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Integrative Transcriptomic and Network Based Framework for Designing Heat-Stress and Protective Topical Therapies in Mammals

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ABSTRACT

Anthropogenic climate change and the urban heat island effect have significantly increased the risk of heat stress in urban mammals. Existing cooling strategies for animals primarily focus on physiological management and often overlook molecular-level mitigation of heat-induced cellular damage. Therefore, this study aimed to employ bioinformatics and computational chemistry approaches to identify candidate bioactive compounds for heat stress mitigation in mammals.

A heat stress-related *Bos taurus* gene expression dataset was analyzed using differential expression analysis; a total of 6273 statistically significant DEGs were identified, of which 1202 were highly differentially expressed ($|\log_2 FC| > 5$). Protein–protein interaction network analysis and functional pathway enrichment analysis were subsequently performed to investigate functional relationships among these genes and associated biological pathways, leading to the identification of nine key DEGs. Several of these genes were also associated with heat stress pathways across multiple mammalian species, demonstrating the broader relevance of the findings.

Molecular docking analysis was conducted to evaluate the interaction potential of selected antioxidative and anti-inflammatory bioactive compounds with proteins corresponding to the top DEGs, UBA52 and RPS9, as well as HSP90, a well-established heat shock protein. Among the tested compounds, epigallocatechin gallate and quercetin demonstrated the strongest binding interactions, leading to the proposal of a formulation incorporating these compounds.

Overall, this study highlights the potential of integrating bioinformatics and computational approaches to identify candidate compounds for mitigating heat-induced cellular stress in urban animals. Further experimental validation is required to confirm these findings.

Keywords:

Heat stress, Transcriptomics, Differential gene expression, Network biology, Topical formulations.

INTRODUCTION

Anthropogenic climate change is a One Health crisis that threatens both animal and human health.[1] This issue is amplified in urban areas due to the urban heat island (UHI) effect, a phenomenon in which cities are warmer than nearby rural areas. Major contributing factors to this phenomenon include the properties of urban infrastructure and reduced vegetation and open space.[3] The UHI effect increases the risk for heat strain and stroke in humans as well as livestock and companion animals.[4] As in humans, mammal heat stroke occurs when an individual's heat dissipation mechanisms are overwhelmed, characterised by core body temperatures above 41°C.[2,5] Mammal heat dissipation methods include evaporative heat loss via panting, as well as non-evaporative mechanisms such as radiation, convection, and conduction from body surfaces.[5] Contributing factors to heat stress include high ambient temperatures, relative humidity, and solar radiation and low wind movement, making it a major issue in Indian cities where these conditions are prevalent.[6] The mortality rate of mammals, specifically canine heat stroke is around 50%.[5] Thus, cooling methods to ameliorate heat dissipation are important to promote their health.

Quantitative research has been conducted on the heat stress in mammals. A recent paper used historical data to calculate mortality rates and identified factors that compound the risk of mortality, such as coat, body size, and breed.[7] Another work collected environmental and dog temperature data across indoor and outdoor rural and urban dogs. They found that traditional measures of heat risk, like heat indices that use weather data, tend to underestimate the risk of heat stress in dogs. They also found that urban outdoor dogs experienced higher peak temperatures and that heat stress was prevalent even at night, consistent with the urban heat island phenomenon.[4]

Research has also been conducted on measuring the biological markers of heat stress. A paper studying the effect of heat stress on hematobiochemical parameters of healthy animals found significant increases in rectal temperature, making it an effective proxy for core body temperatures, as well as changes in blood and elevated cortisol and liver enzyme levels.[6] Another work used infrared thermography to measure surface temperatures of canines of different coat types. Short-coated dogs had higher mean temperatures, indicating an insulating effect of fur. Surface temperature was correlated to ambient temperatures but not rectal temperature (a proxy for core body temperature).[8] A recent study used large-scale veterinary data to revise the VetCompass clinical grading tool for heat-related illnesses in dogs and categorise the extent of heat stress based on mortality rates.[9]

Some research has been conducted on cooling techniques. A study compared passive cooling, partial water immersion, and the application of evaporative isopropyl alcohol on canine paw pads to treat exercise-induced hyperthermia in working dogs. They found passive cooling to be the least effective and partial water immersion to be the most effective among these methods. Isopropyl alcohol was associated with increased heart rates due to irritation as well as injury and flammability risks. However, they noted uncertainty about the extent of water immersion and the temperature of the water, as well as a lack of portability compared to alcohol.[10]

