

## RESEARCH ARTICLE

**PROTECTIVE EFFECT OF GINGER (ZINGIBER OFFICINALE) AND CLOVE (SYZYGIVM AROMATICUM) AGAINST INDUCED HEPATOTOXICITY BY DIMETHOATE IN EXPERIMENTAL ANIMALS**Adel Fadhel Hussein Ali<sup>1,\*</sup>, and Galal Mohammed Salem Assakaf<sup>2</sup><sup>1</sup> Dept. of Biology, Faculty of Yafea University, University of Lahej, Lahej, Yemen<sup>2</sup> Dept. of Physiological Science, Faculty of Medicine and Health Sciences, University of Aden, Yemen\*Corresponding author: Adel Fadhel Hussein Ali; E-mail: [afha7878@gmail.com](mailto:afha7878@gmail.com)

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**Abstract**

Usage of pesticides is world wide spread have harmful effect on human health via environmental or occupational exposure, that pollute water, soil and food. This experimental study aims to investigate possible antioxidant and protective activities of ginger (*Zingiber officinale*) and clove (*Syzygium aromaticum*) aqueous extracts against induced injury of liver hepatocytes using the organophosphate Dimethoate (DM) in rabbits. Thirty adult male rabbits from local market were divided into five groups (6 animals in each). (1) control rabbits, (2) rabbits were given DM (20 mg/kg B. w daily dose 1/20 of half the lethal dose), (3) rabbits were given ginger extract (400 mg/kg B.W) daily dose and then DM, (4) rabbits were given clove extract (100 mg/kg B.W) daily dose and then DM, (5) rabbits were given ginger with clove extracts and then DM as previous dose for 30 days. At the end of the experiment animals were anesthetized and then sacrificed. The blood sample was collected for biochemical analysis. Dimethoate resulted in significant increase in the activity of aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) compared with control. Significantly increased level of both total and direct bilirubin were observed. However, the total protein and albumin were decreased. The ginger and clove extracts either separately or combined in groups 3,4 and 5 showed significantly improvement and decrease activity of liver enzymes AST, ALT and ALP. On the other hand, decreased in total bilirubin compared with DM group were recorded. While increased the serum levels of total protein and albumin were observed. It could be included that ginger and clove extracts were effective in protecting against induced hepatotoxicity by treating liver functions. Therefore, we recommend these two plants by moderate amounts to be added in the daily meals and drinks.

**Keywords:** *Zingiber officinale*, *Syzygium aromaticum*, Dimethoate, Liver functions.**Introduction:**

Usage of pesticides is becoming worldwide in agriculture for pest fight and crop protection from insects, fungi and rodents. Pesticides such as Dimethoate (DM) is an organophosphate insecticide extensively used in agricultural fields and home gardens [1]. Humans and animals are chronically exposed to low doses of DM residues that exist in foods, drinking water, and feeds [2,3]. The liver integrity is threatened by several toxic external agents (drugs, industrial products, heavy metals, etc.), which can cause cirrhosis, steatosis and necrosis, which are generally grouped under the term hepatotoxicity [4]. The most affected class of people are

those directly exposed to pesticides such as handlers and sellers. The acute exposure to DM primarily induces the inhibition of acetylcholinesterase in target tissues, which consequently accumulates acetylcholine and subsequently activates cholinergic muscarinic and nicotinic receptors [5]. The toxicity of DM causes adverse effects on many organs and systems, as evidenced by clinical manifestations, biochemical and histological alterations [6,7]. The molecular mechanism that may be responsible for the toxicity of DM involves oxidative stress by disrupting the antioxidant defense systems and by producing lipid peroxidation mediated by reactive oxygen species (ROS) in different tissues [5,7-9].

These free radicals can alter any type of molecules or cells, causing very serious damage whether in structural or functional level [10]. ROS levels in the body are controlled by antioxidant molecules (glutathione) or enzymes (SOD, catalase). However, ROS levels can be regulated by the uptake of exogenous antioxidants, among them natural antioxidant secondary metabolites, such as polyphenols, flavonoids, carotenoids, ascorbic acid, or allicin [11].

The traditional medicinal system based on the use of herbal remedies still plays an important role in the health care system. In recent decades, medicinal plants have been gaining wider acceptance due to the perception that these plants being natural products have lesser side effects and improved efficacy than their synthetic counterparts [12].

The use of herbal medicines is progressively rising among the general public, they are appreciably effective, inexpensive and have minor side effects [13].

