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Effects of Alcoholic Extract Nigella Sativa on Blood Clotting Markers in Male Rats Models with F2 G20210A Mutation

Zainab Tawfeeq Al-Joubouri
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq

Sadeq Mohammad Jaafar almosawi
Department of Medical physiology, Faculty of Medicine, University of Kufa, Iraq

Ali A. Al-fahham
Faculty of Nursing, University of Kufa, Iraq

Corresponding Author: Ali A. Al-fahham

Abstract

Despite its well-documented antioxidant and anti-inflammatory activities, there are no sufficient data exists regarding duration of Nigella sativa effects on blood coagulation. This experiment observed the effects of a sixty-day oral intoxication of alcoholic Nigella sativa extract on four key hemostatic indicators—prothrombin time, fibrinogen, D-dimer and platelet count—with respect to the veins of normal male rats treated or otherwise and rats carrying the F2 G20210A mutation as a model of increased thrombotic risk. Thirty mature male rats were divided into three groups: control, normal rats treated with N. sativa extract, and rats crossing the G20210A mutation into their genome and therefore in theory at higher risk of thrombosis. After 60 days of treatment, blood was taken for coagulant analysis. The treated normotensive rats displayed a significant prolongation of PT and reductions in fibrinogen and D-dimer levels, pointing to an anticoagulant action of the extract. However, the group disadvantaged by the mutation presented with smaller PT, greater fibrinogen, higher D-dimer and peaked platelet levels, thus manifesting a hypercoagulative profile. The results generally indicate that long-term exposure to alcoholic Nigella sativa may to some extent influence coagulation in such a way as to lessen the tendency toward clot formation and that

when there is a prothrombin gene mutation present, a strong prothrombotic condition still prevails. Altogether, these results emphasize the potential of Nigella sativa to act as a natural modulator of coagulation, particularly in the absence of genetically derived hypercoagulable circumstances.

Keywords: Nigella sativa, Prothrombin Time, Fibrinogen, D-Dimer, Platelet Count

Introduction

Hemostasis is a physiological process that maintains vascular integrity, limiting blood loss in response to tissue injury. This highly regulated process depends on the synchronized contribution of vascular endothelium, circulating platelets and plasma coagulation proteins which ultimately form a firm blood clot. Perturbations of this fine balance can result in pathological bleeding or thrombosis that is a cause for significant morbidity and mortality globally (Palta et al., 2014). As the understanding of hemostatic dynamics has evolved, distinctive biomarkers (such as prothrombin time/international normalized ratio [PT/INR], fibrinogen level, D-dimer level, and platelet count) have become relevant in determining targets for clotting evaluation in clinical and experimental models (Bannoud et al., 2024).

Prothrombin (factor II) is a vitamin-K–dependent protein, which is produced in the liver and is central to the coagulation cascade by being converted to thrombin, a serine protease that plays a pivotal role in the transformation of fibrinogen into fibrin during clot formation. Perturbations in prothrombin activation, either 'over' or 'under,' produce marked differences in clotting function and incidence of thrombosis (Whelihan et al., 2012). The prothrombin time (PT) test is still broadly used for monitoring the effectiveness of the extrinsic coagulation pathway, even though various drawbacks such as PT inaccuracy and insensitivity have been reported to suggest that other coagulation markers are required, particularly when conducting research (Tripodi, 2016).

Fibrinogen an acute-phase glycoprotein is the integral precursor of fibrin, a requisite substrate for clot development. High fibrinogen levels are highly correlated with inflammation, CVD and hypercoagulability, while hypofibrinogenemia causes altered clot stability and increased bleeding tendency (Undas & Ariëns, 2011). D-dimer is a fibrinolysis product that results from the breakdown of cross-linked fibrin with plasmin, and thus it is speculated to be a sensitive marker of ongoing clot turnover (or activation) and fibrinolytic activity. Its diagnostic utility for hyperthrombotic conditions is well established and new developments have emphasized its broader role in risk evaluation and surveillance of thrombosis (Adam et al., 2009).

One of the most commonly used hematological parameters counted in order to afford hemostatic equilibrium is platelet. The primary hemostatic function of platelets is initiated by adhesion, activation and aggregation, but they also supply the phospholipid surface essential for thrombin formation. Alterations in the number or activity of platelets has a direct impact on the efficiency of blood coagulation (Machlus & Italiano, 2013).

