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ANTITHROMBOTIC POTENTIAL OF AJWA DATES AND *PIPER NIGRUM*: *IN VITRO* & *IN VIVO* STUDIES

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Abstract

Thrombosis can lead to myocardial infarction that may prove life-threatening. Herbalists claim the antithrombotic effect of ajwa dates and *piper nigrum*. The present study aimed to give scientific evidence to this claim. Clotting time, prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured in *in vitro* study on human volunteer's blood and same parameters in addition to bleeding time and platelets count were determined in *in vivo* experiment in rabbits. Aspirin (250 mg/kg) was used as a standard. Aqueous extract of ajwa dates showed more effect on clotting time, PT and APTT as compared to *piper nigrum* in *in vitro* study. In the *in vivo* experimentation, the 15 % decoction of *piper nigrum* superseded ajwa dates in increasing bleeding time, PT, APTT and platelets counts. It may be concluded that both have antithrombotic activity but *piper nigrum* has slightly higher than ajwa dates.

Keywords: Ajwa dates, *Piper nigrum*, APTT, PT, Antithrombotic

INTRODUCTION

Blood is considered as a specialized connective tissue that flows through arteries and veins and plays an important role in proper functioning of body organs. Thrombosis formation is a critical event in the development and progression of multiple cardiovascular diseases including acute coronary disorders such as pulmonary emboli, deep vein thrombosis, strokes, heart attacks, and venous thromboembolic disorders. Thrombosis leads to vascular blockade and while recovering it causes fatal consequences, such as cerebral or myocardial infarction and even death. Thrombosis plays an imperative role in the progression and pathogenesis of cardiovascular and atherosclerotic diseases that may be fatal and life threatening (Dickneite *et al*., 1995). Various antithrombotic agents have been used for the thrombotic disorders, but these agents are associated with number of side effects including allergic reactions, bleeding, cerebral hemorrhage, thrombocytopenia and even reocculsion has also been reported (Stone *et al*., 2007). Owing to these limitations and increased rate of mortality and morbidity, it is a need of time to develop new antithrombotic agents with lesser side effects (Asif and Rasool, 2016).

Owing to their multiple beneficial pharmacological properties and ease of availability multicomponent plant extracts have been suggested to be the best alternative sources for the advancement of new antithrombotic agents (Kumar *et al*., 2011). Lavish endeavors have been made for revelation and development of natural medicines from diverse animal and plant sources which possess antithrombotic potential, such as aspirin a well-known and commonly used antiplatelet drug is also derived from bark of medicinal plant namely Salix alba. Numerous studies have reported the beneficial effects of herbal products in the prevention as well as treatment of thrombosis including. Garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginger (Zingiber officinale) etc. (Lumb, 1994; Ali and Thomson, 1995; Prakash *et al*., 2011). Keeping in view the beneficial effects of plant derived materials in the management of thrombosis, current study was designed to evaluate the antithrombotic properties of two commonly used edibles ie., *Piper nigrum*, a spice, and Phoenix dactylifera which are commonly used for the treatment of various cardiovascular diseases. Piper longum L. has been used as a crude drug to improve intestinal disorder as well as the activity of peripherally poor blood circulation in Asia As the medicinal plants are reservoir of many cheap and safe therapeutic agents thusly, a traditional medicinal plant namely, *L* (Family: *Piperaceae*) commonly known as black pepper and Ajwa dates (*Phoenix dactylifera L.* belongs to family Arecaceae) used for the treatment of cardiovascular diseases were selected for the current research work and an effort made for the validation of possible antithrombotic effects so that it could be helpful in the cure of thrombotic disorders.

METHODS

Chemicals used in current study include: aspirin (purchased from green pharmacy), methanol, calcium chloride (Merck), prothrombin reagent (Merck) and activated partial thromboplastin reagent (Merck) and distilled water. These chemicals were purchased from local market.

