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Evaluation of anti-emetic activity of *Syzygium aromaticum* extracts in chick and rat models of emesis

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ABSTRACT: Emesis, a multifaceted physiological response, presents significant challenges in clinical scenarios such as chemotherapy-induced nausea and vomiting. Novel antiemetic interventions are imperative to enhance patient care and well-being. This study investigated the antiemetic potential of *Syzygium aromaticum* (clove) extract using a comprehensive approach involving phytochemical analysis and animal models of emesis. Using chick model of emesis, male chicks aged 4 days were assigned to groups receiving different treatments, including copper sulfate, clove extract and metoclopramide. The effectiveness of treatments were assessed by monitoring retching frequency and changes in feed intake. Similarly, albino Wistar rats were used modelling emesis in rat and were categorized into groups receiving cisplatin, clove extract and metoclopramide as a standard control. Emetic responses were evaluated through pica behaviour assessment. In the chick model, a single dose of the following drugs was administered, either orally (p.o) or intra-peritoneally (i.p) as following: Copper sulfate at 50mg/kg p.o., Clove extract at 50mg/kg p.o, and Metoclopramide at 100mg/kg i.p. For the rat animal model, a once-daily dosage of cisplatin at 3 mg/kg i.p, metoclopramide at 2.5 mg/kg i.p., and clove extract at 100 mg/kg p.o. for three consecutive days were administered. Phytochemical analysis unveiled the intricate composition of clove extract, with eugenol standing out as a prominent constituent (71.56%). In the chick model, clove extract reduced copper sulfate-induced retching, displaying an impressive 84.63% inhibition rate. Similarly, in the rat model, the extract significantly alleviated cisplatin-induced pica behavior, with a statistically significant reduction in retching instances ($P < 0.05$). Clove extract's multifaceted antiemetic effects, attributed to eugenol and other bioactive components, provide promising insights for managing emesis. The study's findings hold translational significance, suggesting clove extract's potential as a complementary antiemetic therapy alongside conventional treatments. The identification of eugenol as a key contributor warrants further investigation into molecular mechanisms, offering hope for effectively managing chemotherapy-induced nausea and vomiting.

KEYWORDS: Clove extract; antiemetic; phytochemical analysis; chick model; rat model; eugenol.

1. INTRODUCTION

The act of vomiting encompasses three phases: the preejection phase, retching, and the actual emetic event. Various triggers for vomiting include exposure to toxins, side effects of medication, cancer treatments, radiosurgery, the initial trimester of pregnancy, and postoperative procedures. This process is facilitated by the combined actions of dopamine type-2, serotonin, and muscarinic cholinergic, histamine, opioids, cannabinoids, gamma - aminobutyric acid, and neurokinin receptors [1]. Despite its importance, emesis can have negative consequences, affecting patients' quality of life. This has sparked an increased effort for improved nontoxic antiemetic treatments. To learn about the causes of vomiting and the ways in which antiemetic drugs work, further research is needed. Drugs such as the opioid apomorphine, which activates

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central dopamine receptors directly, and xylazine, an alpha-2 adrenergic agonist with a well-documented emetic action in cats, are just two examples of the many that have been investigated [1-2]. Hydrogen peroxide and other potential vomiting inducers have also been studied for their effects on the ninth cranial nerve [3-5]. However, several items are not suggested for causing emesis in dogs and cats because of their possible toxicity. These include ipecac syrup, sodium chloride, and powdered mustard [6].

Our research aims to find new ways to alleviate emesis, especially in the context of cisplatin-induced vomiting. As an alkylating medication, cisplatin is used to treat cancer. It has been shown to effectively halt cell division, which kills tumour cells. On the other hand, it causes extreme sickness and vomiting, which can be a major problem for patients receiving therapy [6-7]. Several studies in the past few years have examined the effectiveness of various drugs in reducing emesis and improving patients' health. The phytotherapeutic method of treating cisplatin-induced vomiting in pigeons was the subject of research conducted by Ihsan Ullah et al. (2022). Researchers found that combination 4 was more effective than metoclopramide in preventing cisplatin-induced vomiting because of its antiserotonergic and antidopaminergic actions [8]. The gingerol compound has also been studied for its potential antiemetic effects; it is found in ginger. Li Tian et al. (2019) examined how gingerol affected cisplatin-induced emesis in rats and minks. Researchers discovered that gingerol prevented rats from eating kaolin and minks from throwing up after receiving chemotherapy [9]. Several animal models have been used to investigate the effectiveness of antiemetic drugs, with strain differences proving to be crucial. DBA/2 mice were found to be useful in preclinical studies for analysing the emetogenic or anti-emetic potential of drugs [10], as discovered by Kouichi Yamamoto et al. (2018), who studied strain differences in cisplatin-induced pica behaviour in mice. Studies on the anti-emetic and anti-inflammatory properties of Luffa cylindrica fruit peel [11-12] show that chick emesis models are useful for evaluating natural antiemetics.