Extensive literature exists on heat stress in cattle. A paper measured skin and respiration heat loss in Holstein dairy cows at different temperatures and humidity levels. They found that, as ambient temperatures rise, the cows rely more on evaporative cooling (respiration and sweating) than conduction, convection, and radiation through skin.[11] Other studies on Brangus and Holstein cattle identified differentially expressed genes and pathways between heat-stressed and non-stressed individuals.[12,13] Blood vessel density was found to be an important factor in thermoregulation.[12]

During the literature review, certain research gaps were identified. Firstly, while extensive research has been conducted on cooling solutions in humans and cattle, limited work exists for the cooling of dogs: no effective solutions, other than water-based ones, have been identified. Moreover, research specifically on cooling in animals is limited; literature has been focused on classifying heat stress or correlating it to mortality rather than minimising it. Thus, it was hypothesised that novel cooling solutions, perhaps through topical formulations, can be discovered using canine physiology and chemical databases.

METHODOLOGY

Data collection

A range of different databases were explored to identify relevant sources of data for identifying heat stress and evaluating potential cooling interventions. Veterinary datasets such as VetCompass provide large scale clinical records but are not open access. Thermophysical and materials databases such as Thermtest, NIST, and slPCMlib were explored for data on thermal properties, and chemical databases including PubChem, ChemSpider, and CompTox were reviewed for information on compound properties and safety. PhDat was also considered for phase behaviour. In parallel, biological databases including NHGRI, NCBI, DoGSD, QTL, the European Variation Archive (EVA), and the Dog Aging Project were investigated for genomic and physiological data relating to dogs and cattle. Ultimately, NCBI's Gene Expression Omnibus (GEO) database[1] was selected as a primary data source as it would allow the identification of differentially expressed genes responsible for heat stress in cattle as a proxy mammalian model, with limitations for direct canine application due to the absence of suitable publicly available canine heat-stress transcriptomic datasets.

Differential expression (DE) analysis

The first dataset selected has been deposited in GEO and is accessible through GEO Series accession number GSE244620. It comprises RNA-sequencing data from skin tissue of *Bos taurus* (Brangus cattle), generated to investigate molecular responses to heat stress.[2] Useful datasets on differential gene expression under heat stress in *Canis familiaris* (dog) were not found. The downloaded gene expression matrix was loaded into a Pandas DataFrame and processed using Python (Anaconda environment). Gene expression values were indexed by gene identifiers, and samples were divided into two groups: heat-stressed and control. $\log_2(x+1)$ normalisation was applied to reduce the influence of extreme values. For every gene, a Welch's t-test was performed to see if the mean expression significantly differs between the stress and control groups. The $\log_2$ fold change ($\log_2$FC) was found by calculating the magnitude of change by subtracting the mean of the control group from that of the test group. The False Discovery Rate

(FDR) adjusted p-values were also found to test the statistical significance of results. The results were visualised using a volcano plot.

Network and pathway analysis

Protein–protein interaction (PPI) network analysis was performed to investigate functional relationships among the identified DEGs. Gene symbols corresponding to the DEGs were submitted to the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database, selecting *Bos taurus* as the reference organism. The high-confidence interaction threshold was applied to retain only strongly supported protein–protein interactions. The resulting interaction network table was exported. Using Cytoscape, the network was analysed.[3] Functional pathway enrichment analysis was subsequently performed using the Reactome database.[4] The list of DEGs was submitted to the Reactome pathway analysis database, and biological pathways were identified and ranked based on statistical significance. The results were exported. To integrate the results from both network and functional analysis, the top genes identified from the network analysis were cross-referenced against the top pathways from functional analysis.

Molecular docking

Molecular docking analysis was performed to evaluate the potential interactions between the top proteins (receptors) identified in the network and pathway analysis and bioactive compounds (ligands) identified in the literature based on antioxidant, anti-inflammatory, cytoprotective, skin-protective, soothing, and cooling properties. Three dimensional protein structures were obtained for the top proteins from the network and pathway analysis, as well as a heat shock protein identified in literature, from the RCSB Protein Data Bank (RCSB PDB) and AlphaFold [5,6,7]. Ligand structures were obtained from PubChem in structured data file (sdf) format. Docking simulations were performed using AutoDock Vina through the Tamarind Bio platform, with grid boxes defined around the target proteins using the x, y, and z coordinates of their centres as well as their height, width, and length dimensions.[8] For each receptor-ligand pair, the binding affinity, i.e. the strength of the interaction, was found. The docking of the top pairs were visualised using PyMOL.