The pathological and mode of action of free radicals can be reversed by the use of antioxidants from herbal extracts. These antioxidants scavenge free radicals and restore the normal physiological function. Natural antioxidants from herbal extracts and their bioactive constituents can be used in liver diseases and significantly accelerate recovery process. [14].

Ginger (*Zingiber officinale*) has been known since Ancient times as one of the most popular spices and is widely used all over the world and is one of the plants of wide international fame [15]. Ginger contains many bioactive ingredients that attribute the healing properties of this plant [16].

Clove (*Syzygium aromaticum*) is among the most valuable spices which are being used for centuries to preserve the food and enhance its flavor. Moreover, numerous medicinal uses of clove has been investigated and identified till date [17].

The effective role of clove in the inhibition of different degenerative diseases is attributed to the presence of various chemical constituents in high concentrations with antioxidant activity [18].

The objective of the present study was to evaluate the antioxidant and hepatoprotective activity of ginger and clove aqueous extracts when used separately or combined against DM induced hepatotoxicity. Hepatoprotective activity of ginger and clove have been evaluated in experimental animals using DM induced hepatotoxicity.

## Materials and Methods

The experimental research method is applied in this study. Thirty adult male rabbits (1000-1300 gm) were brought from the local market, they were housed in cages

in the animals houses at the Faculty of Education University of Aden at room-temperature.

Dimethoate (40% DM pure) (purity 40%) was purchased from AGROW ALLIED VENTURES. PVT.LTD India. Ginger and clove obtained from local supper market, other chemicals and equipments used were of analytical grade.

## Preparation of extracts:

Five hundred grams of ginger rhizomes and one hundred grams of clove buds were used. After drying properly, they were cut into small pieces and ground to powder in a ball mill, the powder was kept in sterile dry firmly closed bottles, which were stored in a dry cool place for one week before aqueous extraction. 10ml of sterile water was added to each bottle of the powder. The extracts were allowed to soak for 48 hours before the mixture were centrifuged at 2000 pm for 10 min. The supernatant was passed through membrane filter, the extract was prepared for use in all experiment according to [19].

The animals were randomly divided into five groups, 6 animals in each group. Group 1 (G1), control group were given water only. Group 2 (G2) were given DM (20 mg/kg B.W) [7]. Group 3 (G3) were given ginger extract (400 mg/kg B.W) plus DM [20]. Group 4 (G4) were given clove extract (100 mg/kg BW) plus DM [21]. Group 5 (G5) were given ginger and clove extracts plus DM like previous doses.

Dimethoate, ginger and clove extracts were administrated by intragastric oral gavage on a daily basis.

After 30 days of research period, the animals anesthetized by ether, then sacrificed and blood samples were collected by direct heart puncture into EDTA coated and non EDTA vials for biochemical analysis.

Serum Alanine-amino transferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), serum total bilirubin and direct bilirubin, serum total protein and serum albumin were examined at Al-Wahiri laboratories in Al-Houta city-Lahej.

Biochemical analysis were performed: serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum alkaline phosphatase (ALP) activity and serum total protein were determined according to the method of [22]. serum total bilirubin (TB) and direct bilirubin (DB) were determined according to the methods of [23]. And Serum albumin were determined according to the method of [24]. Biochemical analysis were by Biochemistry Analyzer, Model BS-120, WK-91106241, factory by MINDRAY Company.

## Statistical Analysis

The data were analyzed using statistical package for social science (SPSS) version 21. Significant differences between mean values of exposed and control groups were statistically analyzed using the independent t-test. Results were considered significant when p-value is <0.05.

## Results:

Serum levels of specific liver enzymes were measured to determine the liver function of each animal. In the liver damage induced by DM administration in G2 recorded significantly elevated in the serum levels of specific liver enzymes such as AST, ALT and ALP compared to control group (P<0.05).

In Tables (4&5) a significant increase levels of both total and direct bilirubin, while levels of both total protein and albumin were decreased as shown in Tables (6&7).

The extracts of ginger and clove supplementation either separately or combined for 30 days have significantly lowered (P<0.05) levels of those marker enzymes, and improved the levels of liver enzymes as shown in Tables (1-3). In Table (4) showed significant decrease in total bilirubin compared to G2. At the same time the serum levels of total protein and albumin were increased compared to G2, as shown in Tables (6&7).

## Discussion:

Medicinal plants continue to be an important therapeutic aid treatment of human kind illness, now a day there is an orientation towards traditional medicine that introduced many herbal drugs. This research highlights the possible curative role of aqueous extracts of the herbal spices, ginger (*Zingiber officinale*) and clove (*Syzygium aromaticum*) against induced hepatotoxicity by dimethoate in mature male rabbits.