Syzygium aromaticum (Clove) has emerged as a valuable spice with medicinal properties, including carminative and stimulant effects. Because of its unique chemical composition, clove essential oil shows promise for a wide range of uses, from food flavouring to antibacterial and antiseptic protection. Additional research into clove's antiemetic potential is necessary [13]. Finding effective treatments for nausea and vomiting requires testing potential drugs in animal models. Long-term cisplatin administration in rats was proposed by Gema Vera et al. (2014) as a model for studying nausea caused by antineoplastic treatment and the effectiveness of new antiemetic drugs [13-14]. S.S. Sharma and Y.K. Gupta (1998) conducted an additional study showing that ginger juice reduced chemotherapy-induced nausea and vomiting in cancer patients [15]. In summary, emesis is a complex phenomenon with potentially life-threatening consequences, particularly for cancer patients undergoing cisplatin chemotherapy. Improving patients' quality of life during treatment requires the discovery of effective and safe antiemetic medications [16].

Our intent is to learn more about what causes nausea and vomiting and how antiemetic drugs such as metoclopramide and cisplatin, as well as more natural drugs such as clove, work to prevent these symptoms. We want to assess the antiemetic potential of these medicines, as well as their impact on emetic behaviour and related side effects, using a range of animal models. Patients in a variety of clinical settings will benefit from the improved care and treatment results that may be achieved through the study's findings on antiemetic approaches. The major objective of this study was to investigate the possible antiemetic effects of clove extract in an animal model, focusing on emesis generated by cisplatin and copper sulfate. The therapeutic relevance of learning whether clove extract is effective as an antiemetic agent against emesis generated by these drugs cannot be overstated. The purpose of this research was to determine whether clove extract might alleviate cisplatin- and copper sulfate-induced emesis in a chick emesis model, providing important information for the development of natural treatments for nausea and vomiting. The results of this study have the potential to contribute to the creation of innovative antiemetic treatments that are both effective and devoid of the negative effects of current commercial antiemetic medications. The study's overarching objective is to improve patient care and outcomes by expanding our understanding of natural antiemetics and their use in clinical practice.

1.1 Drug Profile

The essential oil of the Myrtaceae plant *Syzygium aromaticum*, sometimes known as clove, is highly prized for the eugenol it contains [17]. *Table 1* contains comprehensive information about clove extract [18-22] which are known to aid in digestion, reduce stress, and clears up acne. Use with care is advised due to the potential for adverse effects such as convulsions and liver damage [23-25]. The antiemetic properties shown in animal models have brought attention to this fragrant spice. *Table 2* summarizes the steps involved in steam hydro distillation, the method used to extract clove by placing dried clove buds in a steam flask and

collecting the distillate at regular intervals. Clove extract is made by repeating the distillate extraction process using n-hexane as the solvent [18-24]. Anticancer Properties: While the exact mechanism is unknown, clove extract is believed to have potential anticancer properties due to its antioxidant and anti-inflammatory effects.

Treatment of Oral Diseases: Clove extract interrupts action potentials, contributing to its effectiveness in the treatment of oral diseases.

Anticandidal & Antifungal: The antifungal properties of clove extract make it effective against candidal infections and other fungal ailments.

Acne Treatment: Its anti-inflammatory and antiseptic qualities are utilized in acne treatment, helping to reduce skin inflammation and infection.

Stress Relief: Clove extract possesses neuroprotective characteristics, which can aid in stress relief by protecting nerve cells from damage.

Joint Pain Relief: The analgesic properties of clove extract provide relief from joint pain, reducing discomfort and inflammation.

Antiseptic for Wounds: Due to its antiseptic properties, clove extract is beneficial for cleaning wounds and preventing infections.

Improves Skin Radiance: The antioxidant qualities in clove extract help in improving skin radiance and overall skin health.

Aids Digestion: Clove extract aids in digestion, although the exact mechanism is not fully understood. Its anti-inflammatory properties may play a role in soothing the digestive tract.

Antipyretic Effects: As an antipyretic, clove extract can help in reducing fever, thanks to its ability to combat inflammation.

