Antithrombotic activity of Curcuma Oil
(i). Studies on the antithrombotic effect of curcuma oil

1. Effect of Curcuma oil on collagen-epinephrine induced thrombosis model

Curcuma oil (CO) was found to be protective in various animal models of cerebral stroke, as the intravascular thrombosis has been implicated as one of the precipitating factor in cerebral stroke; Effect of curcuma oil was evaluated against collagen-epinephrine induced thrombosis model in mice at various doses. The curcuma oil was administered @500mg/kg both 1 hour and 24 hour prior to the thrombotic challenge. In another group curcuma oil was administered @1g/kg, 1 hour prior to pulmonary thromboembolism. Significant protection was observed at 500mg/kg, 24 h dose from that of aspirin treated groups (38±4% vs 63±3%) (Fig 1C). Therefore, further studies done to assess the efficacy profile of curcuma oil in other models of thrombosis

Fig 1C: Effect of curcuma oil on collagen-epinephrine induced thrombosis in mice at various doses (for *: p<0.05 from aspirin treated groups).
2. **Effect of curcuma oil on bleeding time**

Curcuma oil was also assessed for its effect on bleeding time. Aspirin increased the bleeding time significantly, however there was only marginal increase in bleeding time was observed in curcuma oil treated mice (Fig 2C).

![Bar chart showing effect of curcuma oil on bleeding time](image)

**Fig 2C:** Effect of curcuma oil on bleeding time in mice.

3. **Effect of curcuma oil on ferric chloride induced thrombosis model**

Ferric chloride induced thrombosis in rats was reduced by curcuma oil. It was found to increase TTO significantly at 500mg/kg, po after 1h and 24h treatment. It also increased TTO at 1g/kg dose when administered 1 hour prior to the application of FeCl₃. In control group TTO was 14.44±1min. However, aspirin (30mg/kg, 1h) and ticlopidine (200mg/kg, 2 h) did not increase TTO. Anticoagulant drugs like warfarin increased TTO consistently. The TTO obtained with the treatment of warfarin 0.1mg/kg and 0.3mg/kg, po, 5 days was 66±26 and 120±0 min respectively. TTO following treatment with heparin 10U/kg, 30U/kg and 100U/kg treatment was 17.98±2, 22.15±2.3 and 33.36±14.7 min.
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respectively. Significant prolongation in TTO was obtained after the pretreatment with curcuma oil which was comparable with that of heparin treated group (Fig 3C).

![Graph showing TTO values for different treatments](image)

**Fig 3C: Effect of curcuma oil on total time of occlusion in FeCl₃-induced thrombosis model in rat (for *: p<0.05 and for **: p<0.01).**

These observations suggest that curcuma oil possess significant antithrombotic activities. Therefore, we investigated in details its mechanism of action by platelet aggregation and coagulation parameter studies.

4. *Ex vivo* effect of curcuma oil on platelet aggregation at 500mg/kg dose

The animals were pretreated with curcuma oil at 500mg/kg and platelet aggregation studies were done by various inducers of platelets on rat PRP. It was observed that curcuma oil significantly reduced platelet aggregation induced by ADP (10μM), collagen (5μg) and thrombin (0.3U) after 1hr (Fig 4Ci). Moreover, increased inhibitory effect was observed after 24 hr of curcuma oil administration (Fig 4Cii). While the aggregations induced by PMA (1.5μM), calcium ionophore A23187 (1.25μg) and arachidonic acid (0.5mM) remained unaffected both at 1hr and 24 hr (Fig 4Ci & 4Cii).
Fig 4Cl: Effect of curcuma oil (500mg/kg, po) on platelet aggregation 1h after treatment (for *: p<0.05 & for **: p<0.01).
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Fig 4Cii: Effect Curcuma oil (500mg/kg, po) on platelet aggregation 24 h after treatment (for ***: \( p<0.001 \)).