Formulation design

To identify safe and effective ingredients for reducing heat stress in mammals, a literature-informed screening approach was carried out to evaluate the suitability of candidate compounds for topical use. Compounds were assessed based on their antioxidant and anti-inflammatory properties, ability to support skin barrier function, and reported use or compatibility with topical formulations. Based on these findings, three prototype formulations representing antioxidant-focused, anti-inflammatory/repair-focused, and cooling-support strategies were designed for subsequent testing using synthetic skin and fur models. Concentration ranges were selected conservatively based on concentration ranges reported in the literature for topical formulations to balance predicted efficacy with reduced risk of irritation or toxicity.

RESULTS

Differential expression analysis

For carrying out the DE analysis of the chosen study, 5 samples were placed in the test and 5 in the control groups. The data was divided into test groups and control groups on the basis of rectal temperatures; those above 39.2°C were used to classify cattle as heat-stressed.[9] A total of 7052 differentially expressed genes (DEGs) were identified as a result of the initial analysis. For further screening, genes were considered significantly differentially expressed if they satisfied the conditions FDR-adjusted p-value < 0.05 and $|\log_2 FC| > 1.0$. Upregulated genes have $\log_2 FC > 1$ while downregulated genes have $\log_2 FC < -1$. The DEGs identified are shown in Figure 1. This filtering resulted in 6273 statistically significant DEGs, out of which 6221 were upregulated and 52 were downregulated. Due to the large number of genes identified, a stricter filtering was carried out. The subset of genes with $|\log_2 FC| > 5.0$ was extracted to identify highly differentially expressed candidates. 1202 DEGs were thus found, consisting of 1201 upregulated genes and 1 downregulated gene The sequential filtering of DEGs identified during the differential expression analysis is summarised in Table 1. It is observed that upregulated genes significantly outnumber the downregulated ones in heat-stressed cattle. All further analysis was conducted on these DEGs.

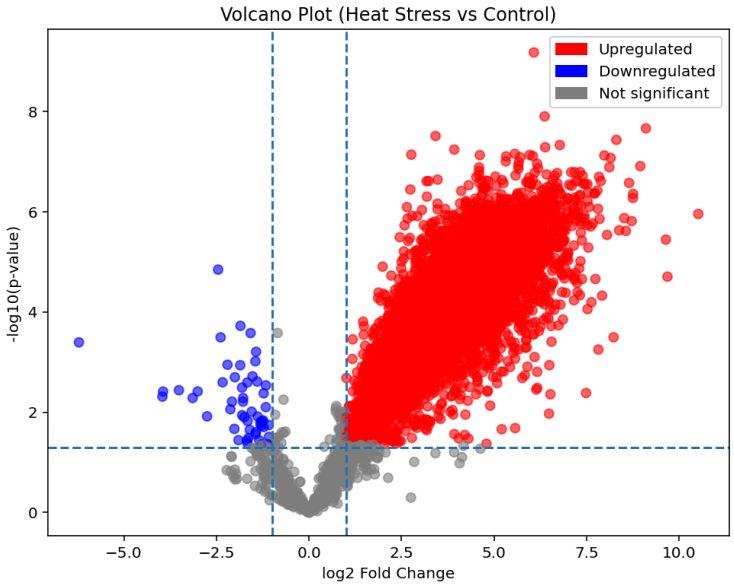


Figure 1. A volcano plot for DEGs in the GSE244620 dataset in heat-stressed versus control *Bos taurus* skin tissue. Each point represents a gene, plotted by $\log_2$ fold change (heat stress vs control) on the x-axis and $-\log_{10}$(p-value) on the y-axis. Genes meeting the significance criteria (adjusted p-value < 0.05 and $|\log_2 FC| > 1$) are highlighted, with upregulated genes shown in red and downregulated genes in blue, while non-significant genes are shown in grey. Vertical dashed lines indicate the fold change thresholds ($\log_2 FC = \pm 1$), and the horizontal dashed line represents the statistical significance threshold (p = 0.05). The plot demonstrates a predominance of upregulated genes under heat stress conditions.

