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Modulation of Inflammatory Signaling by Caffeic Acid in LPS-induced Gut-thyroid Axis Disruption in Female Mice Model

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Abstract

The lining of the gastrointestinal tract acts as a crucial protective barrier that prevents harmful microbes and various toxins from entering the blood stream and spreading throughout the body. However, when this barrier is compromised, as often happens during conditions like gut inflammation, these noxious agents can escape from the gut and enter the general circulation. Since the thyroid gland is linked to the gut *via* the blood stream, it becomes highly vulnerable to inflammation triggered by exposure to these toxins. In this investigation we explored how the bacterial endotoxin like Lipopolysaccharide (LPS), found as an integral part of certain bacteria, induces inflammation in both the gut and the thyroid in female Swiss albino mice. When this endotoxin was injected into the animal, notable tissue damage was observed in the jejunum and ileum sections of their intestine. At the same time, the thyroid gland also showed clear signs of inflammation and structural damage. Further analysis revealed increased levels of inflammatory markers, specifically the cytokines Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) in the blood of LPS-injected mice, indicating an active inflammatory response affecting both organs. Additionally, the hormone levels of Tetra-iodothyronine (T4) and Tri-iodothyronine (T3), which are critical for thyroid function, are disturbed and confirm thyroid inflammation. In conclusion, our study underscores the significant impact of gut-derived inflammation on thyroid health, highlighting the critical role of the intestinal barrier in maintaining systemic immune balance.

Major Findings: The observed gut and thyroid inflammation following LPS administration, accompanied by elevated pro-inflammatory cytokines and disrupted thyroid hormone levels, reinforces the existence of a gut-thyroid axis. Importantly, the successful therapeutic intervention with caffeic acid not only ameliorated tissue damage but also restored hormonal and inflammatory homeostasis. These findings suggest that targeting gut-integrity through antioxidant-rich dietary compounds like caffeic acid may represent a promising strategy to mitigate inflammation-induced thyroid dysfunction and support overall endocrine and gastrointestinal health.

Keywords: Caffeic Acid, Cytokine, Gastrointestinal Tract, Inflammation, Lipopolysaccharide, Thyroid

Abbreviation: LPS- Lipopolysaccharide; CA- Caffeic Acid; i.p.- Intraperitoneal; GIT-Gastrointestinal tract; IL-6- Ineterleukin-6; TNFα- Tumor Nacrosis Factor-α; H and E- Hematoxline and Eosin

1. Introduction

It is widely recognised that gut or gastrointestinal tract (GIT) function as a complex neuroendocrine immune organ, this means it plays a critical role not only in digestion but also in communication between the nervous, endocrine and immune systems. This structure include specialised enteroendocrine cells that release hormones the enteric nervous system which regulate gut activity independently of the brain and various immune

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component, such as Gut Associated Lymphoid Tissue (GALT) that helps or involve in the protection of the body from the infectious agents and maintain overall immune balance[1]. The epithelial lining of the gutmucosa contains specialized cells that help preserve the intactness of the epithelial lining and support gut homeostasis[2]. The GIT is vulnerable to arranging external toxins including infectious agents that can impair its integrity[3].

Under normal conditions GI tract sustains a balanced and diverse population of gut microbiota which can influence host health with both supportive (*e.g.*, *Akkermansia*) and detrimental (*e.g.*, *Salmonella*) community[4]. Owing to literature an assortment of variables notable enteropathogenic infections and excessive antibiotic use have been shown to alter the gut barrier integrity that might contribute to increased accumulation of detrimental intestinal endotoxin such as Lipopolysaccharide (LPS). LPS is an integral constituent of cell wall of Gram-negative bacteria, is typically present at low concentration in both the gut and systemic circulation under normal, homeostatic conditions[5, 6]. Escalated oxidative stress and pro inflammatory cytokines are the result of a string of inflammatory reaction set forth by elevated LPS level, which collectively impair gut barrier function by disrupting one or more of its structural components[7,8].

The GIT barrier is composed of intestinal epithelial cells linked by tight junctions and a protective mucus layer, which functions as an anti-microbial defense system containing various antibacterial peptides secreted by paneth cells[9,10]. Under condition such as medication, and alcohol consumptions LPS-injected damage of GIT mucosal lining increases the chance of gut disorders[11-14]. The disruption of GI tract epithelial barrier increases its permeability allowing the translocation of endotoxin and by this way the level of LPS in systemic circulation elevated[15]. Through systemic circulation, the elevated level of LPS can reach other organs like thyroid, where they can induce the commencement of follicular cells in thyroids to release pro-inflammatory cytokines to instigate or prompt thyroid inflammation[16]. Several studies have associated thyroid disorders with GI tract dysfunctions[17].

Thus, the connection between nutrient absorption the (gut) and the hormone regulation (thyroid), which is referred to as gut-thyroid axis, could prove a valuable and promising role in emergence and maturation of enormous thyroid diseases including Autoimmune Thyroid Disorder (AITD), Graves disease, Hashimoto thyroiditis[18]. The gut-thyroid axis refers to as a bidirectional or 2-way relation between gut health and thyroid function[19]. A healthy gut can support or help to absorb various essential or key nutrients like zinc, selenium, iodine, which are vital for the proper production and activation of thyroid hormone. Conversely, thyroid hormone affects gut motility and barrier function[20]. Disruption of this axis; intestinal barrier disruption or increased intestinal permeability (leaky gut syndrome), have been linked to thyroid disorder like Hashimoto's thyroiditis is now raised to prominence into noteworthy health concern[21]. The potential to beneficially modulate the Gastrointestinal Tract (GIT) for treating gut-related disorders has gained growing attention. GIT epithelial barrier preservation strategies such as supplementation of phenolic compound have become common practice[22].

Caffeic Acid (CA) is a vital phytochemical or plant sourced phenolic compound that offers or provides health advantages to the host or overall host's wellness, when consumed in appropriate amounts[23,24]. To date most research has focused on exploring the role of CA in inflammation. CA has demonstrated effectiveness in alleviating inflammatory bowel disorder in both clinical trials and experimental animal studies and their related evaluation[25]. In the condition of leaky gut CA helps to restore the gut integrity by reducing inflammatory mediators and enhances the transcription level of tight junction proteins like; occludin, claudins, which helps to restore the gastrointestinal tract integrity[26,27]. The anti-inflammatory potential of CA though has been demonstrated through limited clinical and laboratory animal studies, the specific mechanism by which CA modulates inflammation within the gut-thyroid axis remained still less or underexplored.

Building upon this background we hypothesise that CA can mitigate LPS-induced inflammation in both the gastrointestinal tract and thyroid gland buy reducing systemic inflammatory cytokines, restoring epithelial barrier integrity and normalising thyroid hormone function. This study aims to fill a critical gap by being among the first to investigate the dual-organ protective effects of CA across the gut-thyroid axis in an *in vivo* LPS-induced inflammation model using female Swiss albino mice. Our findings could open new therapeutic avenues for managing systemic inflammatory disorders through gut targeted dietary interventions particularly in thyroid dysfunction linked to compromised intestinal health. This study is novel in demonstrating that LPS-induced

intestinal barrier dysfunction not only compromises gut structure and immune regulation but also propagates systemic inflammation that reaches and affects the thyroid gland.

