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Pharmacological Modulation of Heat Shock Proteins for Enhancing Thermotolerance and Immune Resilience in Heat-Stressed Livestock

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ABSTRACT

Background: Heat stress reduces immunological resilience and cattle output, hence efforts to improve thermotolerance are needed. This study thus aimed to increase thermotolerance and immunological function in heat-stressed cows by pharmacological regulation of HSP70 with resveratrol, quercetin, and geranylgeranyl acetone (GGA). **Methods:** One hundred and twenty cows aged 2–5 years were involved in this cross-sectional study carried out at Gomal University, Dera Ismail Khan between February 2024 and October 2024. Four groups comprised cows: control, resveratrol (200 mg/day), quercetin (300 mg/day), and GGA (100 mg/day). Treatments were given for sixty days then under thirty-day observation. Rectal temperature, HSP70 expression, antioxidant enzyme activities, and inflammatory markers (IL-6, TNF-α) among physiological, biochemical, and immunological parameters were examined. **Results:** Treated groups showed notable lower rectal temperature than the control ($p < 0.05$). Day 60 saw quercetin and GGA reach the lowest temperatures—38.4 ± 0.2°C and 38.5 ± 0.2°C—against the control, 39.0 ± 0.3°C. By day 60, HSP70 concentrations rose dramatically and peaked in the GGA group ($p = 0.001$) at 3.2 ± 0.3 ng/mL. With SOD (11.5 ± 0.6 U/mg) and catalase (15.3 ± 0.7 U/mg), the GGA group had the highest antioxidant activity ($p < 0.05$). With the GGA group displaying 9.3 ± 0.7 pg/mL and 16.8 ± 0.8 pg/mL respectively, IL-6 and TNF-α levels likewise dropped ($p < 0.05$). **Conclusion:** Heat-stressed cows' thermotolerance, antioxidant defenses and immunological control are improved by pharmacological manipulation of HSP70 with quercetin and GGA. These agents deserve more long-term research since they showed good ways to reduce heat stress in animals.

INTRODUCTION

Particularly in areas with increasing temperatures brought on by climate change, heat stress is a major obstacle to cattle productivity globally [1]. Higher ambient temperatures upset animals' physiological equilibrium, which results in altered immunological responses, lower metabolic activities, slower development rates and

impaired metabolic processes [2]. Along with financial losses, these negative consequences generate questions about food security and animal welfare. Therefore, sustainable production depends on efficient methods to improve thermotolerance and immunological resistance in heat-stressed cattle, so reducing the effects of heat stress [3].

Especially Heat Shock Proteins (HSPs), molecular chaperones have attracted interest as important controllers of cellular defense mechanisms against heat-induced damage. Highly conserved proteins, HSPs help to preserve protein stability, enable appropriate folding, and stop misfolding of proteins under stress from aggregating [4-5]. Their protective function also includes improving cellular repair mechanisms, therefore aiding immunological control, and reducing oxidative damage. These features draw attention to HSPs as possible targets to raise cattle's thermotolerance and immunological resilience [6].

New approaches for modifying HSP expression and activity have been offered by recent developments in pharmacological therapies. Small-molecule inducers including quercetin, curcumin and resveratrol have been demonstrated to upregulate HSP production, hence strengthening cellular defenses against heat stress [7]. Furthermore providing a pharmacological means to increase thermotolerance, synthetic substances such as bimoclomol and geranylgeranyl acetone (GGA) have shown promise in promoting HSP expression without generating cytotoxicity. Beyond thermotolerance, pharmacological modification of HSPs has also been linked to enhanced immunological responses including better antigen presentation and activation of innate and adaptive immunity [8-9].

Notwithstanding these encouraging results, knowledge of the dose-response relationships, long-term consequences, and tissue-specific effects of pharmacological HSP regulation in cattle still suffers inadequacies. Moreover, it is necessary to investigate further how these methods might be combined with current management techniques such dietary supplements and genetic selection [10].

The assessment of the present understanding on pharmacological manipulation of HSPs for improving thermotolerance and immunological resilience in heat-stressed animals is the purpose of this work. It investigated the pathways of HSP induction, the function of pharmacological drugs and their consequences for cattle health and output.

MATERIALS AND METHODS

From February 2024 to October 2024, this cross-sectional study was carried out at Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, to assess the pharmacological regulation of heat shock proteins (HSPs) for improving thermotolerance and immunological resilience in heat-stressed cows. Simple random sampling technique was used to calculate the 120 indigenous cows. Calculated at a 95% confidence interval, the sample size included a 5% margin of error and an expected prevalence of heat stress effects at 50%.