Table 1. Clove Extract

Property	Clove Extract
Plant Profile	*Syzygium aromaticum* (Family: Myrtaceae)
Composition	Predominantly eugenol
Metabolism	Metabolized to homo vanilic acid and 4-hydroxy-3-methoxy mandelic acid
Route of Elimination	-Rapid absorption -Excreted in urine
Half-Life	Plasma: 14 hrs; Blood: 18 hrs
Toxicity	Acute oral toxicity LD50: 2650 mg/kg (rats) Lethal dose in humans: 3.75 g/kg body weight Contact may cause irritation, contact dermatitis, inflammation of lips, mouth ulceration, hypersensitivity
Adverse Effects	Seizures, liver damage, fluid imbalance, bleeding disorders (with high doses)

Table 2. Extraction process of clove

Extraction Method	Steam Hydro Distillation
Plant Material	Dried Clove Buds
Solvent Used	n-Hexane
Steps	1. Clove buds placed in a steam flask 2. Steam distillation conducted over time intervals (3, 4, 5, and 6 hours) 3. First drop of distillate marks the start of counting time 4. Collected distillate further extracted with n-Hexane in a separatory funnel 5. Clove extract obtained by evaporating the n-Hexane

The phytochemical study of clove extract plays a crucial role in identifying its bioactive compounds, as summarized in *Table 3* [18 and 27]. Various qualitative tests were conducted to detect the presence of specific phytochemical classes in the extract. The color test for eugenol confirmed its presence, exhibiting characteristic reactions such as disappearance of yellow color in the bromine test and formation of precipitates in the permanganate test. Furthermore, the phenol test revealed the presence of an OH- group in the extract [28-29].

In-depth qualitative tests for other compounds were also performed. Carbohydrates were detected using the Molisch test and the reduction of Fehling's solution, resulting in reddish-violet rings and brick-red

precipitates, respectively. Proteins were identified through the Biuret test, showing a violet or pink colouration upon reaction with NaOH and CuSO4, as well as Million's test, indicating the formation of white precipitates turning brick red upon warming or dissolving [19, 30]. Steroids were detected using the Salkowski reaction, displaying greenish-yellow fluorescence, and the Liebermann-Burchard reaction, producing blue to blood-red colouration. Glycosides were confirmed through the Keller-Killani test, Borntrager's test, and Legal test, exhibiting reddish-brown, reddish, and blue color solutions, respectively [29-31].

Table 3. Phytochemical study of clove extract

Phytochemical Tests	Presence of Compound(s) Detected
Eugenol Color Test	Eugenol detected
Carbohydr Test	Detected with Molisch Test (reddish-violet ring) and Reduction of Fehling's Solution (brick-red precipitates)
Proteins	Detected with Biuret Test (violet or pink color) and Million's Test (white precipitates turning brick red or dissolving)
Steroids	Detected with Salkowski Reaction (greenish-yellow fluorescence) and Liebermann-Burchard Reaction (blue to blood-red color)
Glycosides	Detected with Keller-Killani Test (reddish-brown color at junction), Borntrager's Test (reddish color), and Legal Test (blue color solution)
Alkaloids	Detected with Dragendroff's Test (orange–brown precipitates) and Wagner's Test (reddish-brown precipitates)
Tannins	Detected with Gelatine Test (curdy white precipitates), Lead Acetate Test (white precipitates), and Ferric Chloride Test (blue–green color)
Saponins	Detected with Foam Test (formation of foam)
Flavonoids	Detected with Dilute Ammonia and Concentrated Sulfuric Acid (yellow colouration)
Cardiac Glycosides	Detected with Keller-Killiani Test (brown ring at interface)
Triterpenes	Detected with Chloroform and Concentrated Sulfuric Acid (red color)
Anthraquinones	Detected with Benzene and Ammonia Solution (pink color in ammonical layer)

Alkaloids were identified using Dragendroff's and Wagner's tests, displaying orange–brown and reddish-brown precipitates, respectively. The presence of tannins was confirmed through the gelatine test, lead acetate test, and ferric chloride test, which yielded curdy white precipitates, white precipitates, and blue–green colour, respectively. Saponins were detected through the foam test, forming foam after shaking with sodium bicarbonate. Additionally, flavonoids were identified when dilute ammonia and concentrated sulfuric acid produced a yellow colouration that disappeared on standing. Cardiac glycosides were confirmed by the formation of a brown ring at the interface in the Keller-Killiani test [29-32]. Triterpenes were detected when chloroform and concentrated sulfuric acid produced a red color, and anthraquinones were identified through a pink color in the ammonical layer after shaking with benzene and ammonia solution. This comprehensive phytochemical study provides valuable insights into the diverse compounds present in clove extract, supporting its potential therapeutic applications. Understanding the bioactive components of clove extract is essential for further research and exploration of its antiemetic effect in animal models, which holds promise for future pharmaceutical advancements. However, it is crucial to recognize that clove oil should be used with caution, as it can cause severe side effects, such as liver damage and fluid imbalance, emphasizing the importance of responsible and informed usage in medicinal applications [33].