Females are significantly more prone to thyroid related disease or disorder such as hypothyroidism, autoimmune thyroiditis and thyroid hormone fluctuations due to hormonal interplay involving estrogen and other hormones[28]. This increased susceptibility to well documented in both clinical and preclinical studies. Therefore, to better replicate the clinical prevalence and patho-physiological relevance of thyroid dysfunction, we selected female Swiss albino mice as the experimental model. This allowed us to evaluate the potential protective role of the tested supplement in a sex that is inherently more vulnerable to thyroid disturbances. Further, by evaluating the protective role of CA can mitigate inflammation across the axis. Specifically, this work reveals how CA reduces circulating pro-inflammatory cytokines, restores thyroid hormone balance, and improves tissue architecture in both organs. These insights present a new perspective on managing endocrine-inflammatory disorders through gut-targeted nutritional strategies and introduce CA as promising candidate for therapeutic modulation of the gut-thyroid axis.

2. Materials and Methods

2.1 Animals

Swiss albino female mice weighing 25 ±2.5 gram and 120 days of age were obtained from the Central Drug Research Institute in Lucknow, India. Animals were accommodated in specific animal cages and kept in an experimental room with a temperature control of 24° C and humidity level of 55±5%. The mice were kept under specific laboratory condition including standard 12:12 light then dark schedule. Water and animal food pallet were continuously accessible to the animals. 24 female mice were used for the experiment after they had been acclimated for a week. Experimentation design includes four groups of the mice (No. 1; control/untreated, No. 2; LPS injected, No. 3; LPS+CA, & No. 4; Only CA exposed) which were managed as; n=6/group. Animals of group no. 2 and group no. 3 were dosed with stressor i.elipopolysaccharide/LPS derived from *Escherichia coli* O26:B6; (CAS 93572-42-0) at the dose of 1000µg/kilo-gram body weight intraperitoneal (i.p.) injection for 5 consecutive days. After LPS exposure, supplementation of Caffeic acid/CA; (CAS 331-39-5; constituting near perfect purity> 99%) at the decided dose of 20,000 µg/kilo-gram body weight, i.p. were administered to the Group no. 3 (LPS+CA) and Group no. 4 (Only dose of CA) for 28 days. Animal of the group no. 1 (untreated) and Group no. 2 (LPS injected) were also sustained for a period of 4 weeks. LPS and CA were acquired from Sigma Chemicals (Bengaluru, India). The treatment doses of Caffeic acid (20,000 µg/kilo-gram body weight) and LPS (1000 µg/kilo-gram body weight); for both individually and co-exposure) were decided taking the reference of previous reports with slight modifications[29,30]. Daily measurement of food-water intake along with body weight of mice was recorded before exposure of treatments. The handling of mice carried out in adherence to the established guidelines and protocol laid out by the Committee Purpose of Controlled and Supervision of Experiment on Animals (CPCSEA) under the ministry of environment and forest Government of India. The protocol of experiment trails was approved by the Institutional Animal Ethical Committee of the University of Allahabad, (approval number: IAEC/UoA/023/2023).

On 34th day, upon completion of the experiment mice were administered 10 mg/kg of pentobarbital anaesthesia to induce unconsciousness for their dissection. The gut and thyroid tissue was removed for histological analysis, weighed, and dried with filter paper before being placed in a fixative solution containing 0.4% paraformaldehyde.

2.2 Study of Plasma Level of Thyroid Hormone

Quantitative determination of T3 and T4 hormone level using animal's sample was carried out by using ELISA kits that were commercially purchased from the Krishgen Biosystems Ltd., Mumbai, India. The planned assay conducted strictly in accordance with protocols provided by the manufacturers to ensure accuracy and reproducibility following the completion of the assay procedure the Optical Density (OD) of each experimental sample was absorbance at 450 nm based on the standard curve, for error free quantification using a Bio-Rad iMark microplate reader (Bio-Rad Laboratories, Hercules, CA), which allowed for precise detection.

2.3 Study of Cytokine Assay

The abundance level of pro-inflammatory cytokines IL-6 and TNF-α were estimated using ELISA kits which were

gathered from Sigma Chemicals (Bengaluru, India). The cytokine concentrations were calculated as per the manufacturer's instruction and interpreting the result by measuring absorbance at 450 nm based on the standard curve, for error free quantification.

2.4 Histopathological Depiction of Gut and Thyroid

Following the anesthesia, both the tissues (gut and thyroid) were promptly dissected, weighed, and further fixed in purely made 4% paraformaldehyde for the period of 24 hours. Following rinsing then sequential dehydration in increasing grade of alcohols concentration (50%, 70%, 90% and 100%), tissues were fixed in clean and pure paraffin wax. Ultrathin sectioning was done at 5-6 µm thickness by a specific microtome cutter and subsequently stretched on albumin-coated microscopic slides, deparaffinized with xylol, and rehydrated through a water passing after the descending grades or series of alcohol.

Sections of gut and thyroid tissues were affixed on histological microscopic slides and thereafter treated with histological stain Hematoxylin and Eosin (H and E) according to the standardised staining protocol established in laboratory[31]. The histopathological examination and microscopic photography were carried out by light microscope (Leica DM 2500, Germany).

2.5 Histomorphometric Examination of Gut and Thyroid

Morphometric evaluation was carried out by using image J software NIH, Bethesda, USA. To quantitatively assess density of enterocytes within the villi, 10 sections randomly selected from different experimental groups to reduce biasness among sampling. For detailed microscopic examination of sections analysed at 40x magnification. Within each selected part of section goblet cells were systematically counted at the non-overlapping sites of each experimental group and subsequently examined. The cell counting was performed using a standardised counting frame measuring 100 * 100 μm^2 in dimension thereby ensuring precision and consistency in quantification across all samples.

Morphometric analysis of another tissue *i.e.* thyroid gland was conducted using the precise software image J this analysis involves determining both the follicle number and the total volume of colloid of all the experimental groups of designed experiment. The thyroid follicle is the gland's functional unit, exist in multiple layer numbers and various sizes each molecule consists of our single layer of epithelial cell and contain colloid which is primarily composed of protein-based substance rich in the glycoprotein thyroglobulin which acts as a storage medium for thyroid hormone synthesis (Fawcett and Jensh)[32]. Total colloid volume was accessed following the methodology outlined by Kot *et al.*[33]. For volume estimation all colloid containing follicles observed across different representative follicle the major and minor axes of the colloid (oriented perpendicular to another) when analysed using image J software. The colloid diameter (d) was then calculated using the equation; $d = \sqrt{a \times b}$, where "a and b" represent, the major and minor axes respectively. To correct the effect of tissue sectioning on spherical structure the measured diameter was set to evaluate the average diameter [D(m)] using the formula: $D(m)=d\times 4/\pi$. The colloid volume [$V_{(col)}$] was subsequently evaluated by applying the formula: $V_{(col)}=[\pi\times D(m)3]/6$. The number of colloid follicles was determined in accordance with Hartoft-Nielsen *et al.*[34]. Standardised frame of evaluation 500 * 500-micron metre square was used for analysis photomicrograph were examined within this frame and all colloid filled follicles present work counted observation were conducted at specific (40x) magnification.

2.6 Statistical Analysis

All the experimentation data were depicted as mean ± SEM. To access statistical difference among groups, one way (ANOVA) analysis of variance was employed, followed by Tukey's post hoc comparison for different comparison using GraphPad prism software. Significance was assessed statistically, when the value was $p < 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

3. Results

3.1 Impact of LPS and CA on Body Weight

The one-way ANOVA result reveals considerable response on body weight ($p < 0.001$) across the experimental groups relative to the untreated set. The LPS injected mice exhibited marked decline in body weight ($p < 0.001$) relative to the untreated group and both the CA co-exposed (LPS+CA; $p < 0.001$) along with only CA fed mice (CA: $p < 0.001$) showed significant effect of phenolic substance as compared to LPS-challenged mice (Figure 1a).