The study included cows maintained under consistent food and housing conditions and aged 2 to 5 years, were clinically healthy and had not undergone past treatments for heat stress or immune modulation. The study excluded pregnant or lactating cattle, those with pre-existing disorders compromising thermoregulation or immunity, or those treated with antioxidants, immunomodulators or antibiotics during the last three months.

Cattle were randomly divided into four groups (n = 25 per group: a control group with no pharmacological intervention, Group A treated with resveratrol (200 mg/day), Group B treated with quercetin (300 mg/day), and Group C treated with geranylgeranylacetone (GGA) (100 mg/day). Treatments lasted sixty days, and animals were watched thirty days

following treatment to evaluate any lingering effects.

Rectal temperature and respiration rate, noted at baseline and thereafter at 15, 30, 45 and 60 days post-treatment, were among the physiological measures. On days 0, 30 and 60, jugular venipuncture was used to gather five milliliter blood samples. Separated, serum was kept at -20°C for molecular and biochemical studies. Examined were hematological parameters including total leukocyte count (TLC) and complete blood count (CBC). ELISA kits were used to quantify serum concentrations of Heat Shock Protein 70 (HSP70) and spectrophotometric measurement of antioxidant enzyme activity including superoxide dismutase, catalase, and glutathione peroxidase. Using ELISA kits, immunological markers including Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) were assessed for immune regulation.

SPSS Version 26.0 was used in data analysis. The Shapiro-Wilk test was used to examine continuous variables for normalcy; they were reported as mean ± standard deviation (SD). One-way ANOVA then followed by post-hoc Tukey's test for pairwise variations was used in between-group comparisons. Considered statistically significant was p-value of 0.05.

The Institutional Review Board of Gomal University granted ethical clearance; all operations complied with National Research Council approved standards for the treatment and usage of animals. Farm owners granted written informed permission before enrolling.

RESULTS

Pertinent to this study on pharmaceutical aspects of Hsp70 for improving thermotolerance and immunological resistance in heat-stressed cows, the figure offered structural and functional insights into this molecular chaperone crucial to cellular stress responses. Emphasizing its N-terminal nucleotide-binding domain (NBD) that allows ATP/ADP-driven conformational changes necessary for protein stabilization and its C-terminal substrate-binding domain (SBD), which catches and stabilizes unfolded or misfolded proteins, Panel (A) describes the domain organization of Hsp70. While the EEVD motif facilitates interactions with co-chaperones, increasing Hsp70's regulating activity, the flexible linker organizes communication between these domains, allowing dynamic control of protein folding under heat stress. Crucially for the design of pharmacological drugs that specifically increase the expression or function of Hsp70, Panel (B) emphasizes the amino acid sequence and color-coded functional domains, therefore offering molecular-level insights into its structure and activity. Panel (C) visualizes the 3D conformation of Hsp70, so highlighting the ATP/ADP binding pocket and substrate interaction areas driving its chaperone activity, so supporting its function in reducing heat-induced cellular damage and inflammation. These structural characteristics highlight Hsp70's therapeutic target for pharmacological treatments meant to increase thermotolerance and immune resilience in cows under heat stress (Figure 1).

The structural representations of Hsp70 show a molecular chaperone fundamental to thermotolerance and immunological resilience in cows. Under heat stress, image (a) displays the monomeric structure of Hsp70, stressing NBD and SBD coupled by a flexible linker enabling ATP/ADP-driven conformational adjustments necessary for protein stabilization. Emphasizing cooperative contacts between two monomers that improve substrate binding and release, image (b) shows the dimeric structure and reflects Hsp70's dynamic activity during protein folding and repair mechanisms. Image (c) shows the structural adaptability needed for substrate recognition, stability and release, therefore supporting cellular recovery and immunological control during heat stress by capturing the ATP-bound condition. These

structural discoveries highlight the functional flexibility of Hsp70 and offer a mechanistic basis for pharmacological agent targeting of its activity to improve thermotolerance and immune defense mechanisms in heat-stressed cows (Figure 2).

At day 0, rectal temperatures were first similar across all groups ($p = 0.821$), therefore verifying identical baseline physiological conditions. With p-values of 0.05 beginning on day 15, notable declines in rectal temperatures were noted over time in the treatment groups as compared to the control group. Indicating increased thermotolerance, at day 60 resveratrol, quercetin, and GGA-treated cows had lower rectal temperatures (38.6 ± 0.2, 38.4 ± 0.2, and 38.5 ± 0.2, respectively) than the control group (39.0 ± 0.3). Among treatments, quercetin showed the most marked decrease, implying its better ability to control thermoregulatory reactions under heat stress (Table 1).