Intriguingly, among these compounds, eugenol emerged as a dominant constituent, constituting 71.56% of the clove extract composition represented in Table 4 [42]. This compound holds significance due to its multifaceted pharmacological activities. Moving beyond the phytochemical analysis, the study delves into the antiemetic effects of the clove extract. Eugenol is recognized for its diverse therapeutic activities, ranging from antimicrobial and anti-inflammatory effects to its potential in alleviating pain and promoting digestive health. Such a dominant presence of eugenol accentuates the potential of clove extract as a valuable source of bioactive compounds with a wide range of health-promoting effects. The significant concentration of eugenol presents a compelling rationale for the subsequent investigation into the extract's antiemetic effects, hinting at the diverse pathways through which it could potentially contribute to the mitigation of emesis.

2. RESULTS

2.1. Phytochemical analysis of clove extract

There are many different phytochemical components that may alter vomiting, as shown by research into the potential antiemetic benefits of *Syzygium aromaticum* (clove) extract. The many chemicals identified by the phytochemical examination of clove extract—including carbohydrates, lipids, alkaloids, flavonoids, tannins, sterols, and triterpenes—attest to the extract's complexity. The variety of compounds found increases its potential medical value, even if proteins, saponins, cardiac glycosides, and anthraquinones were noticeably absent. The substance's complex chemical make-up provides a solid foundation for future research into its possible antiemetic capabilities. The presence or absence of the identified phytochemicals is shown across a variety of assays, and the results are summarized in Table 4.

Table 4. Composition of clove extract

Compound	Concentration (%)
p-cymene	0.9
S-Hexene-2-one	0.67
Thymol	0.87
Eugenol	**71.56**
Eugenol acetate	8.99
Caryophyllene oxide	1.67
Guaiol	0.9
Benzene-1-butyheptyl	0.55
Nootkatin	1.05
Isolongifolanone	0.86
Hexadecanoic acid	0.5
9,17 Octadeca-dienal	0.24
Octadecanoic acid butyl ester	0.33
Phenol	0.98
Dodeca trienoic acid-3,7,11 trimethyl ester	0.38
Vitamin E acetate	0.43

Table 5 summarizes the primary phytochemical study of the clove extract, which indicates a broad array of components that may subtly impact its potential therapeutic effect. Multiple investigations may help determine whether particular components are present in the extract, which may provide light on the extract's chemical complexity. The discovery of several bioactive chemicals in it—including sugars, lipids, alkaloids, flavonoids, tannins, sterols, and triterpenes—provides the foundation for our confidence in its pharmacological potential. Because of its selective composition, the extract does not include any proteins, saponins, cardiac glycosides, or anthraquinones. Multiple testing techniques improve the validity of the results, showing that a complex interaction of bioactive chemicals may be responsible for its antiemetic benefits. These results serve as a critical foundation, guiding the subsequent exploration of the clove extract's antiemetic properties and contributing to a deeper understanding of its potential therapeutic applications.

Table 5. Results of the phytochemical analysis of clove extract

Test		Result
Carbohydrate	1.Molisch test	+
	2.Fehling's test	-
Proteins	1.Biuret test	-
	2.Million's test	-
Steroids and sterols	1.salkowsky test	+
	2. Liebermann Burchard test.	+
Glycosides	1.Killer-killani test	+
	2.Borntrager's test	+
	3.Legal test	+
Alkaloids	1.Dragendroff's test	+
	2.Wagner's test	+
Tannins test	1.Gelatin test	-
	2.Lead Acetate test	-
	3.Ferric chloride test	+
Saponins	Foam test	-
Cardiac Glucosides	Keller – Killiani test	-
Triterpenes test		+
Anthraquinones test		-

2.2. Anti-emetic activity of clove extract in chick model of emesis

Utilizing a chick model, the antiemetic activity was evaluated, providing insights into its potential to mitigate copper sulfate-induced retching. Acute emesis in humans may be replicated in chicks, making them a useful model for testing plant extracts for their ability to prevent or alleviate vomiting. The evaluation of anti-emetic action in chicks is shown in Table 6 and Figure 1, demonstrating the effectiveness of the clove extract in lowering the incidence of vomiting. Table *6* depicts the results of an investigation of the antiemetic activity of clove extract, which adds to our understanding of its potential therapeutic application and expands the scope of the study. To mimic the acute emesis seen by people, this study used a chick model to test the extract's effectiveness in preventing vomiting caused by copper sulfate. The emetic reaction was clearly seen in the large number of retches displayed by the toxic control group administered copper sulfate. In contrast, metoclopramide, a popular antiemetic medicine, dramatically decreased the amount of retches in the control group, demonstrating its efficacy in preventing emesis. Clove extract with copper sulfate confirmed the usefulness of this paradigm in a clinical trial. The reduction in the number of retches in this group signifies the extract's potential antiemetic effects. The decrease in retches indicates its capacity to counteract the emetic response induced by copper sulfate. The percentage inhibition underscores the magnitude of this effect, demonstrating that clove extract significantly curtailed the frequency of retching by 84.63%. The robustness of these findings is underscored by the statistical significance ($P < 0.001$) observed through unpaired Student's t test, affirming the antiemetic potential of clove extract. These outcomes align with the phytochemical composition highlighted earlier, with compounds such as eugenol potentially contributing to its antiemetic properties. The study's ability to demonstrate substantial inhibition of emetic responses through clove extract administration strengthens its candidacy as a potential natural remedy for managing nausea and vomiting, especially in settings such as chemotherapy where these symptoms pose significant challenges.