5.  Ex vivo effect of Curcuma oil (1g/kg) on platelet aggregation

Furthermore, Curcuma oil was studied at higher dose 1g/kg. The animals were pretreated with Curcuma oil @1g/kg and platelet aggregation studies were done by various inducers of platelets on rat PRP. Aggregation was induced by increasing concentrations of ADP, thrombin, calcium ionophore A23187 and PMA to assess if the Curcuma oil has competitive inhibition on platelet activation and aggregation. Curcuma oil inhibited ADP-induced platelet aggregation dose dependently and no inhibition was found at the higher concentration suggesting that the inhibition was competitive in nature (Fig 5Ci). However, in case of thrombin induced platelet aggregation, the inhibition obtained was irrespective of the concentrations of the inducer. It suggests that Curcuma oil can also inhibit thrombin induced platelet aggregation at higher dose in a non-competitive manner (Fig 5Ci3). Furthermore, we observed that Curcuma oil did not inhibit A23187, arachidonic acid, or PMA-induced platelet aggregation suggesting it has no effect on intracellular calcium mobilization, generation of TxA\(_2\) or protein kinase C activation (Fig 5Cii, 5Civ & 5Cv).
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Fig 5C: Effect of Curcuma oil (1gm/kg, po) on (i) ADP (ii) calcium ionophore A23187 (iii) thrombin (iv) arachidonic acid and (v) PMA induced platelet aggregation ex vivo at various concentrations of the inducers.
6. *Ex vivo effect of Curcuma oil on coagulation parameters*

In addition to platelets, various plasma proteins also play a major role in haemostasis. Effect of Curcuma oil was therefore, studied on coagulation cascade. Thrombin time, prothrombin time and activated partial thromboplastin time was in similar range as in the control group, both at 1 hr and 24 hr at 500mg/kg, po (Fig 6Ci & 6Cii). Antithrombotic effect exhibited by Curcuma oil is thus platelet mediated.

![Graph showing the effect of Curcuma oil on coagulation parameters.](image)

**Fig 6Ci:** *Ex vivo effect of Curcuma oil (500mg/kg, po, 1h) on coagulation parameters.*

![Graph showing the effect of Curcuma oil on coagulation parameters.](image)

**Fig 6Cii:** *Effect of Curcuma oil (500mg/kg, po, 24h) on coagulation parameters ex vivo.*
(ii). Discussion

Natural products from both plant and animal sources have been of great use in treating various diseases and disorders from the ancient times. With the advancement of modern techniques and with better understanding of the etiology of the diseases, these natural products have regained their importance. Most of the present day promising drugs in use have either been isolated from the natural sources as active principles or have been synthetically modified to improve the efficacy.

Likewise, in case of antiplatelet, anticoagulant and antithrombotic therapies, origin of many of these drugs could be traced to the natural sources. Aspirin developed in 1899, is the largest selling drug which has been derived from salicin, a secondary metabolite produced in the willow leaves. Aspirin irreversibly acetylates the serine 529 residue of COX1 and inhibits the synthesis of TxA2 (Vane and Botting, 2003). Moreover, heparin which is the first anti-coagulant drug was discovered by McLean accidentally (Best, 1959). Heparin binds to the antithrombin III and forms a complex and thereby accelerates the process of thrombin inhibition. Another most used anticoagulant is warfarin, which is derived from the from sweet clover plants (Melilotus alba and M. officinalis). It was discovered along with dicumarol (3, 3-methylenebis-9 [4-hydroxycoumarin]). Hirudin, a 65-amino acid containing polypeptide derived from the medicinal leech Hirudis medicinalis, potently inhibits thrombin (Markwardt, 1994). Streptokinase and Urokinase, which act by the dissolution of intravascular clot, are also naturally derived products (Guest, 1954), which activate plasminogen to augment the formation of plasmin. Streptokinase is derived from streptococci, while Urokinase is a proteolytic enzyme synthesized by human kidney.