With the development of coagulation medicine, it has become increasingly appealing for natural hemostatic drugs to be discovered. One of these agents, Nigella sativa or black seed, has been a focus for numerous scientific studies due to its variety of pharmacologic functions. Nigella sativa (NS), formerly known as Kalonji, has been used historically in the Middle East and Asia for respiratory, metabolic and inflammatory diseases, although modern pharmacological investigation on NSA reveals antioxidant, anti-inflammatory, immunomodulatory and hematological properties (Ahmad et al., 2013; Gholamnezhad et al., 2015). Its main bioactive component thymoquinone has been associated with important biological activities in terms of oxidative stress, inflammation and cellular signaling cascades (Muralidharan-Chari et al., 2016).

Recently, there is a growing body of evidence emphasizing the hemological effects of N. sativa extract(s). In experimental studies with rat model, the plant has been shown to protect or restore haematological parameters against chemical and inflammatory challenge. For example, N. sativa seed extract was found to have protective effect on red and white blood cell indices as well as reducing the deterioration of hematological status in chemically administered models of toxicity (Dalli et al., 2025). Furthermore, N. sativa supplementation in humans and animals has been shown to elicit beneficial effects on general hematological profiles and systemic biomarkers, emphasizing their biological significance in blood physiology (Mohtashami & Entezari, 2016; Abbas et al., 2024).

However, in this increasing literature on the focusing of effects of Nigella sativa on coagulation biomarkers is lacking from these most important well-known coagulation factors including prothrombin time (PT), fibrinogen, D-dimer and platelet count. However, most previous studies were limited to the general members of hematological than specific clotting profiles leading to literature gap whether and how the extracts from N. sativa have an influence on the coagulation cascade as well as fibrinolytic pathways. Moreover, differences in the biological properties of N. sativa are noted due to their extraction process, as alcoholic extractions give a different profile of phytochemicals compared to an aqueous or oil preparation. Phenolic compounds and alkaloids are the two main groups of secondary metabolites

concentrated by alcoholic extracts, and they may elicit distinct modulatory effects toward physiological pathways such as coagulation and platelet function (Ahmad et al., 2013).

The purpose of this study is to determine the impacts of alcoholic Nigella sativa extract on prothrombin, fibrinogen, D-dimer and platelet count as essential coagulation factors in male rats. By evaluating these major coagulation indicators, this study attempts to understand whether N. sativa could affect the coagulation system and whole hemostatic equilibrium. The present study aimed to elucidate the experimental modulatory role of Nigella sativa in clotting mechanisms.

Methods

Experimental Animals

This experiment was performed using 24 healthy adult male rats with an average weight of 250 g. Animals The animals were supplied and maintained in the Animal House of College of Veterinary Medicine, Baghdad University. The control environmental conditions were ensured, for a controlled 12:12-hour light–dark cycle in well-ventilated cages with free access to standard chow and water. All animals adapted for two weeks prior to experimentation. Ethical approval of the study was obtained from the Institutional Animal Care and Use Committee (IACCUC), College of Veterinary Medicine, University of Baghdad (Approval No: N.P.G.311).

Seeds of Nigella sativa were collected from local markets in Najaf, Iraq and taxonomic certificate was provided by the department of agriculture (Ministry of Agriculture). Alcohol extract was obtained in a traditional manner by drying and pulverizing of seeds, extraction over 95% ethanol, expelling the solids sedimented on filtration and further concentration of the solution till dryness. New portions of the extract were freshly prepared during the course of study to assure stability of its active ingredients.

The rats were randomly divided into three groups (n = 10 each) following acclimation. Group 1 was the untreated control and received distilled water alone for the entire experimental period. Group 2: Control (Normal rats) receiving alcoholic Nigella sativa extract by oral route. Group 3: these were the rats with genetically produced tendency to hypercoagulability using F2 G20210A mutation model of hypercoagulation (experimental). Rats that were used in the mutation-model studies were bred from a standardized breeding colony and precluded based on genetic analysis.

Then each group was split in an additional two treatment groups according to the length of exposure, one between-the results were measured at 30 days and the other at 60 days. Rats in G2 and G3 both received the alcoholic extract of Nigella sativa. The treatment rats were administered 200 mg/kg body weight/day of alcoholic extracted Nigella sativa. Body weight was measured weekly, and the volume applied was adjusted to ensure accurate dosing per body weight. Observations were made on all animals daily to assess behavior, activity and feeding habits and any evidence of toxicity.

Throughout the study, blood samples were obtained by cardiac puncture at termination of each treatment period under light ether anesthesia. For coagulative tests, samples were collected in citrate tubes; for blood cell count tests, samples were taken in EDTA tubes. PT and fibrinogen were detected by an automatic coagulation analyzer with the kit method according to the manufacturer's instructions. D-dimer: A rat-specific ELISA, and platelet count: An automatic hematology analyzer were used to measure D-dimer and platelet counts, respectively. Prompt testing of all laboratory specimens was carried out to prevent degradation of preservation.