Table 6. The anti-emetic effect of clove extract

Toxic control: Copper sulfate		
Classification of chick	**Chick weight**	**Retches**
Head mark	45 g	97
Body mark	45 g	91
Wing mark	40 g	104

Standard: Metoclopramide +Copper sulfate		
Classification of chick	**Chick weight**	**Retches**
Head mark	40 g	5
Body mark	35 g	8
Wing mark	40 g	9

Test: Clove extract +Copper sulfate		
Classification of chick	**Chick weight**	**Retches**
Head mark	45 g	22
Body mark	45 g	9
Wing mark	40 g	14

Group	**Number of retches (Mean±SEM)**	**% inhibition**
Toxic control (Copper sulfate)	97.6±2.849	-
Standard group (Metoclopramide+Copper sulfate)	7.3±1.196*	92.52
Test (Clove Extract+Copper sulphate)	15±3.757*	84.63

* P < 0.001 is significantly different from control value using unpaired student's t test.

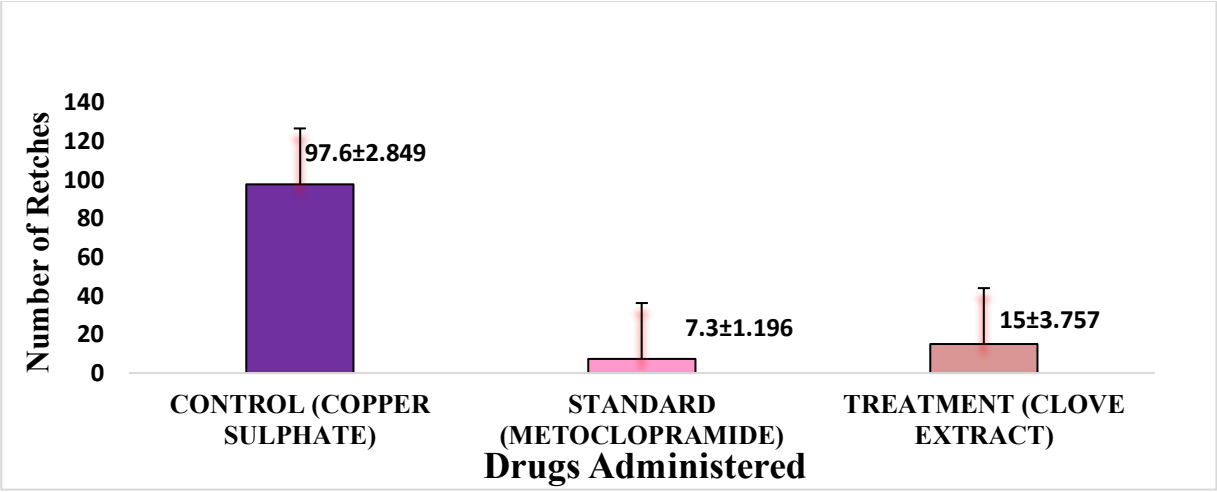

Figure 1. Number of Retches for Control, Standard and Clove Extract in Chick Model of Emesis

The average number of vomiting episodes per chick in the control group was 15, and an extract of clove (*Syzygium aromaticum*) was shown to be most effective in preventing vomiting episodes (84.63 percent). With metoclopramide, the typical treatment, the average number of retching episodes was reduced by 92.52% to 7.3. Control subjects vomited an average of 97.6 times (Figure 1). Since oral copper sulfate induces emesis by peripheral action through excitation of visceral afferent nerve fibres of the GIT, it follows that clove extract (*Syzygium aromaticum*) has protective effects against copper sulfate-induced retching in young chicks. Emesis caused by copper sulfate has also been linked to peripheral 5-HT4 [37-38]. The findings are

statistically significant and on par with those obtained using metoclopramide as a reference medicine, but the underlying mechanism of action and offending chemicals remain unknown. Therefore, eugenol could be responsible for the antiemetic action. This means that these findings need to be replicated in additional experimental models, and the associated action of the compound(s) is needed to further identify the anti-emetic phytochemicals [39-41]. To further confirm the anti-emetic activity of clove extract (*Syzygium aromaticum*), the following activity was tested in rats.