Table 1. Sequential filtering of differentially expressed genes

Serial Number	Filtering stage	Criteria applied	Number of DEGs
1	Initial DE analysis	Welch's t-test performed on all genes	7052
2	Statistically significant DEGs	FDR-adjusted p-value < 0.05 and $\|\log_2 FC\| > 1.0$	6273
3	Highly differentially expressed genes	$\|\log_2 FC\| > 5.0$	1202

Network analysis

On the STRING database, only interactions with confidence > 0.700 were selected. The network of interactions is shown in Figure 2. For each of the 833 genes (nodes) identified on Cytoscape, key topological parameters were computed, including degree centrality, betweenness centrality, closeness centrality, and clustering coefficient. Degree centrality represents the number of direct connections (edges) a node has with other nodes in the network and was used to identify genes with extensive interactions. Betweenness centrality refers to the number of shortest paths between pairs of nodes that pass through a given node, indicating its role in communication between different regions of the network. Closeness centrality measures the inverse of the average shortest path length from a node to all other nodes, reflecting how efficiently a gene can interact with or influence the network. The clustering coefficient describes the extent to which a node's neighbors are interconnected; a high value indicates that a gene is part of a tightly-connected local module, which may correspond to a functional group of proteins involved in the same biological pathway. The top 10 genes with the highest degree centrality values were selected for further analysis, as they are likely to play central roles in the heat stress response network. These genes and their parameters are shown in Table 2.

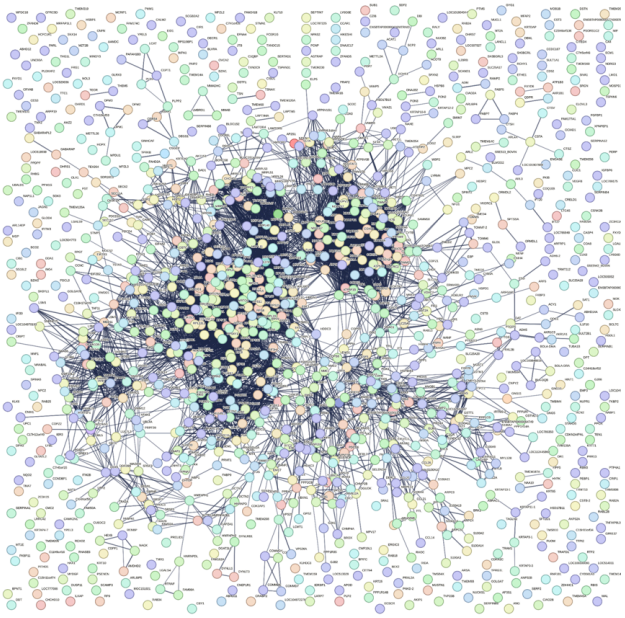


Figure 2. A gene-interaction network of DEGs under heat stress in *Bos taurus*. The network, produced using the STRING database and data from the GSE244620 dataset, comprises 833 nodes. Nodes represent proteins encoded by DEGs, while edges represent predicted protein-protein interactions with confidence > 0.700.

Table 2. Top 10 DEGs under heat stress in *Bos taurus*, identified from PPI network analysis based on the topological parameters.

Serial Number	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree Centrality	Gene Name
1	0.1073442093	0.3692307692	0.3767241379	145	UBA52
2	0.06172666775	0.3876829884	0.4818082789	136	RPS9
3	0.01929417086	0.3631205674	0.5152616279	129	RPL23
4	0.01360389967	0.3626062323	0.5332185039	128	RPL11
5	0.04192011898	0.351326624	0.4562580645	125	RPS27A
6	0.03569839226	0.3828514457	0.5720092128	122	RPS18
7	0.006454945505	0.3563805104	0.6152923538	116	RPS11
8	0.01531691149	0.3545706371	0.5823629871	114	MRTO4

Serial Number	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree Centrality	Gene Name
9	0.01040013187	0.3526170799	0.6117062568	114	RPS8
10	0.003432227903	0.3447037702	0.6256792424	114	RPS24

Pathway analysis

The functional pathway enrichment analysis revealed that these genes were related to a number of pathways in *Bos taurus*. 4 of these were found to be statistically significant ($p<0.05$): GTP hydrolysis and joining of the 60S ribosomal subunit (R-BTA-72706), formation of a pool of free 40S subunits (R-BTA-72689), SRP-dependent cotranslational protein targeting to membrane (R-BTA-1799339), and ZNF598 and the Ribosome-associated Quality Trigger (RQT) complex dissociate a ribosome stalled on a no-go mRNA (R-BTA-9954716). Cross-referencing between pathway-associated genes and network-derived hub genes was performed computationally using spreadsheet-based filtering methods. Hub genes identified through PPI network analysis were cross-referenced with pathway-associated genes using exact gene-symbol matching in a reproducible spreadsheet-based workflow. As a result, the significant genes found in *Bos taurus* were UBA52, RPS9, RPL23, RPL11, RPS27A, RPS18, RPS11, RPS8, and RPS24.