Most of the plant products contain several chemical substances having different properties which they may synergize or antagonize the beneficial effects of the various components. Various natural formulations and their aqueous extracts, alcoholic extracts or active components have been investigated in various animal models and in vitro test systems. Curcuma oil is the oily extract of Curcuma longa. It was identified to be
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protective against cerebral stroke at CDRI. Further studies were therefore undertaken to investigate its effect against thrombosis. Curcuma oil is an herbal component which might have various chemical entities having salutary effects on haemostasis.

As thrombosis is one of causative factor in thrombotic cerebral stroke, we evaluated the effect of curcuma oil on various thrombosis models. Curcuma oil (500mg/kg) conferred significant protection against pulmonary thromboembolism (Fig 1C), indicating antithrombotic effect of curcuma oil, however only marginal increase in bleeding time was observed (Fig 2C). Moreover, in ferric chloride induced thrombosis in rats, curcuma oil (500mg/kg; and 1g/kg) exhibited prolongation in time to occlusion (TTO) which suggests its modulatory effect against intravascular thrombosis (Fig 3C). The prolongation in TTO was comparable to that of heparin, which dose dependently increased TTO (Fig 3C). Effect of curcuma oil on ferric chloride induced thrombosis is equivalent to that of heparin. However, heparin action is mediated through clotting factors and effect of curcuma oil is through platelets.

*Ex vivo* evaluation of platelet aggregation at 500mg/kg at both 1 h, 24 h and at 1g/kg after treatment with curcuma oil revealed that it acts on platelets through various platelet-surface receptors thus ADP, collagen and thrombin induced activation and aggregation of platelets. Moreover, antiplatelet action of curcuma oil was enhanced following 24hr of treatment (Fig 4Ci & 4Cii). Curcuma oil mediated platelet inhibition seems to be competitive against ADP and non-competitive against thrombin (Fig 5Ci & 5Ciii). Curcuma oil-mediated antithrombotic effect was not mediated by anticoagulant action (Fig 6Ci & 6Cii).

However, it is clear that curcuma oil mediated antithrombotic action thus seems to be due to the inhibition of platelet activation. The novel entities of curcuma oil should be isolated which can be garnered for antithrombotic effects.

In addition to curcuma oil, various natural products from plant sources have shown platelet inhibitory effects, anticoagulant effects and antithrombotic effects. The
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Oily derivatives of *Ocimum sanctum* have been reported to increase blood clotting time and its percent increase was comparable to that of aspirin (Singh et al., 2001). Further studies revealed that this oily derivative inhibit COX and LOX pathways (Singh and Majumdar, 1997). Arjunolic acid is a triterpene isolated from the bark of *Terminalia arjuna* have been found to exhibit anti-platelet activity (Sumitra et al., 2001). Inflammatory diseases also lead to the intravascular thrombosis. The constituents of *Allium sativum* have been found to inhibit neutrophil functions and free radical generations (Sankaranarayanan et al., 2007). Aged garlic (*Allium sativum*) extract (AGE) has the ability to reduce platelet aggregation (Steiner et al., 1998; Rahman and Billington, 2000). Aqueous extract of garlic have been found to inhibit ADP, collagen, arachidonate (AA), epinephrine and calcium ionophore A23187 induced platelet aggregation in a dose-dependent manner due to multiple actions (Srivastava, 1984; Srivastava, 1986a). Allicin, a unique thiosulfinate in garlic possess platelet inhibitory effects. Moreover, it inhibits the release reaction at 10µM concentration (Mohammad and Woodward, 1986). Another alcoholic extract of garlic is Ajoene, (E, Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, which has been found to inhibit platelet aggregation and release reactions induced by all the known agonists. Moreover, it inhibited platelet-vessel wall interaction under low shear rate flow and prevented fibrinogen binding to GPIIb/IIIa and thrombus formation (Apitz-Castro et al., 1992; Apitz-Castro et al., 1994).

Curcuma oil isolated at CDRI seems to be potential candidate drug molecule for stroke and antithrombotic activity of this substance seems to be beneficial in addition to its neuroprotective action.