The results revealed significant increases in liver enzymes (AST, ALT & ALP) activity (P<0.05) after administration of DM, such elevation was recorded by other studies [25].

Dimethoate cause liver injury and leakage of cytosolic proteins from hepatocytes and other body organs into blood [26]. Dimethoate is an organophosphate insecticide known to produce oxidative stress in human and animal cells [27].

The results are in accordance with [28]. Their results showed elevated activities of AST, ALT and ALP into circulation after hepatic cell damage, other study carried out by [29], recorded an increase in AST.

A study done by [30] and [31] showed that oral administration of DM cause an exceptional rise in liver enzyme activity (AST, ALT & ALP) in serum of DM

treated experimental animals, which is consistent with the present study. Such results may indicate degenerative changes and hypofunction of liver [32]. As well as, hepatic cell necrosis [33]. And reflects the presence of rupture in hepatocytes plasma membranes [34].

Administration of DM to laboratory animals showed elevation of serum levels of direct and total bilirubin compared to control animals, this results are similar to study done by [35]. The increased in total bilirubin in treated rabbits may be an indicator of hyperbilirubinemia—a useful index for the severity of hepatocellular dysfunction [36].

Dimethoate administration to G2 caused significant increase in levels of total protein and albumin (P<0.05), such results were recorded with other study [37] and in agreement with a study by El-Hadary and Ramadan who reported that treatment with CCl<sub>4</sub> leads in a decrease in serum total protein [38].

low levels of total protein and albumin may be due to liver cell damage. Liver cells are central place for protein synthesis and damage of liver reduces the rate of protein synthesis and liver injury reduced the rate of protein synthesis and hence the levels of protein in the circulation [39].

Ginger and clove are among the natural products that possess large number of active components as antioxidants anti-inflammatory.

Treatment with ginger is effective in the prevention of oxidative damages induced by dimethoate objectified by lowering serum ALAT, ASAT and bilirubin concentrations. These results are in consistent with previous study demonstrating the scavenging effect and an inhibitory action of ginger on superoxide anion production in liver [40].

This study showed a decrease in liver enzymes activity in serum after treatment with ginger alone or combined with clove extracts, such findings are in consistent with other studies [9,41].

[42] conducted an exceptional study of antioxidant and hepatoprotective effect of clove on ethanol induced hepatotoxicity, the study showed decrease in AST, ALT and ALP in serum, they concluded the presence of phenolic components in clove that act as free radical scavengers.

Ginger and clove are hepatoprotectors due to their important scavenging activities of free radicals, suppress lipid peroxidation and DNA destruction, that could restore hepatocyte structure and function leading to cell membrane stabilization [43]. Ginger and clove extracts had restored the level of total protein and albumin in treated animals. This results recorded with other study [38].

Ginger and clove renders protection against oxidative stress induced by DM metabolism. There could be many reasons behind one might be their richness in phenol

contents. Phenols are well known to inhibit lipid peroxidation and reduce oxidative stress.

**Table (1):** Average of serum AST in male rabbits treated with ginger, clove, dimethoate and /or their combination.

Animal groups	N	Mean	±SD	±SE	t-Value	P-Value	Significance
Group1 (G1)	6	65.33	22.85	9.33			
Group 2 (G2) DM	6	119.50	20.21	8.25	-4.349	0.001	HS
Group 3 (G3) ZO + DM	6	84.33	8.12	3.31	-1.919 3.956	0.084 0.003	NS* S**
Group 4 (G4) SA + DM	6	76.17	23.95	9.78	-0.802 3.388	0.441 0.007	NS* S**
Group 5 (G5) ZO + SA+DM	6	78.00	18.24	7.45	-1.061 3.734	0.314 0.004	NS* S**

SD=Standard Deviation; SE=Standard Error; DM= Dimethoate; ZO= Zingiber officinale; SA= Syzygium aromaticum; HS= High Significant; NS= Non-significant; S= Significant;\* In compare with control (group 1); \*\* In compare with dimethoate (group2).