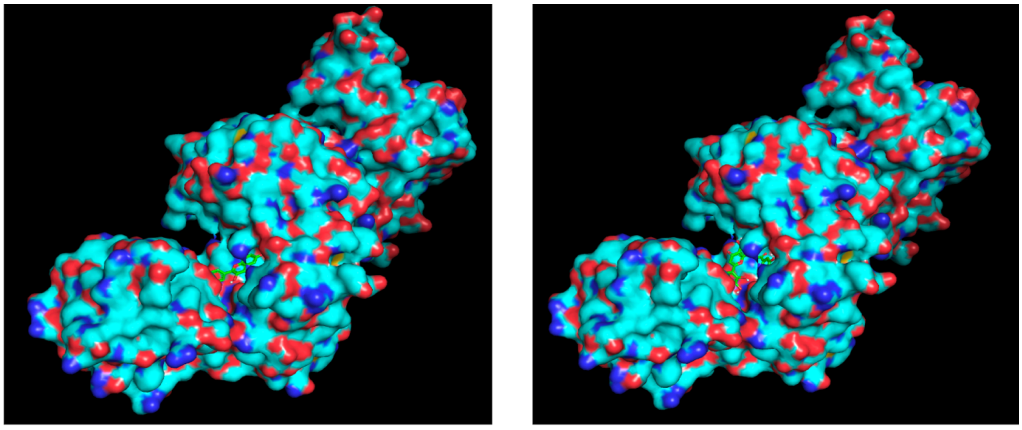
Molecular docking

Molecular docking analysis was performed to evaluate the interaction potential of selected bioactive compounds with proteins associated with heat stress response. Molecular docking is a computational technique used to estimate the binding orientation and interaction strength between a ligand and a receptor. The 7 ligands identified from literature were Quercetin, Curcumin, Epigallocatechin Gallate (EGCG), Ferulic acid, Niacinamide, Panthenol, and Resveratrol. They were docked against the target proteins, UBA52, RPS9, and HSP90. The binding affinities for all the protein-ligand pairs are shown in Table 3. More negative binding affinity values indicate stronger predicted ligand–protein interactions and greater thermodynamic stability of the docked complex. EGCG and quercetin consistently exhibited the strongest binding affinities across all three target proteins. EGCG was selected as it is known for suppressing inflammatory cascades, while quercetin was chosen for its antioxidant and anti-inflammatory properties. The docking visualised for these ligands using PyMOL is shown in Figures 3–5.

Table 3. Predicted binding affinities of selected ligands against target proteins.

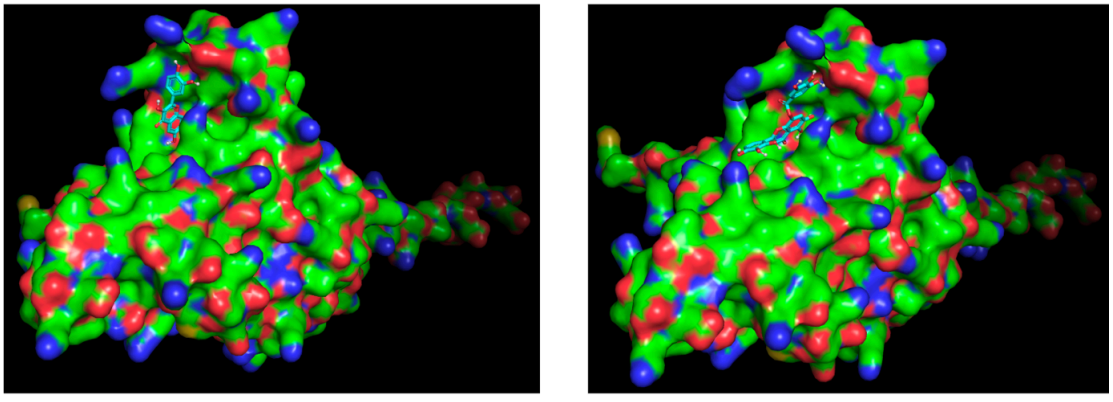
S. No.	Ligand Name	Binding affinity for UBA52 (kcal/mol)	Binding affinity for RPS9 (kcal/mol)	Binding affinity for HSP90 (kcal/mol)
1	Quercetin	-8.263	-6.726	-7.398
2	Curcumin	-7.068	-6.397	-5.965

