Assessing the Impact of Clove (Syzygium Aromaticum) Extract on Intestinal and Hepatic Histological Alterations in Broiler Chickens Infected with Salmonella typhimurium

Saba A. Ali [1], Neeran F. Hassan [2], Sa'adeya Ali Al- Gnami [3], Hanaa abdulabbas Abdulameer[4]

[1] [2,4]Department of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq. Email: saba.ali@qu.edu.iq

[3]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Al-Qadisiyah, Al-Diwaniyah, Iraq

KEYWORDS
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ABSTRACT
This study investigated effects of aqueous and ethanolic clove extracts in small intestine and liver of chickens exposed to Salmonella typhimurium. Two hundred fifty-day-old chicks were divided into five groups. Group 1 served as the control and received a basic diet.Group 2 was orally infected with Salmonella typhimurium. Group 3 received same infection but was treated with Ciprofloxacin. Groups 4 and 5 were infected but received treatments of aqueous and ethanolic clove extracts, respectively. The findings revealed that Group 1 exhibited normal small intestine, while Group 2 displayed severe abnormalities, including flattened mucosa, damaged villi, and inflammation. Group 3 showed improvements, increased cell growth and goblet cells in the small intestine. Group 4 displayed nearly normal intestinal features. Group 5 had fully restored intestinal health. In the liver, Group 1 showed typical hepatic structure, whereas Group 2 exhibited signs of inflammation and hepatocyte necrosis. Group 3 displayed severe hepatocyte damage, while Group 4 demonstrated restored liver structure with slight sinusoidal dilation. Group 5 had a well-preserved hepatic architecture with minor inflammation. Administration of 2 mg/kg of aqueous and ethanolic clove extracts demonstrated alleviate histological alterations in small intestine and liver caused by Salmonella typhimurium infection. Notably, the aqueous extract is more effective than ethanolic extract in reducing liver and intestine damage.

1. Introduction

Infectious diseases continue to pose a significant threat to global health, resulting in substantial illness and death worldwide [1]. Poultry serves as a major reservoir for bacteria and acts as a source of disease transmission. Pathogenic microorganisms can cause infectious diseases with high mortality rates in both animals and humans [2]. Live poultry play a central role in transmitting human salmonellosis, especially among children. Therefore, it is essential to emphasize the importance of raising awareness, providing health education, and implementing comprehensive interventions, ranging from mail-order hatcheries to agricultural feed stores, to reduce the risk of transmission. The microorganism responsible for Salmonellosis in humans is *S. typhimurium*. These zoonotic Salmonella infections can occur either through direct contact with infected poultry or indirectly via materials in their environment. Moreover, these infections can also be contracted by consuming food or beverages prepared in a contaminated environment [3-6]. Antibiotics are effective at inhibiting microorganism growth. However, the use of multiple antibiotics can lead to the development of multi-drug resistance (MDR) [7] MDR has become a pressing global public health concern in recent times [8,9]. In recent years, numerous studies have explored the potential of medicinal plants as sources of antimicrobial agents for combating both infectious and non-infectious diseases [10]. Ancient medicinal practices have long recognized the antimicrobial properties of plant extracts, which are rich in bioactive compounds with powerful antimicrobial benefits. Many combinations of medicinal herbs have been assessed for their antimicrobial properties, with a particular emphasis on their effects against cariogenic bacteria [11,12].

Clove (Syzygiumaromaticum) is a fragrant floral bud extensively utilized in Africa, Asia, and various global regions, commonly as a key ingredient in flavorful dishes. It falls under the Myrtaceae family and is renowned for its abundance of bioactive compounds [13,14]. Clove exhibits a wide range of properties, including antioxidative, fungicidal, viricidal, anti-diabetic, anti-inflammatory, antithrombotic, anesthetic, analgesic, and arthropod-repelling characteristics [15].