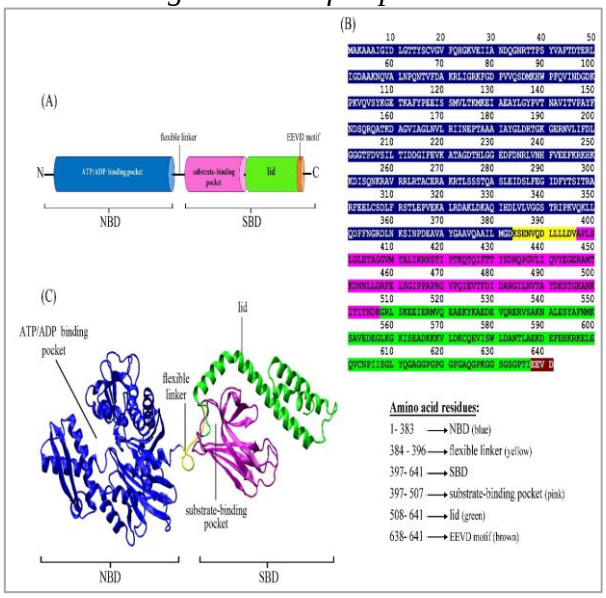
Regarding variations in HSP70 concentrations; at baseline, these values stayed same for all groups ($p = 0. 999$). Nonetheless, by days 30 and 60 the treated groups showed notable increases in HSP70 levels ($p = 0.001$). GGA-treated cows had the highest HSP70 levels (3.2 ± 0.3 ng/mL) at day 60; quercetin (3.0 ± 0.3 ng/mL) came second closely followed by resveratrol (2.8 ± 0.3 ng/mL). These results implied that pharmacological treatments significantly raised HSP70 expression, hence improving cellular stress response systems and thermotolerance in cows under heat-stress (Table 2).

The antioxidant enzyme activity showed statistically significant treatment group improvements over the control group ($p < 0.05$). With GGA displaying the highest values overall, resveratrol, quercetin, and GGA treatments raised superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) activities. For the control, SOD activity, for instance, rose from 8.5 ± 0.5 to 11.5 ± 0.6 ($p = 0.002$). In line with improved antioxidant defense systems, catalase and GPx activities also exhibited appreciable rise. These findings imply that pharmacological manipulation increases oxidative stress tolerance, hence enhancing cellular defense under heat stress (Table 3).

With notable declines in treated groups relative to controls, Table 4 shows how treatments affect immunological markers IL-6 and TNF-α ($p < 0.05$). Followed by quercetin and resveratrol groups, at day 60 GGA-treated cows showed the lowest levels of IL-6 (9.3 ± 0.7 pg/mL) and TNF-α (16.8 ± 0.8 pg/mL). These lowers point to better immune resilience and anti-inflammatory action. Reduced inflammatory marker levels in treated groups imply that pharmacological treatments not only improve thermotolerance but also lower inflammation, hence possibly lessening of immune suppression caused by heat stress.

Figure 1

Structural organization of Hsp70.