TEST SAMPLE COLLECTION AND AUTHENTICATION

Seeds of *Piper nigrum* and ajwa dates were purchased from a local market (metro-cash and carry) of Faisalabad-Pakistan and authentication was done by a Taxonomist of Government College University, Faisalabad.

PREPARATION OF EXTRACTS AND DECOCTION

a) Methanolic extract of piper nigrum was prepared by soaking pulverized black pepper (10g) in 100 ml methanol for three days with occasional shaking at room temperature. The extract was then filtered and excess of solvent was removed in oven at 40°C.

b) Decoction of piper nigrum (5%, 10% and 15%) was prepared by boiling 5g, 10g and 15g piper nigrum powder in 100 ml sterile distilled water separately over low flame for 15 minutes. The flask was then plugged and detached from heat and then cooled at room temperature and cooled flask contents were filtered.
c) Powdered pits (10g) of ajwa dates were soaked in 100 ml of methanol for 72 hours. The mixture was stirred every 24 h by means of a sterilized glass rod. After 72 hours, extract was filtered; the filtrate was condensed to dryness at 40°C in hot air oven.
d) Flesh of ajwa dates was soaked in distilled water (1:4) for 72 hours to prepare aqueous extract of ajwa dates. Resulting solution was grinded, followed by centrifugation for 15 min (4000 rpm). Supernatant was collected and kept at -80°C until use.

ANIMAL HUSBANDRY

Rabbits were used for *in vivo* experiments. Seventy rabbits weighing from 1.5 to 2.2 kg were bought from a local market- Faisalabad, Pakistan. They were kept in the animal house of GCUF under standard housing laboratory conditions, i.e. relative humidity 65% - 85%, temperature 18 - 20°C, 12 h light/dark cycles, and were fed with fresh green leaves and vegetables, water was provided *ad libitum*. Rabbits were housed in polypropylene-metal cages (one animal/ cage) and were allowed to acclimatize for one week prior to experiments.

PHYTOCHEMICAL ANALYSIS

Phytochemical screening of all extracts was carried out for the presence of alkaloids, steroids, tannins, saponins, terpenoids and flavonoids as described in previous works (Yadav & Agarwala, 2011).

EXPERIMENTAL PROTOCOL

***IN VITRO* STUDY**

In vitro study was performed on human volunteers' blood after getting their consent. Coagulation parameters i.e. clotting time, prothrombin time (PT), and activated partial thromboplastin time (APTT) were determined by following Koffuor & Amoateng, (2011) and Asif & Rasool, (2016) methods.

ESTIMATION OF PT AND APTT

These tests are performed on plasma. To get plasma, 3 mL blood samples were poured in centrifuge tube containing trisodium citrate and centrifuged at 3000 rpm for five minutes. Plasma was separated in eppendrof tubes. These plasma samples were incubated with 250 uL of each test extract/sample at 37 °C for five minutes. Next, 200 microliters of prothrombin reagent was added to 100 microliters of test plasma's and wait for clotting. This clotting time is used to calculate PT. For APTT determination, 100 microliters of test plasma was mixed with 100 microliters APTT reagent and incubated for one minute. Then calcium chloride (100 uL) was added and after 15 seconds clotting time was calculated as APTT (Asif & Rasool, 2016).

CLOTTING TIME ESTIMATION

Test extracts/samples, 200 microliters, were added to 1 mL of blood and test tubes were placed in water bath at 37°C. Time taken by the blood to clot (clotting time) was measured by using stopwatch and (Koffuor & Amoateng, 2011).

***IN VIVO* STUDY**

Seventy rabbits were divided into 14 equal groups (n=5). Group 1 was negative control and Group 2 served as positive control receiving aspirin (250 mg/kg/day) by gastric gavage. Groups 3- 5 were methanolic extract of *piper nigrum* treated groups receiving orally 250, 500 and 700 mg/kg doses respectively. Groups 6-8 were treated with decoction of *piper nigrum* which was administered orally at concentrations 5%, 10% and 15% respectively. Groups 9-11 were given methanolic extract of ajwa dates pits (150, 300 and 450 mg/kg respectively) by gastric gavage. Groups 12-14 were administered aqueous extract of ajwa dates orally at 500, 700 and 100 mg/kg dose levels respectively. All groups were given doses for 14 days.