3.2 Impact of LPS and CA on Food and Water Intake

In the result of one-way ANOVA, food intake was pronounced drop in mice injected with LPS (P< 0.001), with reference to untreated outcome. Beneficial effect of CA was found in co-exposed group (LPS+CA; p < 0.001), only CA group (p < 0.001), as compared to LPS. In case of only CA fed, the food intake shows significant result (p < 0.05), with untreated outcome. The post hoc analysis reveals that the water intake was pronounced declined in LPS (p < 0.001) groups as compared to control, whereas in co-exposed group (LPS+CA; p < 0.01) and in only CA (p < 0.001) reflects noticeable change versus the LPS, but both the group (co-exposed, only CA) remains equivalent to control (Figure 1b).

3.3 Impact of LPS and CA on Gut and Thyroid Weight

Analysis of variance result depicts that the weight of gut was pronounced drop in mice injected with LPS (p < 0.001) with reference to the untreated. With exposure of CA pronounced surge in the gut weight in both the co-exposed; LPS+CA (p < 0.001), and only CA (p < 0.001) exposed group of mice compared with the LPS treatment group,

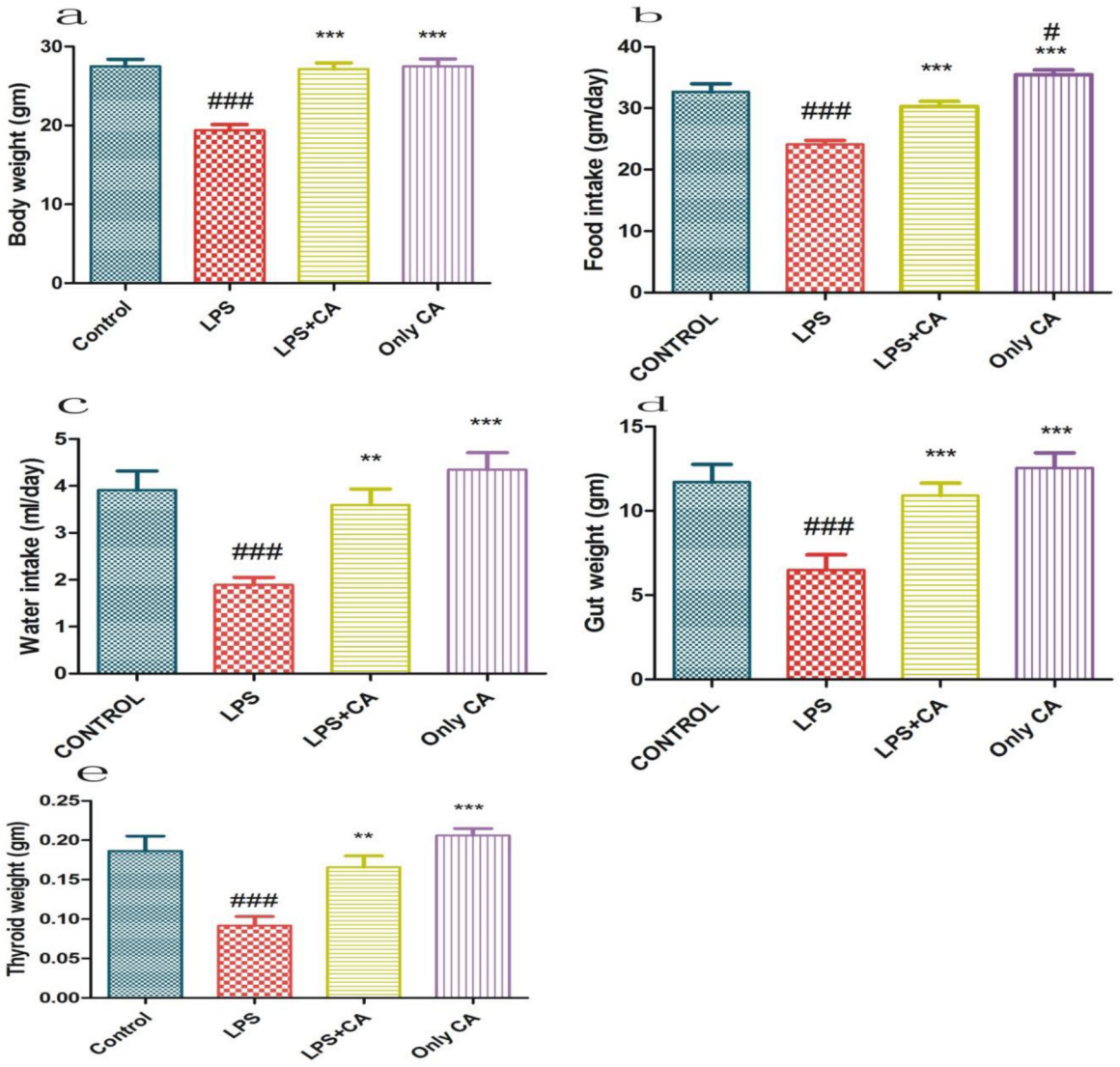


Figure 1. Graph showing; animal body weight **(a)** Food intake **(b)** Water intake **(c)** Gut weight **(d)** Thyroid weight **(e)** In different experimental groups as well as control.

Values are expressed as mean ± SEM.*$P < 0.05$ and ***$P < 0.001$ compared to LPS group; ##$P < 0.01$ and ###$P < 0.001$ in comparison with Control.

restoring it to levels comparable to the untreated outcome. Similar result obtains with thyroid weight as well where LPS treated mice shows toxic effect with the observation by showing the output with considerable drop in thyroid weight ($p < 0.001$) with reference to the untreated group. Co exposure of CA reflects improvement by maintaining the thyroid weight ($p < 0.01$) versus the LPS group, and similar with untreated group. Moreover, only treatment of phenolic compound also represents significant result ($p < 0.001$) as compare with LPS (Figure 1c, d).

3.4 Impact of LPS and CA on Thyroid Hormone

The result of plasma level of T3 hormone pronounced drop in LPS injected group versus the control group. In the phenolic compound co-exposed set of animals (LPS+CA; $p < 0.001$) the condition of T3 level, pronounced surge versus LPS. Whereas the result output shows equivalency between co-treatment and control. Simultaneously, only CA fed mice also exhibit equal results with control group but significant higher ($p < 0.001$) result obtains versus LPS. The ANOVA test shows improved results in suppression of inflammation in thyroid by maintaining the T4 hormone level as well. In case of T4 hormone, post-hoc test reveals the inflammatory effect of LPS, with the abnormal increase of T4 level ($P < 0.001$), in relation to untreated group. CA supplemented groups: LPS+CA, Only CA ameliorates these effects ($p < 0.001$), with reference to LPS, suggesting its protective role on thyroid during inflammation or stress condition. Whereas similar