2.3. Anti-emetic activity of clove extract in rat model of emesis

Transitioning to rat models, the investigation deepens with a focus on cisplatin-induced emetic responses. The study assessed pica behavior as an indicator, shedding light on the extract's potential antiemetic properties. The toxic effects of cisplatin were evident in the rats, inducing pica behavior characterized by irregularities in nerve cell arrangement and ileum morphology (Figures 3 and 4). Interestingly, administration of clove extract demonstrated a remarkable capacity to mitigate these effects, suggesting a protective influence on nerve cells in the area postrema and on the mucosal integrity of the ileum.

In Table 7, we present the results of a rat model study investigating the potential protective effects of clove extract against cisplatin-induced emetic responses. The evaluation's broad scope highlights the extract's potential to mitigate cisplatin's side effects over a wide range of times. The severe emetic responses generated by cisplatin are reflected in the higher incidence of vomiting in the toxic control group after treatment. This study demonstrates the efficacy of the model in capturing essential characteristics of chemotherapy-induced nausea and vomiting. There was a substantial reduction in vomiting episodes across all time periods in the regular metoclopramide group, supporting the drug's established efficacy in the treatment of emesis. Clove extract was shown to reduce nausea and vomiting in a cisplatin-induced nausea and vomiting study. Notably, there was a substantial decrease in retching instances across various time intervals, with a statistically significant effect observed at several time points ($P < 0.05$). This suggests that clove extract administration yielded notable protection against cisplatin-induced emesis. These findings collectively resonate with the antiemetic effects observed in the chick model and align with the extract's multifaceted nature. The protective influence on both the area postrema and the ileum, as highlighted in earlier discussions, complements the potential mechanisms through which clove extract counters emetic responses. The outcomes from this rat model offer translational relevance, indicating that clove extract could hold promise in mitigating chemotherapy-induced nausea and vomiting in clinical scenarios. These findings pave the way for further exploration into the underlying mechanisms, signaling pathways, and potential roles of key compounds such as eugenol. By building upon the evidence garnered from various models, this study underscores the potential of clove extract as a holistic approach to managing emesis, contributing to improved patient well-being during chemotherapy regimens. Similarly, after 24 hours of receiving cisplatin, mice exhibited an initial acute episode of pica behaviour (Figure 2). Pica behaviour in rats treated with metoclopramide was considerably reduced (P 0.05) for the whole 24 hours after cisplatin treatment. However, there is a dramatic drop in kaolin concentration after 24 to 72 hours. Clove extract (*Syzygium aromaticum*) pretreatment substantially lowered (P 0.05) cisplatin-induced kaolin intake in rats over the course of 72 hours. Similar to the control group, the pica conduct of the rats did not change.

Table 7. Pica induced by cisplatin in rats

Groups	Normal control	Toxic control	Standard	Test
Time	**(mg)**	**Cisplatin (mg)**	**Metoclopramide (mg)**	**Clove extract (mg)**
6 hrs	1±0.57	635±7.93	1054.33±3.28	807.66±2.96
12 hrs	3.33±0.33	613.33±3.52	1052.33±2.84	753±3.05
18 hrs	43.33±0.88	571±5.50	1034.66±4.05	718±1.25
24 hrs	24.33±2.33	508.33±2.33*	719.66±1.76**	650±1.15***
30 hrs	25±2.88	259.66±4.91	673.33±1.76	623.33±1.76
36 hrs	51±2.08	237±3.21	645.33±3.52	554.33±1.20
42 hrs	18.33±0.88	218±1.73	560±1.73	472.33±2.02
48 hrs	19±2.08	155±2.64*	449.33±4.05**	445±3.05***
54 hrs	14±1.52	74.66±2.96	232.33±1.76	344.33±2.40
60 hrs	20.33±2.028	52.33±2.02	205.33±3.18	287±2.08
66 hrs	25.66±2.0	21.33±1.85	155±2.64	224±2.88
72 hrs	29.33±1.76	14.66±1.45*	189.33±1.45**	103±2.64***

Value were expressed as Mean± SEM (n=4), P < 0.05 is significantly different from control. P < 0.05 is significantly different from Toxic control, P < 0.05 is significantly different from toxic control