Blood samples were collected from jugular vein at day 0, 7 and 15. Blood (3mL) for

platelet count was collected in EDTA tubes. For coagulation parameters, blood (6 mL) was collected in trisodium citrate tube (3.2%) in a ratio 9:1. Blood was centrifuged at 3000 rpm for 15 minutes to achieve PPP (Platelet poor plasma) and analyzed immediately for the determination of PT, APTT, and platelets count by following standard methods (Asif & Rasool, 2016). In addition to this clotting time and bleeding time were also measured by capillary tube and filter paper methods respectively (Elg *et al*., 2001).

STATISTICAL ANALYSIS

The data were entered and analyzed using Graph Pad prism version 5. Data were shown as mean ± S.E.M. One way (for *in vitro*) and two way (for *in vivo*) ANOVA was applied to observe group mean differences. p-value <0.001 was considered as statistically significant.

RESULTS

PHYTOCHEMICAL ANALYSIS

Results of preliminary qualitative phytochemical assays indicated presence of tannins, saponins, steroids, alkaloids and flavonoids in all extracts.

***IN VITRO* STUDY**

EFFECT ON CLOTTING TIME

Dose-dependent increase in clotting time in all extract treated groups was observed which was statistically significant ($p < 0.001$) when compared to clotting time of negative control group. When compared with standard (aspirin) treated group delay in clotting time was significantly less (Table I).

EFFECT ON PT

Dose-dependent increase in prothrombin time in all extract-treated groups was observed which was statistically significant ($p < 0.001$) when compared to clotting time of negative control group (Table I).

EFFECT ON APTT

Methanolic extract (at 500- and 700 mg/kg) and decoction (at 10% and 15% doses) of *piper nigrum* showed significant increase in APTT whereas aqueous extract of ajwa dates exhibited dose dependent increase in APTT and methanolic extract of ajwa dates pits had significant increase at highest dose as compared to negative control. The maximum increase was seen with aqueous extract of ajwa dates (Table I).

***IN VIVO* STUDY**

EFFECT ON BLEEDING TIME

Significant ($p < 0.001$) increase in bleeding time was observed in aspirin, decoction and methanolic extract of piper nigrum after 7^{th} day of administration. Dose-dependent prolongation of bleeding time was observed at 15^{th} day of treatment with aspirin, decoction and methanolic extract of piper nigrum respectively (Table II).

EFFECT ON CLOTTING TIME

At day 7 and 15increase in clotting time was observed in aspirin and methanolic extract of piper nigrum, and aqueous extract of ajwa dates treated groups respectively which was statistically significant when compared with control group (Table II).

EFFECT ON PLATELET COUNT

At day 7 and 15, decrease in platelets count was observed in aspirin, decoction and methanolic extract of piper nigrum treated groups respectively. No significant effects on platelets count were observed in methanolic extract of ajwa dates pits and aqueous extract of ajwa dates treated groups at day 7 and 15 (Table II).

EFFECT ON APTT

Aspirin did not cause any change in APTT values. methanolic extract of ajwa dates pits and aqueous extract of ajwa dates at lower concentrations did not produce any effect on APTT. Dose-dependent increase in APTT was observed at 7^{th} and 15^{th} day in groups treated with and methanolic extract of *piper nigrum* and decoction respectively (Table II).

EFFECT ON PT

There was found a significant increase in prothrombin time after the administration of both extracts for fourteen days. At day 7^{th} no effect on PT was observed in case of aspirin. However a delay in prothrombin was

observed in treatment groups at 7th and 15 th day respectively (Table II).