3	Epigallocatechin Gallate	-9.785	-7.786	-8.081
4	Ferulic acid	-5.905	-5.026	-5.199
5	Niacinamide	-4.745	-4.427	-5.463
6	Panthenol	-5.645	-4.809	-4.939
7	Resveratrol	-7.157	-6.361	-6.341



(a) Quercetitn (b) EGCG

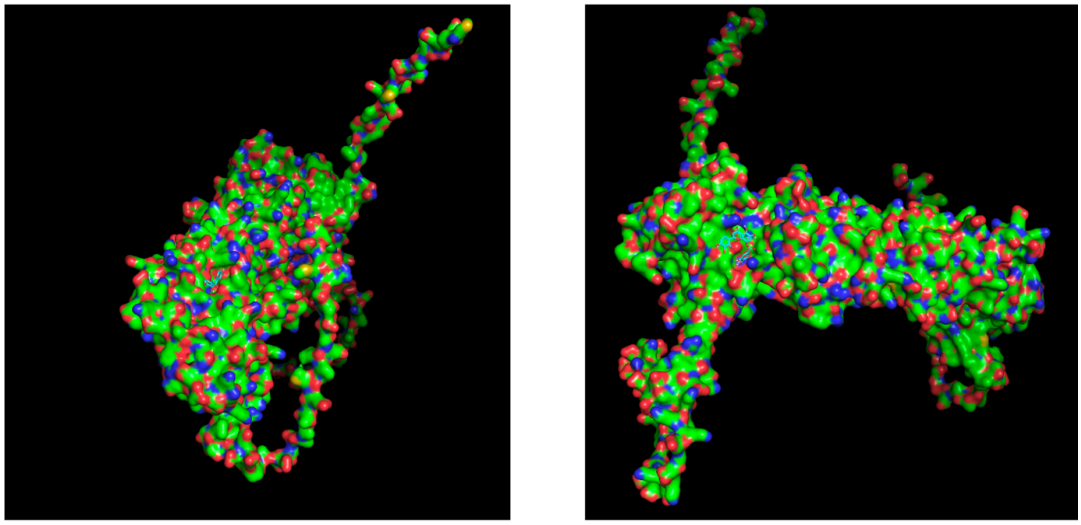
Figure 3. Molecular docking interactions of (a) quercetin and (b) EGCG with the UBA52 protein. The protein surface and ligand was visualised using PyMOL. The ligand structure can be seen as sticks in the binding site of the protein. Among the ligands, quercetin and EGCG have the most negative binding affinities of -8.263 and -9.785, respectively.



(a) Quercetitn (b) EGCG

Figure 4. Molecular docking interactions of (a) quercetin and (b) EGCG with the RPS9 protein. The protein surface and ligand was visualised using PyMOL. The ligand structure can be seen as sticks in the

binding site of the protein. Among the ligands, quercetin and EGCG have the most negative binding affinities of -6.726 and -7.786, respectively.



(a) Quercetitn (b) EGCG

Figure 5. Molecular docking interactions of (a) quercetin and (b) EGCG with the HSP90 protein. The protein surface and ligand was visualised using PyMOL. The ligand structure can be seen as sticks in the binding site of the protein. Among the ligands, quercetin and EGCG have the most negative binding affinities of -7.398 and -8.081, respectively.

Formulation Design

Among the compounds examined, quercetin, curcumin, resveratrol, EGCG, ferulic acid, niacinamide, panthenol, glycerol, allantoin, and ascorbic acid emerged as the most promising candidates because of their complementary biological functions and favorable safety characteristics reported in the literature. Antioxidant compounds such as quercetin, ferulic acid, and ascorbic acid were prioritized for their potential to minimize oxidative damage associated with heat stress. Niacinamide and panthenol were selected because of their known roles in skin repair and soothing effects, while glycerol was incorporated due to its ability to improve hydration and potentially enhance thermal dissipation. The antioxidant-containing formulation consisted of Quercetin (0.3%), Ferulic acid (0.5%), Ascorbic acid (1%) and Glycerol (5%) to prevent oxidative damage due to heat stress. The anti-inflammatory and repair formula included curcumin (0.2%), niacinamide (3%), panthenol (2%) and allantoin (0.3%), which were formulated to soothe inflammation and promote tissue repair. A third formulation identified to improve the hydration and thermal dissipation was developed with cooling properties, with the presence of glycerol (5%), panthenol (2%) and niacinamide (2%).