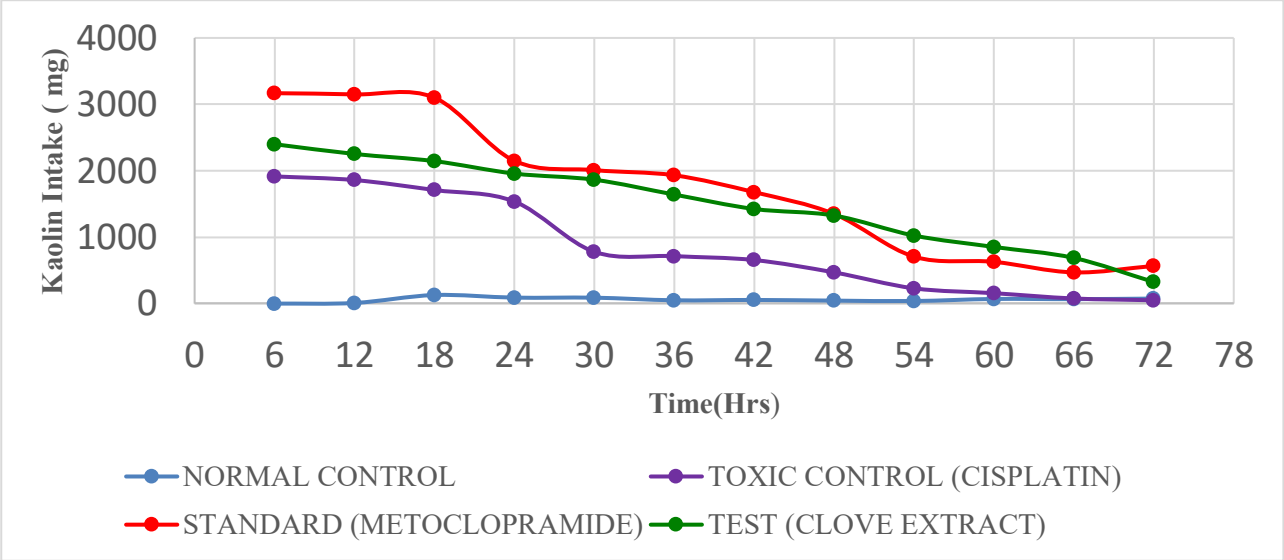

Figure 2. Pica induced by cisplatin in rats

3. DISCUSSION

The cumulative evidence gleaned from these analyses underscores the multifaceted potential of clove extract as an antiemetic agent. Its ability to mitigate emetic responses through both neuroprotective mechanisms in the area postrema and mucosal protective effects in the ileum presents a comprehensive approach to managing emesis. Notably, the presence of eugenol within the clove extract emerges as a potential contributor to these effects, although further research is needed to elucidate the exact mechanisms and responsible compounds. These findings hold significant implications for addressing challenges associated with chemotherapy-induced nausea and vomiting. Clove extract has been shown to reduce emetic reactions in both chick and rat models, suggesting that it may have therapeutic utility. Because of its potential to protect nerve cells and maintain mucosal health, clove extract is being studied as a potential complement to standard antiemetic drugs. Our comparison study also suggests a dose-dependent association, suggesting that optimal dosing needs more research. Clove extract's antiemetic actions show promise in improving the quality of life for those receiving chemotherapy, demonstrating its translational potential.

In summary, this research employs both chicks and rats as animal models to comprehensively investigate the potential antiemetic effects of clove extract, copper sulfate, and metoclopramide. The study analyzes various parameters to gain valuable insights into how these compounds could reduce emetic responses triggered by cisplatin and copper sulfate. Throughout the research, strict adherence to ethical principles, meticulous planning, systematic treatment procedures, and thorough statistical analysis are ensured. This extensive investigation into clove extract's antiemetic properties underscores its significance as a natural therapeutic agent for managing emesis. Its intricate chemical composition, primarily driven by eugenol, presents diverse potential therapeutic uses. Additionally, future research opportunities could explore further the observed protective effects on nerve cells and the preservation of mucosal integrity in the area postrema and ileum. Clove extract's multifaceted approach in preventing emetic reactions offers promise as a potential antiemetic medication, particularly for individuals dealing with chemotherapy-induced nausea and vomiting.

4. CONCLUSION

After extensive research, it became clear that clove extract (*Syzygium aromaticum*) showed value in reducing copper sulfate- and cisplatin-induced nausea and vomiting by acting as an antiemetic. Extensive testing in this study revealed the extract's multifaceted antiemetic actions, providing new insight into its possible utility as a natural cure for treating chemotherapy-induced nausea and vomiting.

An in-depth phytochemical study was the first step in this investigation; it uncovered the complex composition of the clove extract. Our study discovered a wide variety of bioactive chemicals, the most abundant of which was eugenol (making up 71.56% of the extract). The extract's medicinal potential is amplified by the high content of eugenol, a compound with proven pharmacological relevance that may contribute to the observed antiemetic effects. Remarkably, these results fit well with our evaluation of antiemetic effects in chick and rat models. The chick model elucidated the extract's potency in countering copper sulfate-induced retching, demonstrating its ability to inhibit emesis. In parallel, the rat model illuminated the extract's capacity to mitigate cisplatin-induced pica behavior, providing insights into its protective influence on the area postrema and ileum.

The culmination of these findings underscores the multifaceted antiemetic efficacy of clove extract. Its intricate chemical composition, spearheaded by Eugenol, serves as a foundation for diverse pharmacological activities. The demonstrated capacity to mitigate emetic responses through both neuroprotective and mucosal protective mechanisms highlights its holistic approach to managing emesis.