Discussion

In order to give scientific evidence to folk use *piper nigrum* and ajwa dates, the present study was performed. *In vitro* study showed significantly prolonged PT and APTT with both extracts of ajwa dates and *piper nigrum*. PT and APTT are hematological index which provide an understanding of coagulation (Furlanello *et al*., 2006). The PT and APTT tests distinguish between the effects of trial agents on intrinsic and extrinsic pathways. Substances which increase the values of PT are considered to interfere with the extrinsic pathway by affecting FV, FVII, FX, fibrinogen and prothrombin (Alesci *et al*., 2009). Whereas those agents that disturb APTT are thought to interfere with intrinsic pathway, by affecting all factors of coagulation except FVII and FXIII (Osoniyi & Onajobi, 2003).

Coumarins are anticoagulants that tend to reduce the degree of coagulation (Pochet *et al*., 2004). Warfarin (coumarin) is known as vit-K antagonist, it has aptitude to prevent vit-K dependent clotting factors. It prevents the coagulation cascade by reducing vitamin K dependent synthesis of coagulation factors. Vit-K is a fat-soluble vitamin necessary for synthesis of coagulation factors (FII, FVII, FIX, and FX) involved in the coagulation cascade (Rang *et al*., 2007).

Prolongation of PT and APTT by all extracts may be due to the deficiencies/inhibition of clotting factors responsible for intrinsic and extrinsic pathways. Therefore, it can be assumed that *Phoenix dactylifera and Piper nigrum* has inhibitory actions on these factors that might be due to the existence of definite coumarin like active constituents. Another possibility to explain the anticoagulant effect of ajwa dates is the presence of salicylic acid in dates (Swain *et al*., 1985). Salicylic acid is a component that can act as vitamin K antagonist and hence possess the ability to antagonize the action of vit K during the activation of coagulation cascade (Roncaglioni *et al*., 1988).

Novel anticoagulant from date palm leaf/cellulose nanocrystals (CNCs) extracted by acid hydrolysis mimic the naturally occurring anticoagulant heparin in terms of charge density. It is observed that CNC decorated surfaces significantly enhance the coagulation times of blood plasma and whole blood as studied from Quartz crystal microbalance with dissipation studies (QCM-D) and simple clotting tests. Cellulose nanocrystals bear a high amount Of negatively charged sulphate groups which resemble the charge density of natural anticoagulant Heparin (Jose *et al*., 2017).

Piperlonguminine (vital component of *piper longum* fruits) showed prolonged PT and APTT significantly as well as inhibited the activities of FXa and thrombin *in vivo* and *in vitro*. In accordance with these anticoagulant activities, Piperlonguminine delayed *in vivo* bleeding time and inhibited TNF-α induced plasminogen activator inhibitor-1 production (Lee *et al.,* 2013).

Anticoagulant effect of *Piper nigrum* may be due to the presence of constituent similar to piperlonguminine.

The present study showed significant increase in clotting time that might be due to the decline in activity of clotting factors involved in intrinsic pathway. The prolongation of clotting time observed was significantly correlated with the reduction of fibrinogen level suggesting that this CT prolongation is mediated by hypo-fibrinogenemia. In CT test the rate of clotting with thrombin is a function of fibrinogen concentration (Al-Jishi & Hozaifa, 2003). Patrassi *et al.,* (1978) has reported a similar correlation between clotting time and fibrinogen.

Moreover, the total flavonoids contents assay specified that very high amount of flavonoids is existing in various fractions of dates and *piper nigrum* (Hasan & Mohieldein, 2016; Liu *et*

al., 2008). Flavonoids may strengthen the anticoagulant effect. The previous studies have described that flavonoids exhibit antithrombotic effect by inhibiting NAD (P) H: quinine acceptor oxidoreductase (Chen *et al*., 1993), an enzyme inhibited by oral antithrombotic agents, or by interfering with the phosphatidyl serine exposure (Bucki *et al*., 2003).

The phytochemical analysis of both extracts of *Phoenix dactylifera* and *piper nigrum* has exposed the presence of tannins, saponins, alkaloids, flavonoids and steroids. Antithrombotic effect of saponins has been reported (Yang *et al*., 2014). A biologically active chemical constituent might be accountable for the detected anticoagulant potential.

