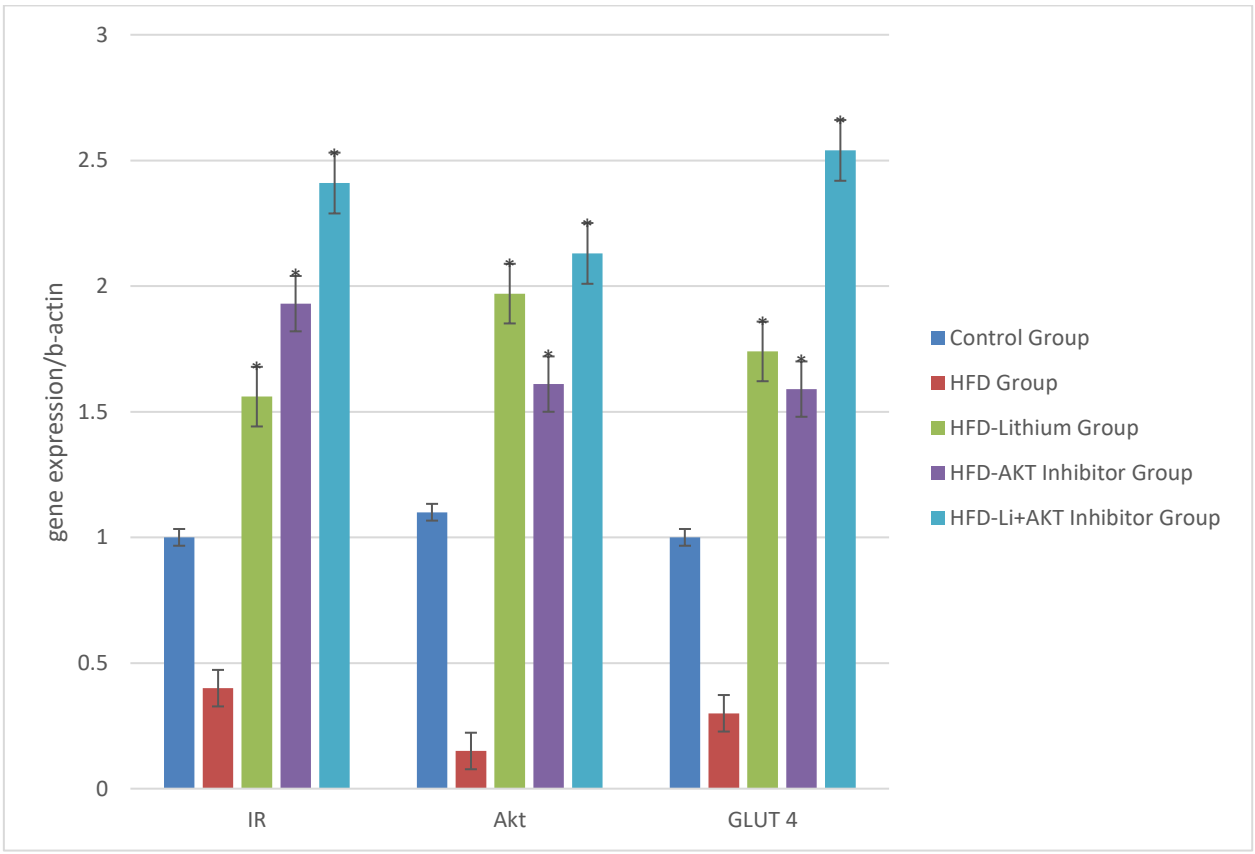

Figure 4. Effects of Lithium, AKT Inhibitor, on IR, Akt, and GLUT 4 expression in HFD- induced rats. Data are expressed as means ± SEM. * $p < 0.05$= indicate significant difference with HFD group.

