

Effect of garlic on platelet aggregation in diabetic patients

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Platelet aggregation and subsequent release reactions have an important role in the genesis of vascular disease. Platelet aggregation is enhanced in diabetic patients. This hyperactivity of the platelets is responsible for the extensive vascular lesions seen in diabetes. Many therapeutic substances have been used to reduce platelet aggregation. Garlic has been described to have an inhibitory effect on platelet aggregation. The present study was conducted on 50 diabetic patients without any complications selected from the various hospitals of Lahore. Fifty healthy subjects were taken as control. A week prior to the test subjects included in the study were advised not to take any drug capable of inhibiting platelet functions. Garlic capsules were administered as 1 capsule twice daily for 14 days. Each capsule containing 500 mg of garlic powder equal to two cloves. Plasma of control and patient groups was collected before and after the garlic therapy and was analysed for rate, intensity and lag phase of platelet aggregation by using Chronology Aggregometer Model 450. Agonists used were ADP and collagen. Pre-therapy and post-therapy values were evaluated using the *t* test and showed a significant reduction in platelet aggregation with *p* value of <0.0001.

Key words: Garlic, platelet aggregation, diabetes, aggregometer, intensity of aggregation, rate of aggregation, lag phases, turbidometrically

Platelets circulate as cytoplasmic discs of 10 fl. Average volume. Platelets have a critical role in the response to injury that involves the process of haemostasis, thrombus formation, vascular and connective tissue healing¹. It has been proposed that platelets may have an important role in the development of atherosclerosis and a similar role has been postulated for platelets in microangiopathy². Platelets contribute to the development of atherosclerosis in at least two different ways. Firstly, the release from activated platelets of chemical mediators that damage the vessel wall or alter its metabolism and secondly repeated micror thrombus formation which augments occlusion of already damaged arteries. When platelets are activated many chemical substances are released which have their local biological effects. Prostaglandins produced by platelets and vascular endothelium may regulatory effects on vessel wall tone³. Hence platelet aggregation and subsequent release have an important role in the genesis of vascular disease. It has been observed that platelet aggregation is enhanced in diabetic patients. This hyperactivity of the platelets could be responsible for the extensive vascular lesions observed in this disorder^{4,5}.

Many therapeutic substances like aspirin, dipyridamole, and herbal remedies have been tried to reduce platelet aggregation and subsequent vascular disease in these patients. Among the herbal remedies garlic is reported to have hypoglycaemic and hypolipaeamic activities and has an inhibitory effect on platelet aggregation⁶. Alicin is a constituent of garlic oil. It has been found to have an inhibitory effect on platelet aggregation^{7,8}. Ajoene (e 4,5,9 trithiadodeca 1,6,11 triene 9-oxide) is a product of rearrangement of alicin. It has been shown to be a potent inhibitor of platelet aggregation⁹. It also has an inhibitory effect on platelet release reaction¹⁰.

Present study was conducted to evaluate the effect of garlic on platelet aggregation in diabetic patients.

Materials and Methods

Fifty diabetic patients without any complications of diabetes were recruited from Mayo Hospital, Services Hospital, and Sir Ganga Ram Hospital. 50 healthy subjects were from the laboratory staff, office workers, and P.G.M.I students. Complete history was taken and detailed clinical examination was carried out a week prior to the test subjects included in the study were advised not to take any drug which was capable of inhibiting platelet function (table 1). If abstinence from drug was not possible such subjects were excluded from the study.

Garlic capsules were prepared by Pakistan medical research council. Garlic capsules 1 gram equal to 4 cloves were given as 1 capsule daily (500 mg = 2 cloves) for 14 days. Before garlic administration platelet aggregation was determined turbidometrically according to the principle described by born in 1962 and then again 14 days after garlic therapy. The experiment was performed in a Chronolg Aggregometer model 450. The Aggregometer detects platelet aggregation by measuring infrared light transmission through a platelet rich plasma prepared by centrifuging citrated blood sample in a siliconized centrifuging tube at 1000 rpm for 10 minutes. Light transmission through the plasma is monitored continuously on a chart recorder. The addition of an aggregating agent results in the formation of increasingly larger platelet aggregates with correspondingly decreasing optical density.