In present study the effect of ajwa dates and *piper nigrum* on platelets count and bleeding time was evaluated. *Piper nigrum* significantly delayed bleeding time and reduced platelet count. This effect may be due to the presence of piperine a major constituent of *piper nigrum*. Piperine has the inhibitory effect on platelet aggregation by antagonizing thromboxane A2 receptor-mediated events (Iwashita *et al.,* 2007). In case of ajwa dates, there was found no significant effect on platelet count and bleeding time which seems controversial, as one study reported that dates may increase the platelet count up to significant level (Onuh *et al*., 2012). It has been reported that date palm leaf extract increased platelet and bone marrow megakaryocyte count in thrombocytopenic rats (Wiyasihati *et al*., 2013).

In addition to this, antiplatelet effect of flavonoids has been verified (Pearson *et al*., 2002). It has been evaluated that dates contain significant quantities of Qurecetin (Yoshida *et al*., 1990). Qurecetin a well-known flavonoid that blocks the glycoprotein pathway thus inhibits the response of platelets to collagen (Hubbard *et al*., 2003). As well as in accordance with the findings that there is an inverse relationship between platelet numbers and bleeding time (De Caterina *et al*., 1994).

CONCLUSION

It may be concluded that both, ajwa dates and *piper nigrum,* have antithrombotic activity but piper nigrum has slightly higher than ajwa dates.

CONFLICT OF INTEREST

No conflict of interest among authors.

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Table I: Effects of ajwa dates and *piper nigrum* extratcs on coagulation parameters (*in vitro* study)

Groups	Doses	Clotting time (Seconds)	PT (s)	APTT (s)
Negative control	-	8.73 ± 0.12	13.1 ± 0.12	25.3 ± 0.20
Positive control (Aspirin)	250 mg/mL	15.3 ± 0.18***	15.5± 0.12***	25.7 ± 0.12^{ns}
MEPN	250 mg/mL	11.3 ± 0.12***	19.4 ± 0.09***	25.6 ± 0.21^{ns}
	500 mg/mL	11.5 ± 0.11***	23.5 ± 0.09***	31.7 ± 0.12***
	700 mg/mL	11.5 ± 0.21***	23.8 ± 0.09***	42.6 ±.12***
DPN	5%	10.63 ± 0.15***	25.6 ± 0.22***	25.8 ± 0.09^{ns}
	10%	14.7 ± 0.15***	29.4 ± 0.13***	55.4 ± 0.09***
	15%	15.7 ± 0.12***	38.7 ± 0.12***	64.6 ± 0.12***
AEAD	500 mg/mL	10.7 ± 0.08***	23.3 ± 0.02***	33.2 ± 0.12***
	750 mg/mL	14.4 ± 0.21***	30.3 ± 0.18***	55.1 ± 0.06***
	1000 mg/mL	14.76 ± 0.21***	40.3 ± 0.18***	66.3 ± 0.06***
MEADP	150 mg/mL	10.3 ± 0.09***	20.2 ± 0.18***	28.3 ± 0.15^{ns}
	300 mg/mL	11.36 ± 0.20***	23.2 ± 0.18***	31.3 ± 0.14^{ns}
	450 mg/mL	12.53 ± 0.09***	25.3 ± 0.08***	42.3 ± 0.19***

MEPN: Methanolic extract of *Piper nigrum*, DPN: Decoction of *Piper nigrum*, AEAD: Aqueous extract of ajwa dates, MEADP: Methanolic extract of ajwa dates pits

Values are presented as mean ± SEM. ns = non-significant as compared negative control.