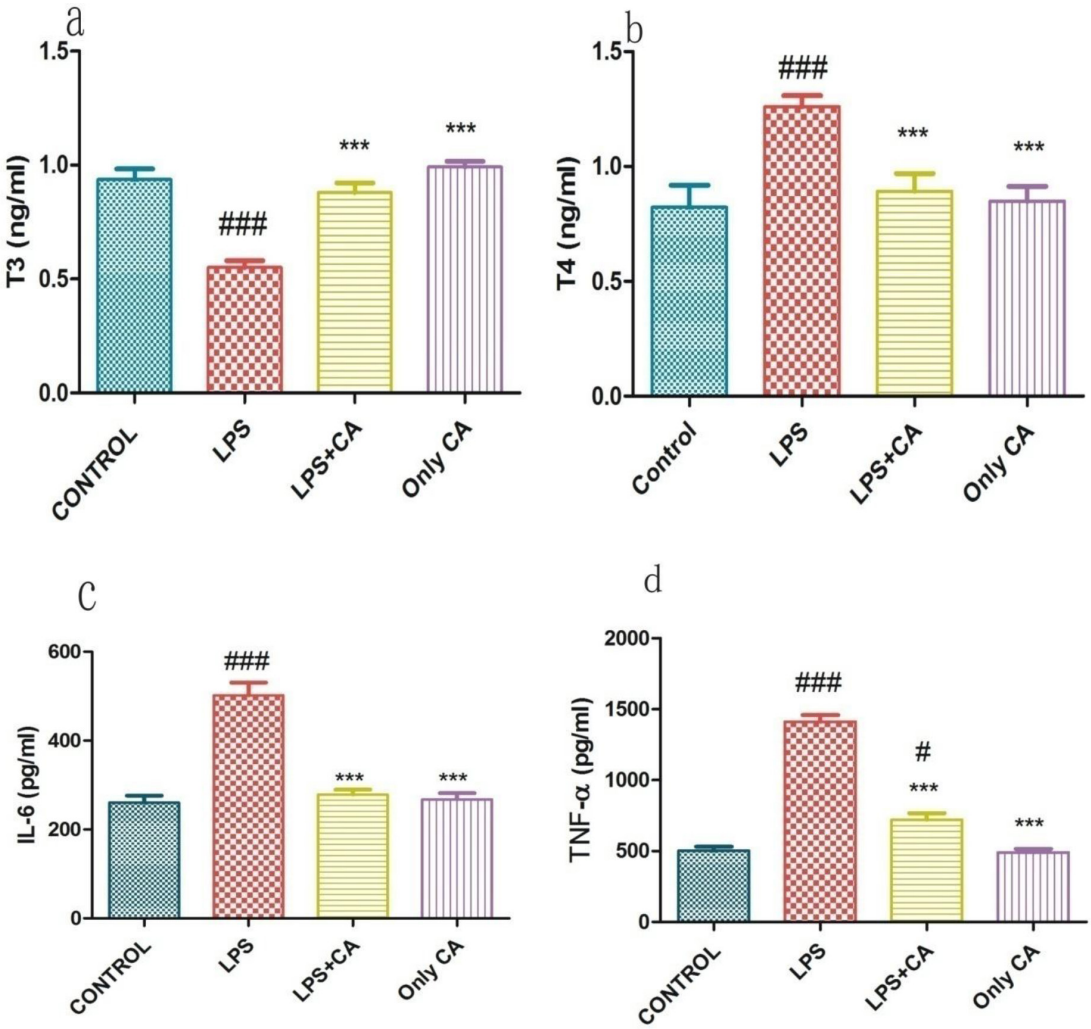


Figure 2. Analysis of plasma assay of T3 hormone **(a)** and T4 hormone **(b)** Cytokine assay of IL-6 **(c)** TNF-α.
Values are expressed as mean ± SEM.*$P < 0.05$ and ***$P < 0.001$ compared to LPS group; ##$P < 0.01$ and ###$P < 0.001$ in comparison with Control group.

quantification observes *via* one-way ANOVA, in only CA supplemented group and control group (Figure 2a, b).

3.5 Impact of LPS and CA on Inflammatory Mediators

The impact of i.p. treatment of LPS and CA administration on plasma level of cytokines IL-6 and TNF-α are illustrated in (Figure 2 c,d). Variance analysis revealed substantial alterations in IL-6 ($p < 0.001$) and TNF-α level ($p < 0.001$) across the experimentation groups with reference to the untreated group. Tukey's post hoc analysis further showed elevated levels of both IL-6 and TNF-α, in LPS+CA ($p < 0.001$) treated groups versus the LPS along with LPS ($P < 0.001$), LPS+CA group shows ($p < 0.05$) level increase as compare with control group. However, the condition of plasma levels of both IL-6 and TNF-α were pronounced drop ($P< 0.001$ for both) in CA co-exposed (LPS+ CA) as compared to group treated with LPS. In solitude CA fed animal, condition of plasma levels of IL-6 and TNF-α consistently comparable to the untreated group (Figure 2c, d).

3.6 Histopathological Characterization of Gut, Thyroid

The histopathology analysis of Jejuno-ilium, part of the GI tract in the control group revealed well-preserved intact tissue armature with continuous layers. In contrast, LPS-exposed mice exhibited pronounced and extensive histopathological alterations; characterized by significant disruption of mucosal epithelial cells, degeneration and