Platelet aggregation was studied by using two aggregation agents viz Adenosine Diphosphate (ADP) in concentrations of 1 μ mole, 2.5 μ mole, and 5 μ mole and collagen in concentration of 2 μ gm/ml and 4 μ gm/ml. After

Completion of the aggregation response the tracing was marked as shown in figure 1. Different aggregation parameters viz intensity of aggregation, rate of aggregation, and lag phase were interpreted as follows.

Table 1. Drugs inhibiting platelet functions

A. Agents that affect prostanoid synthesis
a. Aspirin
b. Other nsoids
B. Phosphodiesterase inhibitors
a. Dipyridamol
b. Methyl xanthines (caffeine , aminophyline , theophyline)
Antimicrobials
a. Penicillins
b. Cephalosporins
c. Others (nitrofurantoin, hydroxychloroquin, membrane stabilizing agents)
Antihistaminics
a. Diphenhydramine
b. Promethazine
Tricyclic antidepressants
a. Imipramine
b. Nortryptiline
Sympathetic blocking agents
a. α Antagonists (phentolamine)
b. β Antagonists (propranolol)
Miscellaneous
a. Clofibrate
b. Phenothiazines
c. Peparin

Intensity of aggregation

As given in figure 1, a horizontal line was drawn through the baseline (a). Another horizontal line was drawn through the plateau region of the curve (b). A third line was drawn through the ascending portion of curve (c). At the point where the line b and c intersected a perpendicular line (d) was drawn extending down to line (a). The intensity of aggregation was defined by the length of the line (d) expressed in terms of percentage read directly from the chart paper.

Rate of aggregation

It was calculated by determining the slope of curve (c) as follows: slope = length in mm of line (d) divided by the time taken in seconds for the transmittence to reach the peak .

Lag phase

This was measured as time in seconds elapsing from addition of aggregating agent to initiation of response .

Results

The various aggregation parameters viz intensity of aggregation, rate of aggregation, and lag phase showed that garlic therapy significantly inhibited platelet aggregation.

Rate of aggregation

When ADP was used in concentrations of 1 μ mol, 2.5 μ mol, and 5 μ mol the value of rate of aggregation was nil, 1.28 \pm 0.10, and 1.71 \pm 0.10 respectively for controls. With ADP concentration of 1 μ mol, for diabetics, the pre-therapy value of rate of aggregation of 1.62 \pm 0.28 was significantly reduced to 1.35 \pm 0.10 after 14 days of garlic administration p value being < 0.001. When ADP was used in concentration of 2.5 μ mol the rate of aggregation was reduced from 2.45 \pm 0.24 to 1.99 \pm 0.26 following 14 days of garlic therapy p value being < 0.001. Similarly, with 5.0 μ mol ADP the rate of aggregation was reduced from 3.41 \pm 0.24 to 2.66 \pm 0.28 following 14 days garlic therapy p value being <0.001 (figure2)

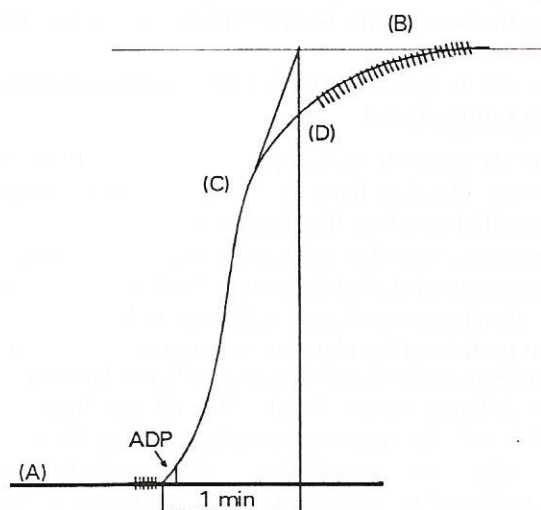
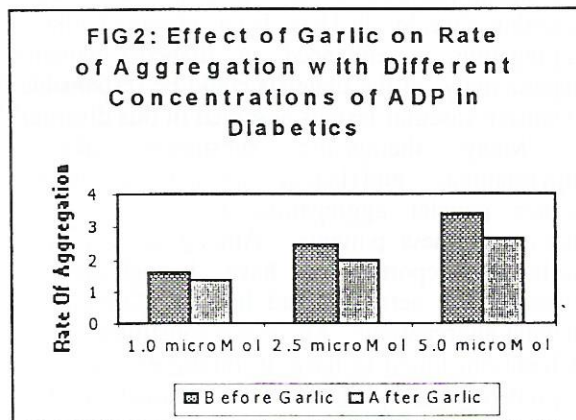
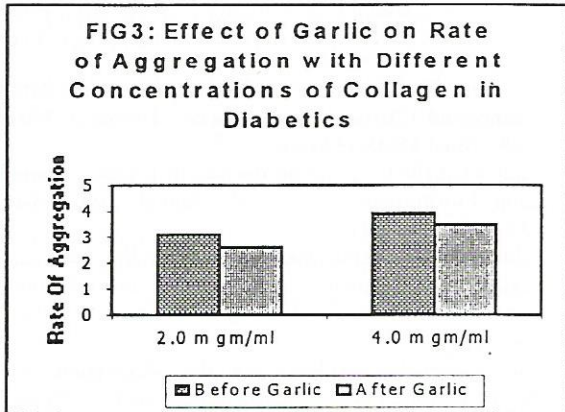