Data were analyzed by SPSS program, version 25. Data were presented as the mean ± SD. Intergroup comparisons among the three groups were performed using one-way ANOVA, followed by post-hoc comparisons to detect significant pairwise differences. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Prothrombin time, fibrinogen concentration and D-dimer upon 30 days treated were not significantly different between the control, Nigella sativa normal rats (G2) and F2 G20210A mutation rat (G3) strains. Although small changes were recorded (mild PT prolongation in G2 and mild fibrinogen rise in G3) they do not exceed the range of normal physiological variations and they are not statistically significant. In conclusion short-term (30-day) alcohol extract of Nigella sativa did not significantly affect the hemostasis studied (as shown in table 1).

Table 1. Blood clotting markers in rats administered with alcoholic extract of Nigella sativa for 30 days

Groups	Prothrombin Time (sec) Mean±SD	Fibrinogen (mg/dL) Mean±SD	D-dimer (ng/mL) Mean±SD
Control	12.0 ± 0.8	345 ± 25	108 ± 12
G2	12.3 ± 0.9	338 ± 22	104 ± 10
G3	11.8 ± 1.0	355 ± 28	112 ± 14
P value	0.32 NS	0.11 NS	0.47 NS

NS: Non-significant at P >0.05

In the G3 group, however, the platelet count was slightly higher than that in the control group (suggesting a possible stimulatory effect of Nigella sativa extract on thrombopoiesis at the high dose). Further, the platelet count decreased in G2 group compared with the other groups, which may have a dose-dependent relationship. No significant difference was observed overall, however (p = 0.07), so these differences could be due to normal biological variation of the animals in the absence of an actual treatment effect (as shown in table 2).

Table 2. Platelets' count in rats administered with alcoholic extract of Nigella sativa for 30 days

Groups	Platelet Count ($\times 10^9$/L) Mean±SD
Control	790 ± 65
G2	740 ± 70
G3	820 ± 60
P value	0.07 NS

NS: Non-significant at P >0.05

Changes in PT values between different groups are striking when compared with each other post 60 days of extract administration, with the G2 group having the most extended clotting time (longer than control), while the G3 offered the minimum clotting time, which may suggest an improvement in coagulation efficiency at higher extract doses, tentatively implying a dose-dependent effect, as shown in Table 3. On the other hand, significantly higher fibrinogen and D-dimer levels were found in G3, intermediate in the control group and significantly lower in G2 ($p < 0.001$). The results indicate a possible enhancing effect on fibrin formation and fibrinolytic activity for Nigella sativa extract at higher dosages but very low suppressive effect at lower dosages (as shown in table 3).

Table 3. Blood clotting markers in rats administered with alcoholic extract of Nigella sativa for 60 days

Groups	Prothrombin Time (sec) Mean±SD	Fibrinogen (mg/dL) Mean±SD	D-dimer (ng/mL) Mean±SD
Control	14.8 ± 1.1 A	350 ± 32 A	420 ± 40 A
G2	15.6 ± 1.3 B	330 ± 28 B	390 ± 36 B
G3	14.1 ± 1.0 A	380 ± 35 A	460 ± 42 A
P value	0.02 S	0.003 HS	0.004 HS

S: Significant at P <0.05: HS; High Significant at P <0.01
A, B different letters refer to significant difference at P <0.05

Platelet counts were significantly different among treatment groups (p = 0.002) post 60 days of treatment. Those in the G3 group showed the greatest platelet number, implying that the higher dose of Nigella sativa extract might induce thrombopoiesis. Platelets would be intermediate in the control group and lowest in G2, suggesting the lower dose may have a slight inhibitory or relatively neutral effect on platelets production. In conclusion, these findings further emphasize a dose-dependent modulation of platelet dynamics by the alcoholic extract (as shown in table 4).

Table 4. Platelets' count in rats administered with alcoholic extract of Nigella sativa for 60 days

Groups	Platelet Count ($\times 10^9$/L) Mean±SD
Control	690 ± 55 A
G2	620 ± 52 B
G3	760 ± 60 C
P value	0.002 HS

HS; High Significant at P <0.01
A,B different letters refer to significant difference at P <0.05

Discussion

In the current study, there was an inconsistent effect of topical application of alcoholic extract of Nigella sativa (NS) in a F2 G20210A prothrombin mutation rats. We found no significant difference between groups for most circulating clotting markers (prothrombin time, fibrinogen, D-dimer) wait 30 days, other than a significant difference in platelet counts (highest in G3, intermediate in control, lowest in G2). At 60 days, however, the extract and genetic paradigm diverged: normal rats treated with extract (G2) had a longer prothrombin time (PT) compared to controls but G3 (mutation carriers) had shorter PT along with higher fibrinogen and D-dimer levels. These results indicate the coexistence of two competing effects, firstly a time-dependent anticoagulant effect of the alcoholic NS extract in otherwise normal rats, and secondly a hypercoagulable phenotype associated with the F2 G20210A model, which becomes apparent over time.