Syzygiumaromaticum boasts a rich reservoir of bioactive compounds with promising therapeutic potential. These compounds can alleviate symptoms such as cough, vomiting, flatulence, nausea, dyspepsia, diarrhea, stomach distension, and gastrointestinal spasms. Moreover, they can function as analgesics, nerve stimulants, and even induce uterine contractions [16].

The essential component present in all clove varieties is eugenol, a phenolic compound, which is particularly concentrated in the flowers and their stalks. Conversely, non-phenolic compounds like karyophilene (sesquiterpene) are primarily found in clove leaves [17]. Clove also boasts an abundance of vitamins, including A (retinol), beta-carotene, and vitamins K, B6, B1, and C [18,19]

In our research, we employed both aqueous and ethanolic clove extracts to investigate their potential protective effects against the detrimental impact of *Salmonella typhimurium* on the small intestine and liver in chickens.

2. Materials and Methods

Plant Material Collection

In 2020, we procured Plantago major, Azadirachtaindica, and Syzygiumaromaticum plants from markets in AL-Diwaniya province. These plants underwent rigorous certification by plant physiology experts at the University of AL-Qadisiyah (Certification No. 115, dated 16/11/2020). Subsequently, meticulous cleaning and mechanical grinding were performed to prepare the plant materials.

Plant Extraction

Plant seed extraction was carried out in accordance with the established techniques outlined by Dziedzic*et al*. (2013)[20]. The preparation of *Salmonella typhimurium* inoculums followed the precise protocol of the colony suspension technique, as specified by EUCAST in 2003[21].

Experimental Design

This study utilized 250-day-old Ross 308 chick strains sourced from a commercial hatchery in Al-Qadisiyah province. The research was conducted at the poultry farm within the College of Veterinary Medicine at the University of Al-Qadisiyah and spanned a duration of 35 days. The poultry coop's floor, walls, and ceiling underwent a meticulous cleaning regimen involving water and sodium hypochlorite, followed by fumigation using a commercial disinfectant. A two-day closure of the poultry coop was observed. All feeding and watering equipment underwent thorough cleaning and disinfection before being provided to the respective experimental groups. The hall grounds were appropriately furnished with wood sawdust as litter material. Lighting and ventilation inside the poultry coop adhered to established guidelines. The chicks were randomly distributed into five groups, each comprising 50 broiler chicks, following a completely randomized design. The feeding protocols for the chicks were as follows: Group 1 (- control): Chicks were exclusively fed a basal diet.

Group 2 (+ control): Chicks were orally administered *Salmonella typhimurium* (200 µl).

Group 3: Chicks were orally infected with *Salmonella typhimurium* (200 µl) and subjected to Ciprofloxacin antibiotic treatment (1 ml/liter of water, twice daily for one week).

Group 4: Chicks were orally infected with *Salmonella typhimurium* (200 µl) and received an aqueous Clove extract treatment (2 gm/Kg B.W) (2 ml administered orally per bird, twice daily for one week) [22].

Group 5: Chicks were orally infected with *Salmonella typhimurium* (200 µl) and treated with ethanolic Clove extract (2 gm/Kg B.W) (2 ml administered orally per bird, twice daily for one week) [22].

Collection of Samples

Intestine and liver tissue samples were detached from the birds and stored in 10% formalin for histological investigation.

Histopathological preparation

Slide preparation was undertaken according to method proposed by Luna, (1968)[23].

3. Results and Discussion

Small Intestine Histology

In the control group, small intestine sections exhibited normal, elongated villi covered by intact brush borders. Additionally, they displayed typical features in intestinal crypts, muscularis mucosa, and the submucosal layer (Fig.1(A1,A2)). In group 2, consisting of chicks orally infected with *Salmonella typhimurium*, small intestine sections revealed severe villous abnormalities. The mucosa appeared flattened with barely discernible villi. Inflammatory cell infiltration in the lamina propria, loss of intestinal glands, and crypt destruction were evident (Fig.1,(B1,B2)). In group 3, where chicks were orally infected with *Salmonella typhimurium* and treated with Ciprofloxacin antibiotic, histological examination showed marked hyperplasia of epithelial cells forming the brush borders of villi. The villi exhibited elongation and extension, and a notable proliferation of goblet cells was observed. The crypts displayed characteristic fibrous morphology (Fig.1(C1,C2)).