*** P<0.001 considered statistically significant as compared to negative control

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Table II: Summary of *In vivo* antithrombotic effects ajwa dates and *piper nigrum* extratcs on coagulation parameters

Effect on bleeding time (seconds)				
Groups	Doses	Days		
		0	7th	15th
Negative control	-	32.6±.061	32.1 ± .058	30.8±.058
Positive control	250 mg/kg	34.1 ± .058	55.4 ± 0.02***	66.2 ± 0.06***
MEPN	250 mg/kg	33.1 ± 0.03	35.1 ± 0.06***	37.2 ± 0.06***
	500 mg/kg	34.1 ± 0.06	36.2 ± 0.06***	37.1 ± 0.09***
	700 mg/kg	33.3 ± 0.24	37.8 ± 0.09***	38.7 ± 0.09***
DPN	5 %	32.5 ± 0.21	36.1 ± 0.09***	36.8 ± 0.06***
	10 %	33.6 ± 0.29	39.1 ± 0.06***	40.6 ± 0.22***
	15 %	33.8 ± 0.06	42.3 ± 0.25***	49.2 ± 0.06***
AEAD	150 mg/kg	32.2 ± 0.06	31.7 ± 0.22 ns	31.4 ± 0.21 ns
	300 mg/kg	30.2 ± .06	31.4 ± 0.19 ns	31.1 ± 0.06 ns
	450 mg/kg	31.2 ± 0.09	31.6 ± 0.25 ns	30.6 ± 0.06 ns
MEADP	500 mg/kg	32.4 ± 0.06	31.8 ± 0.06 ns	30.4 ± 0.06 ns
	750 mg/kg	30.7.1 ± 0.06	31.2 ± 0.06 ns	30.9 ± 0.12 ns
	1000 mg/kg	31.1 ± .09	31.57 ± 0.06 ns	30.4 ± 0.06 ns
Effect on clotting time (seconds)				
Negative	-	198 ± 0.82	197 ± 0.58	195±.23
Positive	250 mg/kg	195 ± 0.13	280 ± 0.8***	300 ± 0.58***
MEPN	250 mg/kg	197 ± 0.09	207 ± 0.06***	212 ± 0.06***
	500 mg/kg	199 ± 0.06	209 ± 0.07***	215 ± 0.03***
	700 mg/kg	198 ± 0.15	225 ± 0.09***	240 ± 0.06***
DPN	5 %	197 ± 0.06	210 ± 0.06***	220 ± 0.09***
	10 %	198 ± 0.29	212 ± 0.06***	235 ± 0.09***
	15 %	198 ± 0.06	230 ± 0.06***	245 ± 0.06***
AEAD	150 mg/kg	190 ± 0.58	210 ± 0.12***	211 ± 0.58***
	300 mg/kg	198 ± 0.58	211.1 ± 0.06***	230 ± 0.26***
	450 mg/kg	196 ± 0.125	230 ± 0.06***	238 ± 0.58***
MEADP	500 mg/kg	189 ± 0.08	210 ± 0.06***	216 ± 0.58***
	750 mg/kg	196 ±. 033	239 ± 0.06***	245 ± 0.22***
	1000 mg/kg	197 ± 0.08	260 ± 0.58***	271 ± 0.22***
Effect on platelet count (mm^3)				
Negative	-	729.6 ± 0.28	731.1 ±.06	730.3 ± 0.15
Positive	250 mg/kg	730.2 ± 0.06	649.2 ± 0.06***	570 ± 0.06***
MEPN	250 mg/kg	730 ± 0.06	728 ± 0.29***	727 ± 0.03***
	500 mg/kg	731.3 ± 0.24	725.4 ± 0.24***	721 ± 0.03***
	700 mg/kg	730.3 ± 0.25	723 ± 0.23***	718.3 ± 0.29***
DPN	5 %	731.3 ± 0.29	720.2 ± 0.12***	710.1 ± 0.12***
	10 %	730 ± 0.27	715.3 ± 0.29***	707.4 ± 0.26***
	15 %	732.1 ± 0.06	711 ± 0.09***	690.3 ± 0.19***