The anticoagulant signal observed in the rats treated with extract is supported by mechanistic data showing that NS constituents, especially thymoquinone (TQ), can modulate coagulation pathways. Impaired initiation of coagulation in vitro by TQ was observed in vivo as extended clotting times in some settings and TQ could also inhibit platelet activation/aggregation in response to some agonists (Muralidharan-Chari et al., 2016). These indirect mechanisms on coagulation initiation and platelet activity would likely induce a higher PT and decrease downstream fibrin generation when exposed chronically, as we demonstrated in our G2 group at 60 days. This seconded antithrombotic property of NS/TQ has been highlighted in several earlier reviews and preclinical studies, based on antioxidant and antiinflammatory actions reducing the systemic and local endothelial and inflammatory drivers of coagulation (Alberts et al., 2024; Tavakkoli et al., 2017).

Whilst others report heterogeneity: NS extracts and TQ reduce platelet counts and aggregation in some models (Yusof, 2017) but preserve or even enhance platelet counts in some toxic-insult models, depending on extract type (oil, aqueous, alcoholic), dose (high, low), duration (acute, chronic), and setting (e.g., baseline inflammation and toxin exposure). The change of platelets was lowest in the G2 and maximum in the mutation group at day 30; however, platelets followed other prothrombotic markers—higher in G3 at day 60—suggesting a scenario where short consistent exposure to NS gives minimal, directionally non-persistent impacts on hematology with prolonged (or an underlying genetic background) exposure unmasking unambiguous directed shifts (Mashayekhi-Sardoo et al., 2020).

The F2 G20210A is a genetically well characterized thrombophilia, which is known to increase prothrombin levels in circulation and leads to thrombotic events in humans. Carriers usually exhibit elevated prothrombin levels and an increased risk of venous thromboembolism (Li et al., 2017) which is also observed in hypercoagulable profiles in animal models designed to emulate this change. The elevated levels of both fibrinogen and D-dimer noted in G3 after 60 days suggest a hypercoagulable, high-turnover state—inflammatory or prothrombotic stimuli cause fibrinogen to rise and when fibrin formation and breakdown are enhanced, D-dimer increases. The shorter PT at 60 days in G3 (compared to G2) also aligns with the phenotype of prothrombin over-expression: increased prothrombin leads to accelerated conversion to thrombin and decreased clotting times in certain assays (Elkattawy et al., 2022).

The opposing trends—NS extract creating an anticoagulant shift while the G20210A mutation promotes procoagulant activity—suggest that NS effects are context-dependent and can be displaced in the presence of a sufficiently robust genetic hypercoagulable background. Our findings indicate that NS has an anticoagulant effect that can progress PT and reduce fibrinogen/D-dimer in normal animals to some extent but cannot overcome an inherent genetic predisposition since that was seen in G3 followed by G2 (normal + extract) in our study. Such an interaction highlights the value of context: plant extracts can shift hemostasis in the direction of anticoagulation under basal conditions, but this may vary in genetically or clinically susceptible individuals. The complex and integrated models of NS in high and low risk patients described previously reinforces the necessity for tailored dose selection and attention to baseline coagulation status (Tavakkoli et al., 2017; Mashayekhi-Sardoo et al., 2020).

The present work has its limitations which need to be acknowledged. First, the selected markers—PT, fibrinogen, D-dimer and platelet count—are informative but incorporating activated partial thromboplastin time (aPTT), thrombin generation assays and direct prothrombin measurements would allow for more detailed mechanistic distinction. Second, the use of an alcoholic extract provides synergy but is not necessarily interchangeable with dose response seen with oil or aqueous extracts, and phytochemical profiling (for example quantifying TQ) will strengthen this ability to link dose with effect.

Conclusion

The results show a time-dependent anticoagulant activity of alcoholic Nigella sativa extract in normal rats (prolonged PT, decreased fibrinogen/D-dimer), whilst hypercoagulable phenotype in rats contain a prothrombin gene G20210A mutation (shorter PT, increased fibrinogen/D-dimer and platelets). Such findings are in concert with mechanistic information regarding the effects of NS and thymoquinone on coagulation and platelet function but highlight that genetic prothrombotic risk can outweigh contextual effects of SS botanicals. Future studies should be performed to quantify active constituents, broaden coagulation testing (to include thrombin generation), and assess if NS can mitigate thrombotic events in genetically predisposed models or a clinical cohort.

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