Table 1. The composition of diet for rats

Standard Diet	**High Fructose Diet**
60 % starch	60 % fructose
21 % protein	21 % protein
5 % fat	5 % fat
8 % cellulose	8 % cellulose
1% vitamins and 5% minerals mix	1% vitamins and 5% minerals mix

Table 2. Experimental design

Groups	
A. Control group (10 rats)	Rats fed on basal diet for 15 weeks.
B. HFD group (10 rats)	Rats fed on HFD for 14 weeks and approved to be affected with Mets
C-Treatment groups	
C_1. HFD-Lithium group (Li) (10 rats)	Rats administered LiCl (120 μg.kg^{-1} body weight, dissolved in NaCl 0.9%) through daily intra-peritoneal injection after HFD induction for 4 weeks (Delangre et al., 2023).
C_2. HFD-AKT Inhibitor group (10 rats)	Rats administered MK-2206 was given orally for 4 weeks on alternate days (120 mg/kg body weight) after HFD induction (Agarwal et al., 2014).
C_3.HFD-Li+AKT Inhibitor (Combination) group (10 rats)	Rats administered LiCl+AKT Inhibitor after HFD induction.

Table 1. Primer sequence used in the study

Gene	**Primer Sequence**	**Reference**
IR	Sense primer: 5′-GCC ATC CCG AAA GCC AAG ATC-3′ Anti-sense primer: 5′-TCT GGC TCC TGA TTG CAT-3′	González et al. (2003)
Akt	Sense primer: 5′-GGA AGC CTT CAG TTT GGA TCC CAA-3′ Anti-sense primer: 5′-AGT GGA AAT CCA GTT CCG AGC TTC-3′	Sharma et al. (2010)
GLUT 4	Sense primer: 5′-GGC CTG TGA GTG AGT GCT TTC-3′ Anti-sense primer: 5′-CAG CGA GGC AAG GCT AGA-3′	Liu et al. (2006)
β-actin	Sense primer: 5′-AAC TCC TTC ACC TTC CCA AAA G-3′ Anti-sense primer: 5′-AAG CAA TGC TGT CAC CTT CCC-3′	Peinnequin et al. (2004)

Table 2. Effect of Lithium, AKT Inhibitor, on chemical and inflammatory biomarker levels in HFD- induced rats.

Biomarker	**Control Group (Mean ± SD)**	**HFD Group (Mean ± SD)**	**HFD-Lithium Group (Mean ± SD)**	**HFD-AKT Inhibitor Group (Mean ± SD)**	**HFD-Li+AKT Inhibitor Group (Mean ± SD)**	**p-value**
Fasting Insulin	1.70 ± 0.10*	9.40 ± 0.04	1.50 ± 0.15*	1.90 ± 0.19*	2.40 ± 0.24*	0.016
Fasting Blood Glucose	5.00 ± 0.10*	22.50 ± 0.05	8.00 ± 0.16*	9.00 ± 0.18*	11.00 ± 0.22*	0.027
HOMA-IR	2.00 ± 0.10*	13.0 ± 0.03	3.40 ± 0.17*	3.20 ± 0.16*	4.60 ± 0.23*	0.009
ALT	35.00 ± 0.10*	82.50 ± 0.15	50.00 ± 0.20*	53.00 ± 0.18*	47.50 ± 0.25*	0.036
AST	30.00 ± 0.10*	99.00 ± 0.13	57.00 ± 0.19*	51.00 ± 0.17*	43.00 ± 0.21*	0.029
TG	150.00 ± 0.10*	345.00 ± 0.12	210.00 ± 0.18*	205.00 ± 0.15*	180.00 ± 0.23*	0.014
IL-1β	31.00 ± 0.10*	58.00 ± 0.20	30.00 ± 0.30*	25.00 ± 0.25*	35.00 ± 0.35*	0.034
TNF-α	38.00 ± 0.10*	62.50 ± 0.19	42.00 ± 0.28*	36.00 ± 0.24*	48.00 ± 0.32*	0.005

Table 3. Effect of Lithium, AKT Inhibitor, on oxidative stress biomarker levels in HFD- induced rats.