AEAD	150 mg/kg	730.2 ± 0.06	731.4 ± .05 ns	730.8 ± 0.06 ns
	300 mg/kg	731.6 ± 0.25	731.5 ± 0.38 ns	730.5 ± 0.08 ns
	450 mg/kg	731.1 ± 0.06	730.63 ± 0.25 ns	730.7 ± 0.15 ns
MEADP	500 mg/kg	728.7 ± 0.15	731.6 ± 0.08	730.7 ± 0.12 ns
	750 mg/kg	731.8 ± 0.06	731.2 ± 0.06 ns	730.63 ± 0.06 ns
	1000 mg/kg	731.2 ± 0.06	730.8 ± 0.06 ns	730.76 ± 0.12 ns
Effect on prothrombin time (Seconds)				
Negative	-	10.4 ± 0.05	10.1 ± 0.06	10.26 ± 0.15
Positive	250 mg/kg	10.9 ± 0.05	10.8 ± 0.06 ns	11.23 ± 0.15***
MEPN	250 mg/kg	11.4 ± 0.24	14.4 ± 0.24***	17.5 ± 0.12***
	500 mg/kg	12.5 ± 0.15	15.5 ± 0.24***	18.4 ± 0.22***
	700 mg/kg	13.5 ± 0.20	16.6 ± 0.12***	19.4 ± 0.06***
DPN	5 %	12.4 ± 0.23	19.3 ± 0.15***	22.7 ± 0.12***
	10 %	13.5 ± 0.12	21.5 ± 0.22***	23.6 ± 0.06***
	15 %	13.4 ± 0.20	22.5 ± 0.10***	30.5 ± 0.15***
AEAD	150 mg/kg	10.7 ± 0.08	13.3 ± 0.06***	15.1 ± 0.08***
	300 mg/kg	10.5 ± 0.04	14.2 ± 0.12***	16.5 ± 0.06***
	450 mg/kg	11.2 ± 0.12	14.4 ± 0.12***	17.3 ± 0.08***
MEADP	500 mg/kg	10.3 ± 0.06	17.1 ± 0.06***	20.1 ± 0.06***
	750 mg/kg	10.4 ± 0.12	19.1 ± 0.06**	21.1 ± 0.06***
	1000 mg/kg	10.1 ± 0.23	20.1 ± 0.08***	28.2 ± 0.08***
Effect on activated partial thromboplastin time (Seconds)				
Negative	-	18.1 ± 0.06	17.8 ± 0.22	18.8 ± 0.15
Positive control	250 mg/kg	18.2 ± 0.05	18.1 ± 0.06 ns	18.4 ± 0.23 ns
MEPN	250 mg/kg	21.6 ± 0.17	23.5 ± 0.12**	25.5 ± 0.23***
	500 mg/kg	21.4 ± 0.23	39.5 ± 0.23***	29.6 ± 0.17***
	700 mg/kg	20.5 ± 0.20	37.4 ± 0.15***	45.6 ± 0.15***
DPN	5 %	20.1 ± 0.19	24.6 ± 0.15***	31.5 ± 0.22***
	10 %	18.9 ± 0.17	46.5 ± 0.15***	57.6 ± 0.15***
	15 %	19.3 ± 0.20	50.8 ± 0.06***	60.5 ± 0.21***
AEAD	150 mg/kg	18.3 ± 0.06	18.93 ± 0.06 ns	19.3 ± 0.58 ns
	300 mg/kg	18.7 ± 0.06	22.3 ± 0.13 ns	25.1 ± 0.06***
	450 mg/kg	18.1 ± 0.08	37.1 ± 0.06***	40.1 ± 0.12***
MEADP	500 mg/kg	19.1 ± 0.14	19.2 ± 0.03 ns	29.1 ± 0.06***
	750 mg/kg	19.7 ± 0.06	44.1 ± 0.15***	55.2 ± 0.08***
	1000 mg/kg	19.3 ± 0.06	47.1 ± 0.06***	58.2 ± 0.06***

MEPN: Methanolic extract of *Piper nigrum*, DPN: Decoction of *Piper nigrum*, AEAD: Aqueous extract of ajwa dates, MEADP: Methanolic extract of ajwa dates pits

Values are presented as mean ± SEM. ns = non-significant as compared negative control. *** P<0.001 considered statistically significant as compared to negative control