DISCUSSION

This paper aimed to use bioinformatics and chemical databases to identify potential cooling solutions for heat-stressed urban mammals due to the heat island effect. DEGs were identified in mammals through

data on Bos taurus. PPI network analysis was used to investigate the functional relationships between these genes, and functional pathway enrichment analysis to find the most statistically significant biological pathways associated with the genes. Cross-referencing these two results, the top genes, UBA52 and RPS9, were selected for further analysis, as well as HSP90, a well-known heat shock protein. Molecular docking was used to identify bioactive compounds with potential protective effects against heat-induced cellular damage. EGCG and Quercetin were found to be the most effective ligands for both identified proteins as well as HSP90, confirming their potential for use in anti-heat stress topical formulations, a result consistent with the compounds' antioxidative and anti-inflammatory properties.

Previous studies have explored the use of bioinformatics in the analysis of heat stress and the use of molecular docking approaches to identify bioactive compounds. The genetic data used in this project originates from a paper which also studied DEGs in Bos taurus . However, this research study uses DEGs identified from the data to find potential cooling solutions and demonstrated its applicability to other mammals. Several studies have investigated cooling formulations. For example, another study has developed a topical analgesic to accelerate heat loss . However, the study focused on humans, while this paper proposes a formulation for mitigating heat stress in mammals. Another paper has the objective of mammal heat stress mitigation, they have focused on water-based and evaporative cooling, while this study has approached the problem through bioinformatics. One study has used bioinformatics, including DE analysis and molecular docking to evaluate phytochemicals against cervical cancer, but this paper has used this methodology to address the heat stress.

Despite the promising findings obtained, this study has several limitations. First, the work was entirely theoretical and relied on publicly available datasets; therefore, the identified ligand-protein interactions represent theoretical predictions but need experimental validation. It does not account for the process of application and for other major factors related to skin like permeability. Furthermore, the dataset was focused on *Bos taurus*. Although it has been suggested that the results are also applicable to other mammals, they may not fully map to thermoregulatory mechanisms in other organisms like dogs. Future work should therefore focus on the experimental validation (and modification, if required) of the proposed formulation. Additional studies involving transcriptomic datasets from other mammals, such as canines, could strengthen the multi-species applicability of this paper's results.

In conclusion, this study demonstrates the potential of integrating transcriptome-based DE, network and pathway analysis with molecular docking to identify not only genes and proteins associated with heat stress but also identify candidate bioactive compounds with potential protective effects and develop a cooling formulation. It addresses the increasingly important issue of animal welfare and well-being in the face of threats like anthropogenic climate change and urban heat.

CONCLUSION

This study demonstrates the potential of integrating transcriptomic analysis, protein–protein interaction network analysis, pathway enrichment analysis, and molecular docking approaches to identify candidate compounds for mitigating heat-induced cellular stress in mammals. By analysing heat stress-related gene expression data from Bos taurus, key differentially expressed genes associated with heat stress pathways

were identified, including UBA52 and RPS9. Molecular docking analysis further revealed that epigallocatechin gallate (EGCG) and quercetin exhibited the strongest predicted interactions with these proteins as well as with HSP90, supporting their potential role in protective topical formulations.

The findings highlight the usefulness of computational biology and bioinformatics in addressing emerging challenges related to anthropogenic climate change and urban heat stress. In addition to identifying molecular targets associated with thermoregulatory stress, this study proposes prototype formulations aimed at reducing oxidative stress, inflammation, and heat-related cellular damage in urban mammals. Although the study was theoretical and based on publicly available datasets, it provides a framework for the future development of scientifically informed cooling and protective therapies for animals exposed to extreme environmental temperatures.

Further experimental validation, including in vitro and in vivo testing, is necessary to evaluate the efficacy, safety, skin permeability, and real-world applicability of the proposed formulations. Future studies involving transcriptomic datasets from additional mammalian species may also strengthen the broader applicability of these findings.

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