In group 4, chicks orally infected with *Salmonella typhimurium* and treated with aqueous Clove extract exhibited small intestine sections with normal, elongated villi covered by intact brush borders. Furthermore, they presented typical features in intestinal crypts, muscularis mucosa, and the submucosal layer (Fig.1(D1,D2)). In group 5, sections of the small intestine from chicks orally infected with *Salmonella typhimurium* and treated with ethanolic Clove extract displayed normal intestinal villi, a regular smooth muscularis, and serosa layers (Fig.1(E1,E2)).

Liver Histology

In group 1, liver sections exhibited a standard hepatic architecture, with hepatocytes arranged radially around the central vein (Fig.2(A1,A2)). In group 2, comprising chicks orally infected with *Salmonella typhimurium*, liver sections revealed the presence of microgranulomas, characterized by an accumulation of inflammatory cells, primarily lymphocytes, along with a few neutrophils. This may be associated with localized hepatocyte necrosis (Fig.2(b1,B2)). In group 3, where chicks were orally infected with *Salmonella typhimurium* and treated with Ciprofloxacin antibiotic, liver sections exhibited severe hydropic degeneration and necrosis of hepatocytes, sinusoidal engorgement with bile secretion, and a disruption of the typical arrangement of hepatocytes (Fig.2(C1,C2)). Group 4, consisting of chicks infected with *Salmonella typhimurium* orally and treated with aqueous Clove extract, displayed liver sections with a standard hepatic architecture. Hepatocytes were organized radially around the central vein, with slight sinusoidal dilation (Fig.2(D1,D2)).

In group 5, chicks infected with *Salmonella typhimurium* orally and treated with ethanolic Clove extract, liver sections showed the standard hepatic architecture. Hepatocytes were arranged radially around the congested central vein, accompanied by mild aggregation of inflammatory cells and sinusoidal dilation (Fig.2(E1,E2)). *Salmonella typhimurium* poses a significant threat, causing enteric infections in both humans and animals [24,25]. While antibiotic treatment remains the prevailing approach for managing Salmonellosis [26], the alarming rise in multi-drug-resistant strains is a growing concern [27]. Intestinal health hinges upon the harmonious functioning of various factors, including the integrity of the intestinal barrier, the level of inflammation, and the composition of the microbiota. The integrity of the intestinal barrier is pivotal, as its compromise can lead to reduced nutrient absorption, inflammation, and the translocation of harmful microbes and their byproducts, such as endotoxins, into the bloodstream [28].

The intestinal barrier is a complex structure composed of the lamina propria, tightly interconnected epithelial cells, and the mucous membrane. Central to its defense and regulatory functions are critical

components, including ZO proteins, Claudins, Occludin, and MUC2 mucin[29]. Notably, studies have highlighted the adverse impact of Salmonellosis on the mechanical integrity of the intestinal barrier, primarily through the downregulation of critical junctional proteins like ZO-1, Claudin-1, and Occludin, as well as MUC2 [30,31].

While inflammatory responses represent an intrinsic mechanism for identifying and eliminating invading pathogens and restoring tissue homeostasis, excessive inflammation can lead to tissue damage [32].This inflammatory response is further potentiated by the production of nitric oxide (NO) during Salmonellosis. NO acts as a stimulant for the host's immune response, but its excessive production results in harmful reactions, including the formation of peroxynitrite. Peroxynitrite, in turn, leads to lipid peroxidation of cell membranes and DNA damage.

Recent investigations have shed light on the substantial NO production within chicken macrophages during Salmonellosis [33], providing insight into the observed small intestine lesions following *Salmonella typhimurium* treatment.