**Table (2):** Average of serum ALT in male rabbits treated with ginger, clove, dimethoate and /or their combination.

Animal groups	N	Mean	±SD	±SE	t-Value	P-Value	Significance
Group1 (G1)	6	50.67	11.67	4.77			
Group 2 (G2) DM	6	69.33	6.92	2.82	-3.370	0.007	S
Group 3 (G3) ZO + DM	6	54.00	9.47	3.86	-0.543 3.203	0.599 0.009	NS* S**
Group 4 (G4) SA + DM	6	56.83	11.34	4.63	-0.928 2.305	0.375 0.044	NS* S**
Group 5 (G5) ZO + SA+DM	6	53.33	2.42	0.99	-0.548 5.347	0.596 0.000	NS* HS**

SD=Standard Deviation; SE=Standard Error; DM= Dimethoate; ZO= Zingiber officinale; SA= Syzygium aromaticum; HS= High Significant; NS= Non-significant; S= Significant;\* In compare with control (group 1); \*\* In compare with dimethoate (group2).

**Table (3):** Average of serum ALP in male rabbits treated with ginger, clove, dimethoate and /or their combination.

Animal groups	N	Mean	±SD	±SE	t-Value	P-Value	Significance
Group1 (G1)	6	105.83	15.09	6.16			
Group 2 (G2) DM	6	166.00	27.28	11.14	-4.727	0.001	HS
Group 3 (G3) ZO + DM	6	109.00	26.88	10.97	-0.252 3.646	0.806 0.004	NS* S**
Group 4 (G4) SA + DM	6	114.33	37.64	15.37	-0.513 2.722	0.619 0.021	NS* S**
Group 5 (G5) ZO + SA+DM	6	107.83	19.00	7.76	-0.202 4.285	0.844 0.002	NS* HS**

SD=Standard Deviation; SE=Standard Error; DM= Dimethoate; ZO= Zingiber officinale; SA= Syzygium aromaticum; HS= High Significant; NS= Non-significant; S= Significant;\* In compare with control (group 1); \*\* In compare with dimethoate (group2).

**Table (4):** Average of serum T. Bilirubin in male rabbits treated with ginger, clove, dimethoate and /or their combination.

Animal groups	N	Mean	±SD	±SE	t-Value	P-Value	Significance
Group1 (G1)	6	0.50	0.09	0.04			
Group 2 (G2) DM	6	0.72	0.10	0.04	-3.993	0.003	HS
Group 3 (G3) ZO + DM	6	0.55	0.10	0.04	-0.889 2.840	0.395 0.018	NS* S**
Group 4 (G4) SA + DM	6	0.57	0.15	0.06	-0.933 2.043	0.373 0.068	NS* NS**
Group 5 (G5) ZO + SA+DM	6	0.53	0.14	0.06	-0.500 2.668	0.628 0.024	NS* S**

SD=Standard Deviation; SE=Standard Error; DM= Dimethoate; ZO= Zingiber officinale; SA= Syzygium aromaticum; HS= High Significant; NS= Non-significant; S= Significant;\* In compare with control (group 1); \*\* In compare with dimethoate (group2).

**Table (5):** Average of serum D. Bilirubin in male rabbits treated with ginger, clove, dimethoate and /or their combination.

Animal groups	N	Mean	±SD	±SE	t-Value	P-Value	Significance
Group1 (G1)	6	0.28	0.08	0.03			
Group 2 (G2) DM	6	0.42	0.10	0.04	-2.638	0.025	S
Group 3 (G3) ZO + DM	6	0.30	0.06	0.03	-0.415 2.445	0.687 0.035	NS* S**
Group 4 (G4) SA + DM	6	0.37	0.12	0.05	-1.431 0.785	0.183 0.451	NS* NS**
Group 5 (G5) ZO + SA+DM	6	0.33	0.05	0.02	-1.342 1.838	0.209 0.096	NS* NS**

SD=Standard Deviation; SE=Standard Error; DM= Dimethoate; ZO= Zingiber officinale; SA= Syzygium aromaticum; S= Significant; NS= Non-significant; \* In compare with control (group 1); \*\* In compare with dimethoate control (group2).

**Table (6):** Average of serum total protein in male rabbits treated with ginger, clove, dimethoate and /or their combination.

Animal groups	N	Mean	±SD	±SE	t-Value	P-Value	Significance
Group1 (G1)	6	7.30	0.45	0.18			
Group 2 (G2) DM	6	5.85	0.84	0.34	3.738	0.004	S
Group 3 (G3) ZO + DM	6	7.18	0.91	0.37	0.282 -2.648	0.784 0.024	NS* S**
Group 4 (G4) SA + DM	6	7.15	1.01	0.41	0.332 -2.429	0.747 0.035	NS* S**
Group 5 (G5) ZO + SA+DM	6	7.22	1.11	0.45	0.170 -2.406	0.868 0.037	NS* S**

SD=Standard Deviation; SE=Standard Error; DM= Dimethoate; ZO= Zingiber officinale; SA= Syzygium aromaticum; (+C)= Negative control; (-C)= Positive control; S= Significant; NS= Non-significant; \* In compare with control (group 1); \*\* In compare with dimethoate (group2).