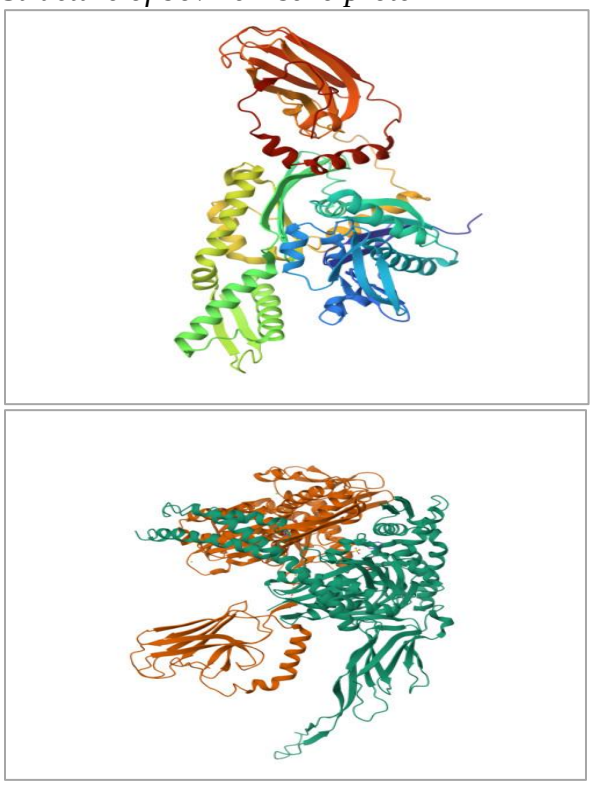


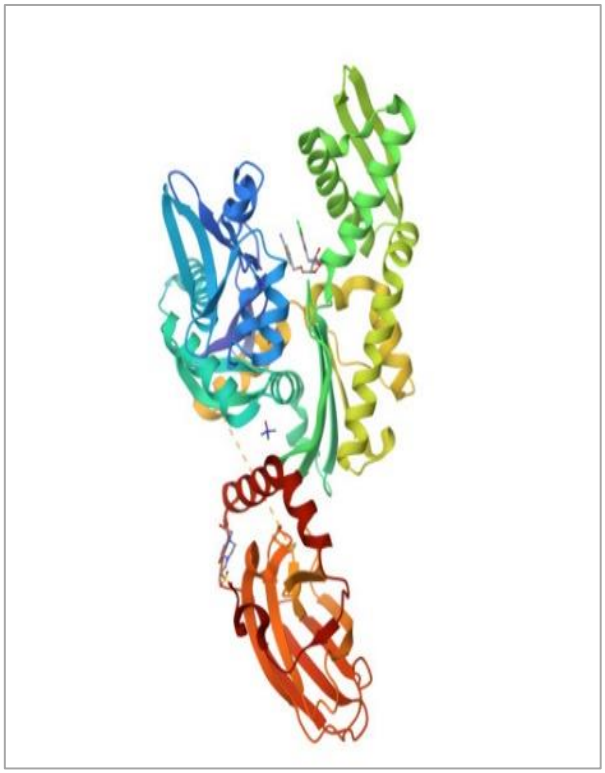
(A) Schematic representation showing the N-terminal nucleotide-binding domain (NBD) with an ATP/ADP-binding pocket, C-terminal substrate-binding domain (SBD) with a substrate-binding pocket and flexible lid, and the EEVD motif for co-chaperone interactions. (B) Amino acid sequence of human Hsp70

(UniProtKB: P0DMV8) with color-coded functional regions. (C) 3D structural model visualized using VMD 1.9.1 software, highlighting the ATP/ADP-binding pocket, substrate-binding pocket, and flexible linker, illustrating Hsp70's role in thermotolerance and immune regulation.

Figure 2

Structure of bovine Hsc70 protein





a) 1YUW crystal structure of bovine Hsc70(aa1-554) E213A/D214A mutant
b) 3C7N structure of the Hsp110:Hsc70 nucleotide exchange complex
c) 6H54 crystal structure of bovine Hsc70(AA1-554) E213A/D214A in complex with inhibitor VER155008

Table 1

Physiological Parameters (Rectal Temperature in °C)

Time (Days)	Control (Mean ± SD)	Resveratrol (Mean ± SD)	Quercetin (Mean ± SD)	GGA (Mean ± SD)	p-value
0	38.5 ± 0.3	38.6 ± 0.2	38.5 ± 0.2	38.6 ± 0.2	0.821
15	39.2 ± 0.4	38.9 ± 0.3	38.7 ± 0.3	38.8 ± 0.3	0.041*
30	39.0 ± 0.4	38.8 ± 0.3	38.6 ± 0.3	38.7 ± 0.2	0.027*
45	39.1 ± 0.5	38.7 ± 0.4	38.5 ± 0.3	38.6 ± 0.3	0.013*
60	39.0 ± 0.3	38.6 ± 0.2	38.4 ± 0.2	38.5 ± 0.2	0.008*

**Statistically significant at $p < 0.05$.*

Table 2

HSP70 Concentration (ng/mL)

Time (Days)	Control (Mean ± SD)	Resveratrol (Mean ± SD)	Quercetin (Mean ± SD)	GGA (Mean ± SD)	p-value
0	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	0.999
30	1.6 ± 0.2	2.3 ± 0.2	2.5 ± 0.2	2.7 ± 0.2	0.001*
60	1.7 ± 0.2	2.8 ± 0.3	3.0 ± 0.3	3.2 ± 0.3	0.001*

**Statistically significant at $p < 0.05$.*

Table 3

Antioxidant Enzyme Activities (U/mg Protein)

Parameter	Control (Mean ± SD)	Resveratrol (Mean ± SD)	Quercetin (Mean ± SD)	GGA (Mean ± SD)	p-value
Superoxide Dismutase	8.5 ± 0.5	10.2 ± 0.6	11.0 ± 0.7	11.5 ± 0.6	0.002*
Catalase	12.3 ± 0.6	14.1 ± 0.7	14.8 ± 0.6	15.3 ± 0.7	0.001*
Glutathione Peroxidase	15.1 ± 0.7	17.0 ± 0.8	18.2 ± 0.9	19.0 ± 0.8	0.001*