In a broader clinical context, these results hold promise for improving the quality of life for individuals undergoing chemotherapy. The extract's potential as a supplementary antiemetic therapy to conventional treatments opens new horizons in patient care. Moreover, the identification of eugenol as a significant contributor warrants future molecular-level investigations to unravel the extract's intricate mechanisms. In summary, the present study not only validates the antiemetic effects of clove extract through robust experimental evidence but also lays the groundwork for further research, offering a ray of hope for individuals facing the challenges of chemotherapy-induced nausea and vomiting. Further study is needed to evaluate the antiemetic activity at the molecular level to further confirm its activity.

5. MATERIALS AND METHODS

5.1. Study Design and Extraction of *Syzygium aromaticum*

In this research, we aimed to explore the antiemetic potential of clove extract (*Syzygium aromaticum*) against induced emesis triggered by cisplatin and copper sulfate. The study follows a meticulous plan encompassing various stages to ensure comprehensive assessment and reliable results. Before commencing the study, essential groundwork was undertaken to ensure research integrity and ethical adherence of Institutional Animal Ethics Committee (IAEC) clearance is obtained and Preparation and review of study protocol and consent forms with certificate number (IAEC/N.Jegan/262/KMCP/2022).

5.2. Chick Model of Emesis

During our study, we utilized chicks as the animal model for our investigation. Specifically, 4-days-old chicks within a weight range of 150-200 grams were selected in the study. The male chicks were divided into three groups, each consisting of three animals. The substances utilized included copper sulfate, clove extract and metoclopramide injection. The chicks were carefully chosen and acclimatized under controlled conditions with a 12/12-hour light/dark cycle. Housed in plastic cages, they had access to rice and water. Institutional Animal Ethics Committee clearance was obtained before commencing the study, adhering to ethical guidelines. A standard diet and hygienic environment were maintained, and a 2-day quarantine period was observed for the chicks. The chicks were categorized into three groups: toxic control, standard control, and treatment control. These groups received varying administrations of copper sulfate, metoclopramide, and clove extract. The substances were carefully administered in specified doses, ensuring accuracy and consistency in the study. One single dose of drug administration of the following was used: Copper sulfate (50mg/kg p.o.), Clove extract (50mg/kg p.o.) and Metoclopramide (100mg/kg i.p). The efficacy of the treatments was assessed by monitoring retching frequency and changes in feed intake. These parameters provided valuable insights into the potential antiemetic effects of clove extract and other substances in the chick model [8,27].

5.3. Rat model of Emesis

We employed Albino Wistar rats as the animal model to further investigate antiemetic effects. These adult rats, aged 6 months with a weight range of 200-250 g, were selected for their suitability. Both male and female rats were included in the study, enhancing its comprehensiveness. Like the chick model, the rats were sourced and acclimatized under controlled conditions [9-10, 19]. A 12/12-hour light/dark cycle, plastic cages, and a standard pellet diet were maintained. Ethical clearance and a 15-day quarantine period ensured the ethical and controlled setup required for the research. The rats were categorized into four groups [25]. Each group received a distinct treatment involving cisplatin, clove extract, metoclopramide, or normal saline. These treatments were administered intraperitoneally and orally as per the designated doses. The drug administration regimen comprised cisplatin at 3 mg/kg administered intraperitoneally, metoclopramide at 2.5 mg/kg administered intraperitoneally, and clove extract at 100 mg/kg administered orally. These administrations were conducted over a three-day period. On the third day, cisplatin was adjusted to 4 mg/kg and given intraperitoneally 30 minutes following each administration of metoclopramide or clove extract. After treatment of cisplatin, rats were perceived at every 6 h uninterruptedly for 72 h for the consumption of kaolin of rats [9,34-36].

To further evaluate emetic responses, we recorded and analyzed the intake of kaolin, a substance that induces vomiting-like behavior. In both animal models, the tested drugs, kaolin with 7% gum arabic were combined together with distilled water to prepare a thick paste. This paste was kept in a tube and partly dried using a dryer. The paste was removed from the tube, and was cut into the same size as the normal feed pellets. Next, it was dried entirely in a dryer [9,34]. Changes in feed consumption and weight alterations provided valuable insights into the effects of the treatments.

5.4. Statistical analysis

Throughout the study, rigorous statistical analysis was conducted. One-way analysis of variance (ANOVA) followed by relevant post hoc tests was employed to assess significance levels. Investigational outcomes are presented as the mean ± S.E.M. The statistical significance of the difference was determined by an unpaired Student's t test. P values of < 0.05 were considered significant and < 0.01 were highly significant. This statistical scrutiny ensures the accuracy and validity of the results obtained..

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