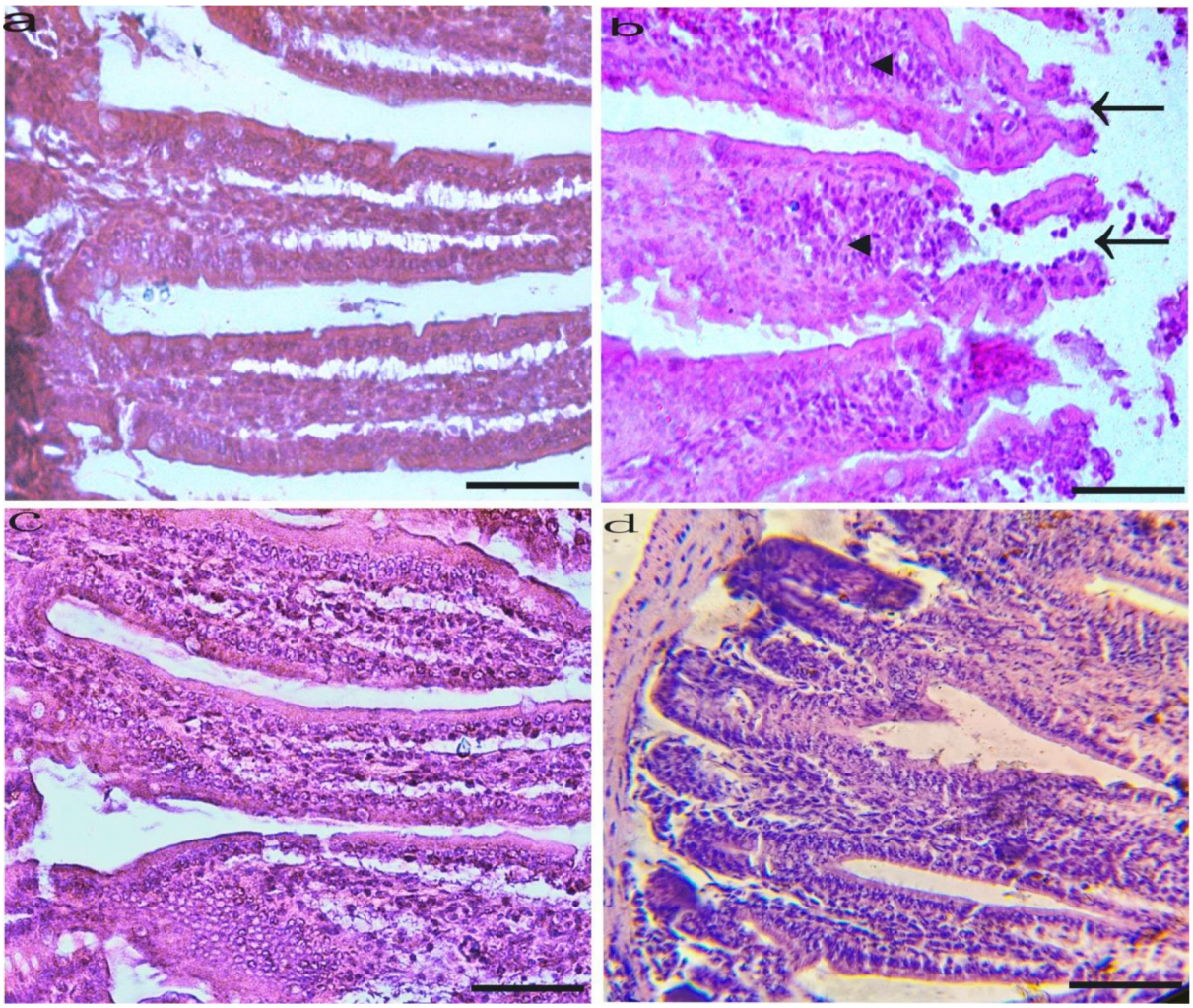


Figure 3. Histopathology of jejunum-ileum of the gut of control **(a)** LPS **(b)** LPS+CA **(c)** CA **(d)** Damage of apical region of villi (➔) condensation of lamina propria (◀). Magnification: ×40, Bar scale = 50.
Values are expressed as mean ± SEM. **P*< 0.05 and ***P*< 0.01 compared to control. # *P*< 0.05 and ##*P*< 0.01 in comparison with LPS exposed group.

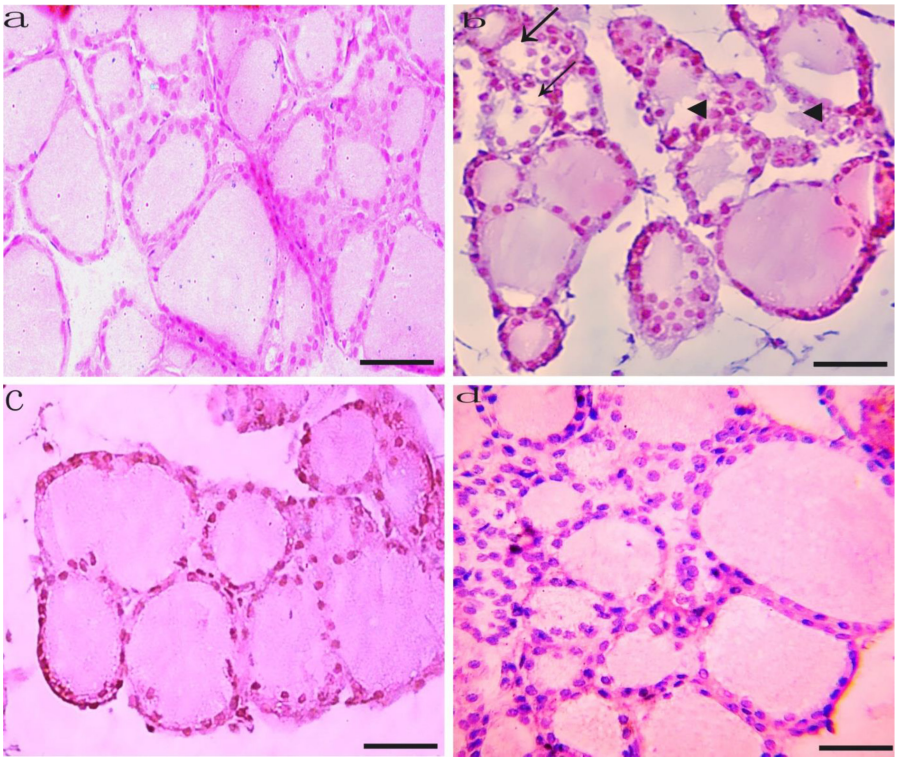


Figure 4. Histopathology depiction of thyroid of Control **(a)** LPS **(b)** LPS+CA **(c)** and CA **(d)** Thyroid follicle devoid of colloid (↙), damage of follicle (◀). Magnification: ×40, Bar scale = 50µm.
(↙) condensation of lamina propria (◀). Magnification: ×40, Bar scale= 50

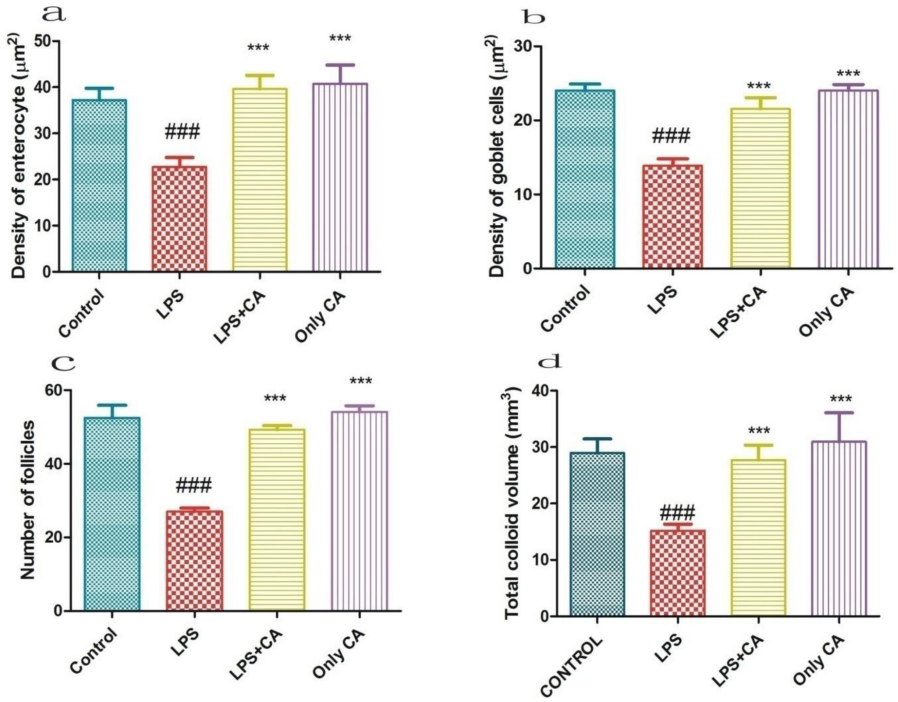


Figure 5. Figure 5. Graph showing the measurement of the density of enterocytes **(a)** Density of goblet cells **(b)** Density of gut and number of follicle **(c)** Volume of colloid **(d)** Volume of the thyroid. Values are expressed as mean ± SEM. *P< 0.05, **P< 0.01 and **P< 0.001 compared to LPS exposed #P< 0.05, ##p<0.01, ###p<0.001 in comparison with control group.

loss of goblet cells, marked infiltration of inflammatory cell into lamina propria, coupled with accumulation and structural disorganization of both lamina propria and enterocytes (Figure 3). Significant decrease in no. of enterocytes ($p < 0.001$) and no. of goblet cells ($p < 0.001$) was registered (Figure 5). In CA co-exposed (LPS + CA) along with the only CA supplemented mice, overall histological architecture that closely resembles to that of the control, with no significant histopathological disintegration observed. In the phenolic co-exposed group (LPS + CA) along with only the CA administered group, histopathological features such as goblet cell vacuolization, movement of inflammatory cells in lamina propria, and degeneration of cell lining were improved as compared to LPS (Figure 3). Density of enterocytes and no. of goblet cells were pronounced drop ($p < 0.001$) in relation to the LPS, that signifies the inflammatory or stress causing effect of endotoxin, which gets normalized upon CA exposure helps to get insight of regulatory role of phenolic compound.