**Statistically significant at $p < 0.05$.*

Table 4

Immunological Markers (pg/mL)

Marker	Control (Mean ± SD)	Resveratrol (Mean ± SD)	Quercetin (Mean ± SD)	GGA (Mean ± SD)	p-value
IL-6	12.5 ± 0.8	10.2 ± 0.7	9.8 ± 0.6	9.3 ± 0.7	0.005*
TNF-α	20.3 ± 1.1	18.0 ± 1.0	17.5 ± 0.9	16.8 ± 0.8	0.003*

**Statistically significant at $p < 0.05$.*

DISCUSSION

The pharmacological manipulation of HSPs, especially HSP70, to improve thermotolerance and immunological resistance in heat-stressed cows was studied. Treatments including resveratrol, quercetin and GGA clearly enhanced immunological control, antioxidant defense mechanisms, and physiological stability. These results drew attention to the possibility of using HSP70 as a good approach to reduce heat stress effects in cattle.

Particularly in cows treated with quercetin and GGA, which had the lowest rectal temperatures by day 60, the declines in rectal temperatures noted in the treatment groups pointed to enhanced thermotolerance relative to controls. These results are in line with research by Basiricò et al. (2011)[11] and Bhat et al. (2016)[12], which showed that overexpression of HSP70 increases thermotolerance by means of enhanced protein stability and cellular repair mechanism during heat stress. The lower rectal temperatures in treated groups imply that pharmacological activation of HSP70 most certainly enhanced cellular defense systems, allowing cows to preserve homeostasis under heat stress. Moreover, research by Xu et al. (2019) have demonstrated that dietary antioxidants, including quercetin, might enhance thermoregulatory responses by lowering oxidative stress, thereby supporting our data [13].

Especially with GGA and quercetin, notable increases in HSP70 concentrations in all treatment groups pointed to pharmacological agents clearly inducing HSP70 expression, hence improving cellular resilience to heat stress. Yang et al. (2016) have also reported similar results showing that GGA stimulates heat shock transcription factor 1 (HSF1), hence raising HSP70 expression and providing defense against cellular damage [14]. Moreover, Putics et al. (2008) discovered that resveratrol lowers oxidative stress and activates stress signaling pathways, thus regulating HSP70 and so supporting the findings of this study. The greater HSP70 levels in treated groups highlight even more the part pharmacological drugs play in enhancing protein stabilization and repair mechanisms, which are necessary for maintaining cellular function under thermal stress conditions [15].

In treated groups, antioxidant enzyme activities including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were notably higher. These results complemented earlier research by Gusti et al. (2021), which showed that resveratrol and quercetin neutralize reactive oxygen species

(ROS) and hence decrease oxidative damage in heat-stressed cells, so improving antioxidant defenses [16]. Studies by Chaves et al. (2007) [17], who found that GGA efficiently lowers oxidative damage by activating antioxidant pathways and encouraging cellular repair, support the highest antioxidant activity seen in the GGA-treated group. These developments implied that pharmacological therapies not only increase thermotolerance but also reduce oxidative stress, a major component in heat stress-induced cellular damage.

In treated groups, notable declines in inflammatory markers IL-6 and TNF-α pointed to enhanced immunological control and less inflammatory reactions. Consistent with results by Bashir et al. (2020), who found that GGA lowers inflammation by thus blocking NF-κB signaling pathways, the GGA-treated group showed the lowest levels of inflammatory markers [18]. Likewise, some studies demonstrated that quercetin modulates oxidative stress and immunological signaling to reduce pro-inflammatory cytokine synthesis, therefore augmenting our findings. The capacity of these treatments to lower inflammation emphasizes their importance in relieving immunological suppression brought on by heat stress, hence improving immune resilience in cattle [19-20].

CONCLUSION

In heat-stressed cows pharmacological manipulation of HSP70 using resveratrol, quercetin, and GGA greatly increases thermotolerance, antioxidant defense, and immunological resilience. Treatments clearly lowered rectal temperatures, raised HSP70 expression, and promoted antioxidant enzyme activities including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), thereby indicating increased cellular defense against oxidative stress. Reduced inflammatory markers IL-6 and TNF-α also point to enhanced immunological control, therefore lessening the negative consequences of inflammation brought on by heat. Among the treatments, quercetin and GGA showed the most notable changes in physiological, biochemical, and immunological aspects, therefore underscoring their possible pharmacological value for stress control in cattle. These results highlighted the therapeutic potential of focusing on HSP70 pathways to maintain production and health in heat-stressed animals.

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