The interplay between *Salmonella typhimurium* and the intestinal barrier's mechanical integrity, inflammation, and NO production underscores the complex pathophysiology of Salmonellosis. Understanding these intricacies is critical for developing effective strategies to combat this persistent and evolving threat.

The liver, a vital organ, stands as a primary target for the deleterious effects of reactive oxygen species (ROS) [34]. The detrimental consequences of ROS accumulation and resultant oxidative stress manifest through the activation of cell death pathways, involving both necrotic and apoptotic mechanisms, ultimately culminating in cellular and tissue damage.

While oxidative stress-induced injury significantly impacts parenchymal cells, other liver cell types, such as Kupffer cells, hepatocyte stellate cells, and endothelial cells, might exhibit heightened sensitivity or visibility concerning oxidative stress-related molecules. This heightened sensitivity amplifies the potential consequences of oxidative stress within the liver.

Notably, oxidative stress can stimulate the production of cytokines, including TNF-α, primarily within Kupffer cells. This cytokine production may further exacerbate inflammation and promote apoptotic processes [35,36].

In a previous investigation, the adverse effects of *Salmonella typhimurium* infection on liver function were evident through the marked elevation of ALT and AST levels in the bloodstream. Intriguingly, even after treatment with ciprofloxacin, oxidative stress markers remained significantly increased. These findings underscore the persistent impact of S. typhimurium infection on the liver.

Furthermore, despite treatment, animals exhibited pronounced tissue damage and inflammation, as indicated by elevated pro-inflammatory cytokine and chemokine secretion levels in both the blood serum and liver, a reflection of the activated macrophages' response [37].

A separate study by Alandiyjany*et al.* (2022)[38] corroborated these observations, revealing that *Salmonella typhimurium* challenge led to a substantial increase in pro-inflammatory cytokines and chemokines, as evidenced by mRNA expression levels in both the bloodstream and liver. The intricate interplay between oxidative stress, inflammation, and liver injury in the context of *Salmonella typhimurium* infection highlights the multifaceted nature of this pathophysiological process. Understanding these intricacies is paramount for devising effective strategies to mitigate the impact of this persistent and evolving threat.

This study underscores the remarkable potential of a clove extract, containing both aqueous and ethanolic components, at a dosage of 2 mg/kg, to ameliorate the condition of the small intestine and liver in chicks subjected to a challenge of 200 µl of *Salmonella typhimurium*. Cloves, abundant in dietary polyphenols, have long been recognized for their diverse medicinal benefits [39].

Cortés-Rojas *et al.* (2014)[40] previously established the potent antimicrobial activity of clove extract within the small intestine. Notably, in both group 4 and 5, histological examination of the small intestine revealed normal and elongated villi adorned with intact brush borders, alongside intact intestinal crypts. These findings align closely with Awad*et al.* (2009)[41], who correlated increased villus length and villus/crypt ratio with heightened epithelial cell turnover and associated longer villi with activated cell mitosis.

Moreover, prior research has illuminated the antioxidant prowess of clove extracts, surpassing that of synthetic antioxidants like butylatedhydroxytoluene (BHT). Impressively, the ethanolic extract of clove has demonstrated potent hepatoprotective activity against paracetamol-induced liver damage in female rats [42].

The liver, as the primary site for nutrient breakdown in the body [43], emerges as a key beneficiary of clove's therapeutic attributes. Both clove essential oil and extracts have exhibited hepatoprotective properties by mitigating liver toxicity [44]. In a pivotal study by Abdel-Rahman *et al.* (2006)[45] the inclusion of 500 mg/kg of clove powder in the diet of mice notably improved hepatocyte histology.Thehepatoprotective influence of clove can be attributed in part to eugenol, a bioactive compound within cloves, which effectively counters thioacetamide-induced liver damage.Thehepatoprotective mechanisms of clove extend beyond its antioxidant properties. Cloves and their primary component, eugenol, have demonstrated efficacy in preventing oxidative and tissue injury [46], suggesting a potential mechanism of action in mitigating liver cirrhosis. The enhanced activity of enzymes like glutathione peroxidase and catalase, integral to peroxide metabolism, has been attributed to eugenol-rich fractions [47]. Furthermore, eugenol's intrinsic antioxidant properties have been substantiated both in vitro and in vivo [48].