The observation of histopathology of thyroid of all the groups gives the proper indication of the toxic effect of LPS and protective impact of CA (Figure 4). LPS can induce necrosis and degeneration of thyroid follicular cells and disruption of follicular epithelium, which are responsible for thyroid hormone synthesis. The follicle becomes disorganized or collapsed; the colloid may appear scanty. There may be congestion as well as edema in the inter-follicular spaces. Dissolution, retraction of colloids, rupture of overall cells was noticed in LPS-injected groups. The number of follicles and volume of colloids get sharply reduced ($p<0.001$) in LPS injected group as compared to untreated group. All the altered features of thyroid get pronounced improved after CA supplementation, show ameliorative effect of phenolic compound. In co-exposed group LPS+CA, the ameliorating effect was seen in LPS, where there is a loss of empty follicles, interstitial spaces between follicles with proper compactness. Morphometric evaluation also pronounced improved after phenolic compound supplementation as compared to LPS, indicating its preventive benefits (Figure 5).

4. Discussion

The current investigation we found that injection of the bacterial endotoxin lipopolysaccharide triggers a strong inflammatory reaction in both the absorptive organ (Gastrointestinal Tract or GIT) and the metabolic organ (thyroid) marked by amplified condition of pro-inflammatory cytokines; IL-6 and TNF-alpha, disturbance in thyroid hormone balance. Importantly administrating Caffeic Acid (CA) for continuous 4 weeks to mice challenged with LPS pronounced reduce these inflammatory responses that signify protective potential of caffeic acid. CA administration led to the marked reduction in pro-inflammatory cytokines level and contributed to the restoration of normal thyroid hormone concentration indicating its potential therapeutic role in modulating inflammation and preserving endocrine immune balance.

The LPS endotoxin is a powerful immunogenic stimulus known to initiate or amplified a robust immune response by specifically binding to toll like receptor 4 (TLR-4), which is expressed on the surface of immune competent cells including monocyte cells[35]. Activation of these immune cells triggers the release of a wide range of inflammatory substances such as pro-inflammatory cytokines, chemokines, and oxidative free radicals have widely documented in literatures[36,37]. The augmented levels of pro-inflammatory cytokines are the biomarker of inflammation[38]. The LPS-injected group manifested a sharp drop in the animal's body weight, food along with water intake, including organ weight; gut and thyroid, which contributed to the amplification of systemic toxicity response due to immune activation triggered by heightened condition of pro-inflammatory cytokines. The LPS injection resulted in the boost of the pro-inflammatory cytokines TNF-α and IL-6, escalating inflammatory responses, and gut inflammation, as mentioned previously[39]. LPS induces Gastrointestinal Tract or GIT inflammation or toxicity was obvious from observations of jejuno-ilium.

LPS exposure led to significant damage to the gut mucosal epithelium characterised by epithelial leakage, damage of goblet cell, mucin disintegration and aberrant mucin accumulation on the peripheral or epithelial surface accompanied by densification of the Lamina Propria (LP). As previously reported leucocyte infiltration within the LP suggested flourishment of immune cells or an inflammatory restitution process[40]. The accumulation of LP may be linked to LPS-instigate chemokine emergence including the enhancement ICAM from fibroblast[41]. LPS exposed models, intestinal inflammation often accompanies thyroid dysfunction, exacerbating systemic inflammation and altering thyroid functioning and related metabolism[42]. Up regulation of pro-inflammatory

mediators plays a contributing action in the initiation of pathogenesis, advancement, and magnitude of systemic inflammation as well as thyroid[43]. In LPS exposed models, systemic inflammation commonly induces thyroid dysfunction, characterized by altered thyroid hormone levels and disrupted thyroid histology, such as follicular cell damage and architectural disarray indicating severity of thyroid inflammation[44]. Few reports are available where LPS, can directly affect thyroid cells through activation of Toll Like Receptor 4 (TLR4/NFκB) pathway. Binding to TLR4 receptor triggers a signalling cascade that leads to NFκB activation and increased production of pro inflammatory cytokines. This inflammatory response impairs thyroid function by reducing thyroid hormone and promoting thyroid damage[45].

In the presented work, we have demonstrated that the exposure of Caffeic Acid (CA), a well-known plant based phenolic compound, maintains body weight, gut and thyroid weight and thyroid hormone level by reducing the inflammation-induced systemic toxicity. CA supplementation mitigates the histopathological alterations of gut and thyroid generated by LPS because of antioxidant and anti-inflammatory properties. Treatment of CA leads to a marked reduction in these plasma conditions of pro-inflammatory cytokines TNF-α and IL-6 indicating its anti-inflammatory effect. CA likely suppresses the flourishment of key-inflammatory pathways, such as NFκB, thereby down regulating the transcription and release of cytokines[46]. CA acts not only as a direct antioxidant but also activates key cellular pathways and modulates gut microbiota. It enhances the Nrf2 pathway, boosting the body's own antioxidant defence by up regulating Enzymes like HO-1 and NQO1. Additionally, it improves gut microbiota composition promoting beneficial bacteria and reducing inflammation *via* microbial metabolites like SCFAs. These combined actions contribute to its strong anti-inflammatory and protective against beyond simple ROS scavenging[47].

CA through its anti-inflammatory properties helps to restore and improve intestinal lining integrity by enhancing proteins of tight junction, reducing the translocation of LPS into systemic circulation[48]. Enhancement of intestinal barrier function is achieved by the up regulation of transcription of tight junction proteins which serve to prevent the translocation of lipopolysaccharide from the gut into systemic circulation thereby reducing inflammation and immune activation. A well-maintained gut mucosal barrier plays a critical role not only in immune defence but also in facilitating the efficient biotransformation and absorption of essential micronutrients like selenium, zinc, iodine. These trace elements are fundamental for the synthesis and regulation of hormone of thyroid glands highlighting the correlation between gut health and thyroid function[49].

CA acts through the stabilization of gut barriers and suppression of local and systemic inflammation[50], which in turn leads to the preservation or restoration of normal thyroid histo-architecture and thyroid hormone biosynthesis by decreasing the inflammatory burden on the thyroid gland. The marked reduction in weight of body, tissue weight (gut and thyroid), food along with water consumption as gathered in this investigation suggested systemic toxicity induced by LPS consistent with previous reports[51]. CA supplementation helps in the maintenance of weight of body and food along with water consumption in the condition of stress or inflammation which possibly attributed to decline in systemic toxicity by modulating inflammatory cytokines and restoring homeostatic signaling pathways involved in appetite and metabolism[52,53].

Thus, caffeic acid plays a dual role by improving the gut barrier, protecting intestinal tissue from LPS-induced damage and aiding in the repair of damaged cells. It also promotes a balanced gut environment and reduces systemic inflammation which in turn supports thyroid health and optimal hormone functioning. These combined effects help to re-establish the gut-thyroid axis in LPS-induced inflammatory conditions. Overall, these findings suggest that CA aid in sustaining the preservation of metabolic stability and may serve as a protective agent against inflammatory stress condition.

5. Summary and Conclusion

The maintenance of gut mucosal barrier integrity is essential for upholding the physiological balance of the gut-thyroid axis, a complex bidirectional communication network that interconnects gastrointestinal and thyroid function. Disruption of this axis particularly through endotoxemic stressor *i.e.* LPS can trigger and amplifies systemic inflammation, further compromising both gut and thyroid health. In this context, CA a naturally occurring phenolic compound has shown significant protective potential. Its ability to mitigate histopathological damage, regulate pro-inflammatory cytokine levels, and stabilise thyroid hormone profiles highlights its therapeutic relevance. Dietary

supplementation with CA may serve as a promising adjunct strategy in the management of inflammatory condition such as Irritable Bowel Syndrome (IBS) and ulcerative colitis as well as autoimmune thyroid disorder including Hashimoto's thyroiditis. Overall, targeting the gut-thyroid axis through anti-inflammatory supplements like CA offers a novel approach to restoring systemic homeostasis and improving healthy gut-thyroid axis.