Biomarker	Control Group (Mean ± SD)	HFD Group (Mean ± SD)	HFD-Lithium Group (Mean ± SD)	HFD-AKT Inhibitor Group (Mean ± SD)	HFD-Li+AKT Inhibitor Group (Mean ± SD)	p-value
MDA	5.00 ± 0.10*	50.50 ± 0.21	15.50 ± 0.31*	13.50 ± 0.27*	19.00 ± 0.38*	0.015
SOD	100.00 ± 0.10*	60.00 ± 0.06	120.00 ± 0.12*	90.00 ± 0.09*	140.00 ± 0.14*	0.016
GPx	90.00 ± 0.10*	55.00 ± 0.07	126.00 ± 0.14*	90.00 ± 0.10*	135.00 ± 0.15*	0.044
CAT	80.00 ± 0.10*	64.00 ± 0.08	120.00 ± 0.15*	96.00 ± 0.12*	128.00 ± 0.16*	0.001

Table 4. Effects of Lithium, AKT Inhibitor, on IR, Akt, and GLUT 4 expression in HFD- induced rats.

Gene	Control Group (Mean ± SD)	HFD Group (Mean ± SD)	HFD-Lithium Group (Mean ± SD)	HFD-AKT Inhibitor Group (Mean ± SD)	HFD-Li+AKT Inhibitor Group (Mean ± SD)	p-value
IR	1.0 ± 0.1	0.40 ± 0.09	1.56 ± 0.09*	1.93 ± 0.1*	2.41 ± 0.12*	0.001
Akt	1.1 ± 0.08	0.15 ± 0.07	1.97 ± 0.07*	1.61 ± 0.08*	2.13 ± 0.11*	0.003
GLUT 4	1.0 ± 0.1	0.30 ± 0.06	1.74 ± 0.09*	1.59 ± 0.09*	2.54 ± 0.12*	0.001

The findings correspond with research by Jung et al. [32] and Lee et al. [22] which indicated that lithium augments insulin signaling by up regulating GLUT 4 expression and enhancing glucose absorption in skeletal muscle. AKT inhibitors are essential in controlling insulin sensitivity by modifying the PI3K/AKT signaling pathway, which is directly implicated in glucose homeostasis and lipid metabolism [2-7]. The effect of lithium in regulating metabolic pathways is well-documented. Rezk et al. [33] revealed that lithium chloride, in conjunction with medicines such as quercetin, augments protection against renal ischemia-reperfusion injury in mice subjected to a high-fructose, high-fat diet. This indicates lithium's capacity to safeguard against oxidative stress and metabolic abnormalities generated by food, aligning with our study's results that lithium markedly diminished oxidative stress markers and enhanced metabolic health. Mikosha et al. [34] emphasize lithium's extensive biological impacts, including its capacity to alter neurotransmitter signaling and provide protection against neurodegenerative disorders, which may also pertain to chronic metabolic diseases like type 2 diabetes.

The findings of our study correspond with those of Graniel-Amador et al. [35] who investigated lithium's efficacy in alleviating the adverse consequences of type 1 diabetes, namely its capacity to diminish bone loss and mechanical sensitivity. AKT inhibitors are progressively acknowledged for their function in regulating insulin signaling pathways. Yaribeygi et al. [36] examined the molecular mechanisms of insulin resistance, highlighting that alterations in the PI3K/AKT signaling pathway are pivotal in the onset of insulin resistance. Nitulescu et al. [37] and Hyman et al. [38] both emphasize the enduring interest in AKT inhibitors for oncological therapy, as the PI3K/AKT/mTOR pathway is frequently dysregulated. Moreover, Guo et al. [39] and Huck & Mochalkin [40] observed substantial advancements in the formulation of ATP-competitive AKT inhibitors for clinical use, especially for disorders characterized by the overactivation of the AKT pathway. Cao et al. [41] investigated the efficacy of dual AKT and ERK inhibitors, highlighting the benefit of simultaneously targeting several pathways for enhanced therapeutic results.

4. Conclusion

In conclusion, our results indicate that both lithium and AKT inhibitors has considerable potential in alleviating the metabolic, inflammatory, and oxidative stress linked to HFD-induced insulin resistance. The combined therapy offered the most extensive antioxidant protection. These findings align with other research that emphasizes the therapeutic potential of modulating insulin signaling and oxidative stress in obesity-related disorders. Future investigations should examine the prolonged impacts of these therapies and their prospective uses in the management of metabolic disorders, including type 2 diabetes and non-alcoholic fatty liver disease (NAFLD).

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