Recent research has uncovered immunomodulatory properties in both aqueous and ethanolic fractions of clove, particularly within macrophages [49]. Moreover, Estevão-Silva *et al.* (2014)[50] demonstrated that eugenol, administered at a dose of 250 mg/kg, significantly reduced leukocyte migration to perivascular tissue, thus underscoring its role in curtailing excessive leukocyte migration during inflammation.

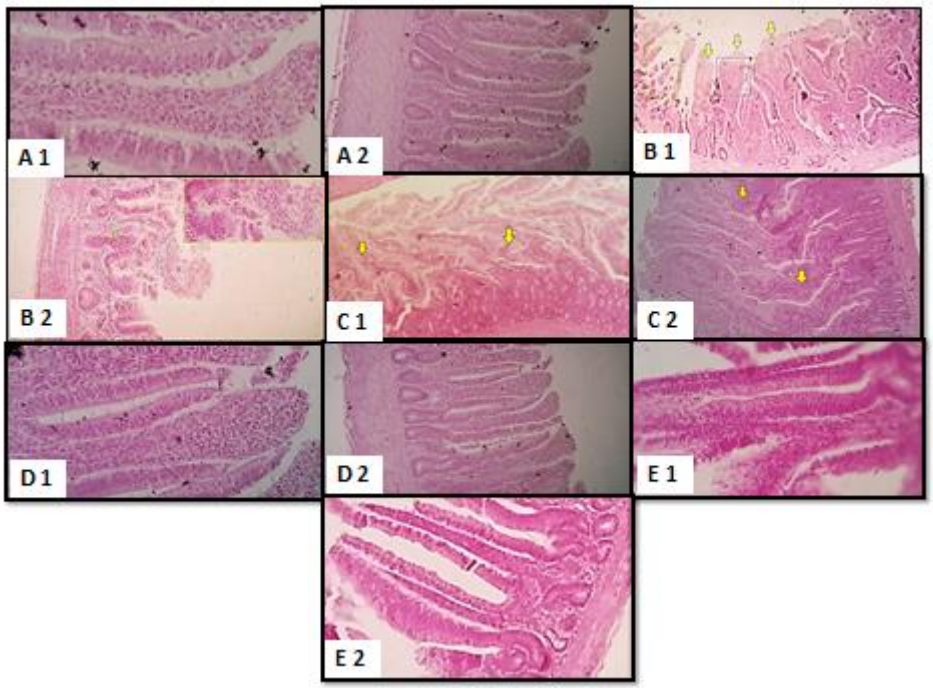

Figure 1, (A1, A2) depicts the small intestine in group 1, revealing well-preserved, elongated villi that are properly coated with intact brush borders. Furthermore, we can observe the presence of typical intestinal crypts, a muscularis mucosae, and the sub mucosal layer.(B1,B2) in group 2 shows severe villous abnormalities in the small intestine. The mucosa appears flat (white box) with barely discernible villi. Inflammatory cell infiltration (black box) is evident in the lamina propria, along with the loss of intestinal glands (yellow arrow) and crypt destruction. The villi exhibit irregular morphology, appearing shorter (yellow arrows) with degenerated epithelial cells (yellow box).(C1,C2)in group 3 displays pronounced hyperplasia of villi brush border epithelial cells, elongation and extension of the villi, increased goblet cell proliferation, and crypts with fibrous morphology.(D1,D2)the small intestine section from group 4 displays normal, elongated villi covered by intact brush borders. Additionally, it exhibits typical intestinal crypts, the muscularis mucosae, and the submucosal layer.(E1,E2)the small intestine in group 5 reveals normal intestinal villi, smooth muscularis, and serosa layers.(H&E stain; 1 X400; 2 X100).