6. Author Contributions

B. M was involved in the formulating the concept and overall design of the study. The responsibility of material preparation, data collection and analysis were sincerely conducted by A.Y., who also prepares the first draft or version of the manuscript. B. M thoroughly reviewed ensures with the study's objective and further gave final approval to the submitted version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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8. References

1. Takiishi T, Fenero CIM, Camara NOS. Intestinal barrier and gut microbiota: Shaping our immune responses throughout life. Tissue Barriers. 2017; 5(4):e1373208. https://doi.org/10.1080/21688370.2017.1373208
2. Herath M, Hosie S, Bornstein JC, Franks AE, Yardin ELH. The role of the gastrointestinal mucus system in intestinal homeostasis: Implications for neurological disorders. Front Cell Infect Microbiol. 2020; 10:248. https://doi.org/10.3389/fcimb.2020.00248
3. Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp Mol Med. 2018; 50(8):1-9. https://doi.org/10.1038/s12276-018-0126
4. Karakan T, Tuohy KM, Solingen GJV. Low-dose lactulose as a prebiotic for improved gut health and enhanced mineral absorption. Front Nutr. 2021; 8:672925. https://doi.org/10.3389/fnut.2021.672925
5. Li W, Andrew D. Factors influencing the gut microbiota, inflammation, and type 2 diabetes. J Nutr. 2017; 147(7):1468S-1475S. https://doi.org/10.3945/jn.116.240754
6. Mohammad S, Thiemermann C. Role of metabolic endotoxemia in systemic inflammation and potential interventions. Front Immunol. 2021; 11:594150. https://doi.org/10.3389/fimmu.2020.594150
7. Suzuki K. Chronic inflammation as an immunological abnormality and effectiveness of exercise. Biomolecules. 2019; 9(6):223. https://doi.org/10.3390/biom9060223
8. Ferro D, Baratta F, Pastori D, Cocomello N, Colantoni A, Angelico F, Ben MD. New insights into the pathogenesis of non-alcoholic fatty liver disease: Gut-derived lipopolysaccharides and oxidative stress. Nutrients. 2020; 12(9):2762. https://doi.org/10.3390/nu12092762
9. Maunder R. Mediators of stress effects in inflammatory bowel disease: Not the usual suspects. J Psychosom Res. 2000; 48(6):569-577. https://doi.org/10.1016/s0022-3999(00)00098-2
10. Kurashima Y, Yamamoto D, Nelson S, Uematsu S, Ernst PB, Nakayama T, Kiyono H. Mucosal mesenchymal cells: Secondary barrier and peripheral educator for the gut immune system. Front Immunol. 2017; 8:1787. https://doi.org/10.3389/fimmu.2017.01787
11. Andrade JAB, Freymuller E, Neto UF. Pathophysiology of enteroaggregative *Escherichia coli* infection: An experimental model utilizing transmission electron microscopy. Arq Gastroenterol. 2010; 47(3):306-312. https://doi.org/10.1590/s0004-28032010000300018
12. Zheng D, Liao H, Chen S, Liu X, Mao C, Zhang C, Meng M, Wang Z, *et al*. Elevated levels of circulating biomarkers related to leaky gut syndrome and bacterial translocation are associated with Graves disease. Front Endocrinol. 2021; 12:796212. https://doi.org/10.3389/fendo.2021.796212
13. MacDonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. Science. 2005; 307(5717):1920-1925. https://doi.org/10.1126/science.1106442
14. Erben U, Loddenkemper C, Doerfel K, Spieckermann S, Haller D, Heimesaat MM, Zeitz M, Siegmund B, Kuhl AA. A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int J Clin Exp Pathol. 2014; 7(8):4557-4576. PMID: 25197329; PMCID: PMC4152019.
15. Ghosh SS, Wang J, Yannie PJ, Ghosh S. Intestinal barrier dysfunction, LPS translocation, and disease development. J Endocr Soc. 2020; 4(2):bvz039. PMCID: PMC7033038. PMID: 32099951. https://doi.org/10.1210/jendso/bvz039
16. Vasu C, Holterman MJ, Prabhakar BS. Modulation of dendritic cell function and cytokine production to prevent thyroid autoimmunity. Autoimmunity. 2003; 36(6-7):389-396. https://doi.org/10.1080/08916930310001603073
17. Vanuytsel T, Tack J, Farre R. The role of intestinal permeability in gastrointestinal disorders and current methods of evaluation. Front Nutr. 2021; 8:717925. https://doi.org/10.3389/fnut.2021.717925