**Table (7):** Average of serum Albumin in male rabbits treated with ginger, clove, dimethoate and /or their combination.

Animal groups	N	Mean	±SD	±SE	t-Value	P-Value	Significance
Group1 (G1)	6	4.97	0.80	0.33			
Group 2 (G2) DM	6	3.75	0.40	0.16	3.329	0.008	S
Group 3 (G3) ZO + DM	6	4.57	0.55	0.22	1.010 -2.931	0.336 0.015	NS* S**
Group 4 (G4) SA + DM	6	4.50	0.70	0.28	1.079 -2.284	0.306 0.045	NS* S**
Group 5 (G5) ZO + SA+DM	6	4.68	0.85	0.35	0.593 -2.420	0.566 0.036	NS* S**

SD=Standard Deviation; SE=Standard Error; DM= Dimethoate; ZO= Zingiber officinale; SA= Syzygium aromaticum; S= Significant; NS= Non-significant; \* In compare with control (group 1); \*\* In compare with dimethoate (group2).

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## مقالة بحثية

## الأثر الوقائي لمستخلصي الزنجبيل والقرنفل ضد سمية الكبد المستحثة بالديمثويت في حيوانات التجارب

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## المُلخَص

الانتشار العالمي الواسع لاستخدام المبيدات الحشرية له تأثير ضار على صحة الانسان بالتعرض المباشر عبر البيئة أو طبيعة العمل، الذي يلوث المياه والتربة والغذاء. هدفت هذه الدراسة للتحقق من الأنشطة المضادة للأكسدة والوقائية المحتملة لمستخلصات كلاً من الزنجبيل والقرنفل ضد الضرر المستحث في الخلايا الكبدية باستخدام الفوسفات العضوي الدايمثويت على الأرانب. ثلاثون من ذكور الأرانب البالغة المشترك من السوق المحلية قسمت إلى خمس مجموعات (ست حيوانات في كل مجموعة): (1) المجموعة الضابطة (2) أرانب أعطيت دايمثويت 20 ملجم/كجم من وزن الجسم جرعة يومية، (3) أرانب أعطيت مستخلص الزنجبيل 400 ملجم/كجم من وزن الجسم جرعة يومية وأعطيت دايمثويت 20 ملجم/كجم من وزن الجسم جرعة يومية، (4) أرانب أعطيت مستخلص القرنفل 100 ملجم/كجم من وزن الجسم جرعة يومية وأعطيت دايمثويت، (5) أرانب أعطيت كل من مستخلصي الزنجبيل والقرنفل وأعطيت دايمثويت بنفس الجرعات السابقة. استمرت التجربة لمدة 30 يوماً، وفي نهاية التجربة تم تخدير الحيوانات، ثم تم التضحية بها، وبعد ذلك تم جمع عينات الدم لأجراء التحاليل البيوكيميائية. أظهرت النتائج أن اعطاء الدايمثويت أدى إلى زيادة كبيرة في نشاط انزيمات الكبد الأسيرتات أمينو ترانسفيراز (AST)، الأنين أمينو ترانسفيراز (ALT) والفوسفاتيز القلوي (ALP) مقارنة بالمجموعة الضابطة. بينما هناك ارتفاع في مستوى كلاً من البيليروبين الكلي والبيليروبين المباشر، في حين انخفض كل من البروتين الكلي والألبومين. أدى استخدام مستخلصات الزنجبيل والقرنفل إما منفصلاً أو مجتمعاً في المجموعات 3، 4 و 5 إلى تحسناً كبيراً في وظائف الكبد إذ انخفض نشاط أنزيمات الكبد AST و ALT و ALP وانخفض البيليروبين الكلي مقارنة مع مجموعة الدايمثويت وحدها. بينما زادت مستويات البروتين الكلي والألبومين. أن مستخلصات الزنجبيل والقرنفل كانت فعالة في معالجة وحماية الخلايا الكبدية من الاكسدة السمية المستحثة وتحسين عمل ووظائف الكبد. لذلك توصي الدراسة باستخدام هذين النباتين بكميات معتدلة وذلك بأضافتها في الوجبات اليومية والمشروبات.

الكلمات المفتاحية: الزنجبيل، القرنفل، الدايمثويت، وظائف الكبد.

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