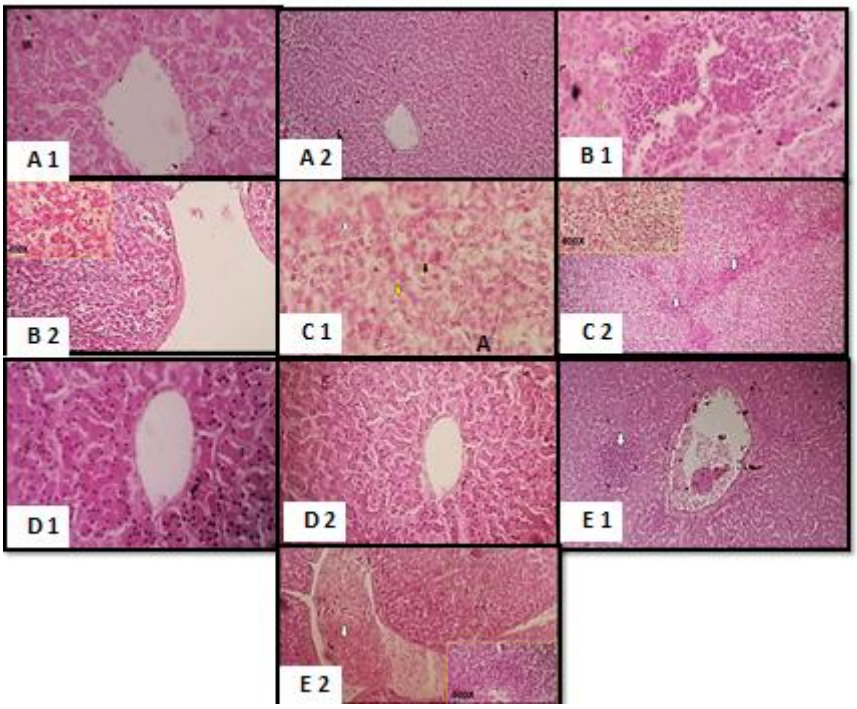

Figure 2,(A1,A2) section of live in group1shows standard hepatic architecture in which the hepatocytes radially around the central vein.(B1, B2), the liver section in group 2, displays a microgranuloma consisting of accumulated inflammatory cells, primarily lymphocytes (white arrows), and a few neutrophils (yellow arrows). This may be associated with small hepatocyte necrosis (white box).(C1, C2) depicts the liver section in group 3, revealing severe hydropic degeneration (black arrow) and hepatocyte necrosis (white arrow). There is also engorgement of sinusoids with bile secretion (yellow arrow) and disruption of the typical hepatocyte arrangement.(D1,D2) the liver section from group 4 exhibits the typical hepatic architecture, with hepatocytes arranged radially around the central vein. There is also a slight dilation of sinusoids observed.(E1, E2), the liver section from group 5 demonstrates the typical hepatic architecture, with hepatocytes arranged radially around the congested central vein (white arrow). Additionally, mild aggregation of inflammatory cells (yellow box) and sinusoidal dilation are observed. The highlighted region represents inflammatory cells.(H&E stain; 1 X400; 2 X100).

4. Conclusion and future scope

This study highlights the promising potential of clove extracts in mitigating liver fibrosis and cirrhosis by bolstering antioxidant defenses and quelling inflammation, as exemplified by the reduced migration of leukocytes. Clove emerges as an effective countermeasure against oxidative stress, ultimately curbing the production of reactive oxygen species (ROS). We used an aqueous and ethanolic extract of clove to investigate its possible protective role against the deleterious effect of Salmonella Typhimurium on small intestine and liver in chicken. An aqueous and ethanolic extract of clove administered at a dosage of 2 mg/kg exhibit the ability to reduce histological alterations in the intestine and liver due Salmonella typhimuriuminfection.Theaqueous extract has more activity in improving tissues of liver and intestine in chickens than ethanolic extract.

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Author Contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval: The students gotten ethical approval from the research Ethical Approval Committee of the University of Al-Qadisiyah.

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