18. Fenneman AC, Bruinstroop E, Nieuwdorp M, Spek AHVD, Boelen A. A comprehensive review of thyroid hormone metabolism in the gut and its clinical implications. Thyroid. 2023; 33(1):32-44. https://doi.org/10.1089/thy.2022.0491
19. Zou H, Xu N, Xu H, Xing X, Chen Y, Wu S. Inflammatory cytokines may mediate the causal relationship between gut microbiota and male infertility: A bidirectional, mediating, multivariate Mendelian randomization study. Front Endocrinol. 2024; 15:1368334. https://doi.org/10.3389/fendo.2024.1368334
20. Knezevic J, Starchl C, Berisha AT, Amrein K. Thyroid-gut-axis: How does the microbiota influence thyroid function? Nutrients. 2020; 12(6):1769. https://doi.org/10.3390/nu12061769
21. Cayres LCF, Salis LVV, Rodrigues GSP, Lengert AVH, Biondi APC, Sargentini LDB, *et al*. Detection of alterations in the gut microbiota and intestinal permeability in patients with Hashimoto thyroiditis. Front Immunol. 2021; 12:579140. https://doi.org/10.3389/fimmu.2021.579140
22. Ramirez BAS, Catalan U, Pedret A, Valls RM, Motilva MJ, Rubio L, Sola R. Exploring the effects of phenolic compounds to reduce intestinal damage and improve the intestinal barrier integrity: A systematic review of *in vivo* animal studies. Clin Nutr. 2021; 40(4):1719-1732. https://doi.org/10.1016/j.clnu.2020.09.027
23. Birkova A, Hubkova B, Bolerazska B, Marekova M, Cizmarova B. Caffeic acid: A brief overview of its presence, metabolism, and bioactivity. Bioact Compd Health Dis. 2020; 3(4):74-81. https://doi.org/10.31989/bchd.v3i4.692
24. Rahman MM, Rahaman MS, Islam MR, Rahman F, Mithi FM, Alqahtani T, *et al*. Role of phenolic compounds in human disease: Current knowledge and future prospects. Molecules. 2021; 27(1):233. https://doi.org/10.3390/molecules27010233
25. Lu Y, Han X. Therapeutic implications of phenolic acids for ameliorating inflammatory bowel disease. Nutrients. 2024; 16(9):1347. https://doi.org/10.3390/nu16091347
26. Zielinska D, Zielinski H, Llopis JML, Nowak DS, Honke J, Bastida JAG. Caffeic acid modulates processes associated with intestinal inflammation. Nutrients. 2021; 13(2):554. https://doi.org/10.3390/nu13020554
27. Chen X, Qiao T, Mao Z, Jia G, Zhao H, Liu G, Huang Z. Caffeic acid improves intestinal barrier functions by regulating colonic bacteria and tight junction protein expression and alleviating inflammation in weaning piglets. Anim Biotechnol. 2023; 34(8):3693-3699. https://doi.org/10.1080/10495398.2023.2200441
28. Naz S, Ahmad M, Abbas F, Zeb A, Muhammad A, Banori A. Gender and age-related differences in the distribution of neoplastic and non-neoplastic thyroid lesions: A cross-sectional study. Khyber J Med Sci. 2024; 17(3):133. https://doi.org/10.70520/kjms.v17i3.506
29. Guo S, Sadi RA, Said HM, Ma TY. Lipopolysaccharide causes an increase in intestinal tight junction permeability *in vitro* and *in vivo* by inducing enterocyte membrane expression and localization of TLR-4 and CD14. Am J Pathol. 2013; 182(2):375-387. https://doi.org/10.1016/j.ajpath.2012.10.014
30. Chiang YF, Lin IC, Huang KC, Chen HY, Ali M, Huang YJ, Hsia SM. Caffeic acid's role in mitigating polycystic ovary syndrome by countering apoptosis and ER stress triggered by oxidative stress. Biomed Pharmacother. 2023; 166:115327. https://doi.org/10.1016/j.biopha.2023.115327
31. Mishra AC, Mohanty B. Effects of lactational exposure of olanzapine and risperidone on hematology and lymphoid organs histopathology: A comparative study in mice neonates. Eur J Pharmacol. 2010; 634(1-3):170-177. https://doi.org/10.1016/j.ejphar.2010.02.014
32. Fawcett D, Jensh R. Bloom and Fawcett's Concise Histology. 2nd Edition. Arnold Publishers. 2002: pp. 257-258.
33. Kot BC, Lau TY, Cheng SC. Stereology of the thyroid gland in Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) in comparison with human (*Homo sapiens*): Quantitative and functional implications. Plos One. 2013; 8(5):e62060. https://doi.org/10.1371/journal.pone.0062060
34. Nielsen MLH, Rasmussen AK, Rasmussen UF, Buschard K, Bock BT. Estimation of number of follicles, volume of colloid and inner follicular surface area in the thyroid gland of rats. J Anat. 2005; 207(2):117-124. https://doi.org/10.1111/j.1469-7580.2005.00442.x
35. Sharma A, Shandilya UK, Sullivan T, Naylor D, Canovas A, Mallard BA, Karrow NA. Identification of bovine serum miRNAs following bacterial lipopolysaccharide challenge. Int J Mol Sci. 2020; 21(21):7920. https://doi.org/10.3390/ijms21217920
36. Alexander C, Rietschel ET. Bacterial lipopolysaccharides and innate immunity. J Endotoxin Res. 2001; 7(3):167-202. PMID: 11581570.
37. Maldonado RF, Correia IS, Valvano MA. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiol Rev. 2016; 40(4):480-493. https://doi.org/10.1093/femsre/fuw007
38. Selleri S, Palazzo M, Deola S, Wang E, Balsari A, Marincola FM, Rumio C. Induction of pro-inflammatory programs in enteroendocrine cells by the Toll-like receptor agonists flagellin and bacterial LPS. Int Immunol. 2008; 20(8):961-970. https://doi.org/10.1093/intimm/dxn055
39. Mohan M, Kaushal D, Aye PP, Alvarez X, Veazey RS, Lackner AA. Focused examination of the intestinal lamina propria yields greater molecular insight into mechanisms underlying SIV induced immune dysfunction. Plos one. 2012; 7(4):e34561. https://doi.org/10.1371/journal.pone.0034561
40. Liu C, Li A, Weng YB, Duan ML, Wang BE, Zhang SW. Changes in intestinal mucosal immune barrier in rats with

endotoxemia. World J Gastroenterol. 2009; 15(46):5843-5850. https://doi.org/10.3748/wjg.15.5843
41. Fenneman AC, Bruinstroop E, Nieuwdorp M, Spek AHVD, Boelen A. A comprehensive review of thyroid hormone metabolism in the gut and its clinical implications. Thyroid. 2023; 33(1):32-44. https://doi.org/10.1089/thy.2022.0491
42. Ganesh BB, Bhattacharya P, Gopisetty A, Prabhakar BS. Role of cytokines in the pathogenesis and suppression of thyroid autoimmunity. J Interferon Cytokine Res. 2011; 31(10):721-731. https://doi.org/10.1089/jir.2011.0049
43. Luca RD, Davis PJ, Lin HY, Gionfra F, Percario ZA, Affabris E, Pedersen JZ, Marchese C, *et al*. Thyroid hormones interaction with immune response, inflammation and non-thyroidal illness syndrome. Front Cell Dev Biol. 2021; 8:614030. https://doi.org/10.3389/fcell.2020.614030
44. Yu C, Wang D, Yang Z, Wang T. Pharmacological effects of polyphenol phytochemicals on the intestinal inflammation *via* targeting TLR4/NF-κB signaling pathway. Int J Mol Sci. 2022; 23(13):6939. https://doi.org/10.3390/ijms23136939
45. Nicola JP, Velez ML, Lucero AM, Fozzatti L, Pellizas CG, Repiso AMM. Functional toll-like receptor 4 conferring lipopolysaccharide responsiveness is expressed in thyroid cells. Endocrinology. 2009; 150(1):500-508. https://doi.org/10.1210/en.2008-0345
46. Zha P, Liu W, Zhou Y, Chen Y. Protective effects of chlorogenic acid on the intestinal barrier of broiler chickens: An immunological stress model study. Poult Sci. 2024; 103(8):103949. https://doi.org/10.1016/j.psj.2024.103949
47. Scapagnini G, Sonya V, Nader AG, Calogero C, Zella D, Fabio G. Modulation of Nrf2/ARE pathway by food polyphenols: A nutritional neuroprotective strategy for cognitive and neurodegenerative disorders. Mol Neurobiol. 2011; 44:192-201. https://doi.org/10.1007/s12035-011-8181-5
48. Ganesh BB, Bhattacharya P, Gopisetty A, Prabhakar BS. Role of cytokines in the pathogenesis and suppression of thyroid autoimmunity. J Interferon Cytokine Res. 2011; 31(10):721-731. https://doi.org/10.1089/jir.2011.0049
49. Zielinska D, Zielinski H, Llopis JML, Nowak DS, Honke J, Bastida JAG. Caffeic acid modulates processes associated with intestinal inflammation. Nutrients. 2021; 13(2):554. https://doi.org/10.3390/nu13020554
50. Sun J, Zhao F, Lin B, Feng J, Wu X, Liu Y, Zhao L, Zhu B, Wei Y. Gut microbiota participates in antithyroid drug induced liver injury through the lipopolysaccharide related signalling pathway. Front Pharmacol. 2020; 11:598170. https://doi.org/10.3389/fphar.2020.598170
51. Pavlikova N. Caffeic acid and diseases- Mechanisms of action. Int J Mol Sci. 2023; 24(1):588. https://doi.org/10.3390/ijms24010588
52. Kadar NNMA, Ahmad F, Teoh SL, Yahaya MF. Caffeic acid on metabolic syndrome: A review. Molecules. 2021; 26(18):5490. https://doi.org/10.3390/molecules26185490
53. Shulhai AM, Rotondo R, Petraroli M, Patianna V, Predieri B, Iughetti L, Esposito S, Street ME. The role of nutrition on thyroid function. Nutrients. 2024; 16(15):2496. https://doi.org/10.3390/nu16152496