Fig 1: a diagrammatic representation of aggregation curve. Horizontal lines drawn through the base line (a) and through the plateau region of the curve (b) line (c) drawn along the steepest ascending portion of the curve and a vertical line (d) representing the intensity of aggregation.



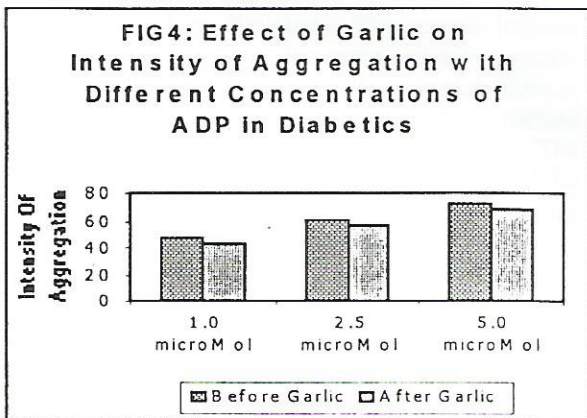
With collagen concentrations of 2 μ gm/ml and 4 μ gm/ml for controls the value of rate of aggregation was 1.39 \pm 0.22

and 1.87 ± 0.29 respectively .with collagen concentration of $2 \mu\text{g}/\text{ml}$ the rate of aggregation in diabetics was highly significantly reduced from 3.08 ± 0.23 to 2.64 ± 0.31 after 14 days garlic therapy p value being <0.001 .when collagen was used in a concentration of $4 \mu\text{g}/\text{ml}$ the rate of aggregation for diabetics was reduced highly from 3.90 ± 0.34 to 3.50 ± 0.30 p value being <0.001 (figure3).



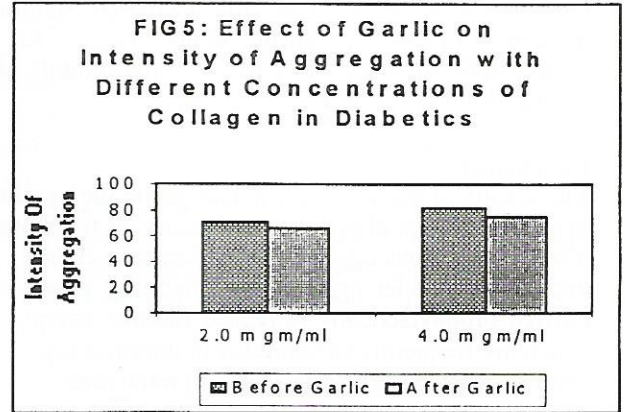
Intensity of aggregation

When ADP was used in concentrations of $1 \mu\text{mol}$, $2.5 \mu\text{mol}$, and $5 \mu\text{mol}$ the value of intensity of aggregation for controls was nil, 49.61 ± 3.87 , and 59.79 ± 3.13 respectively. In diabetics with ADP concentration of $1 \mu\text{mol}$ the value of intensity of aggregation was reduced from 47.55 ± 4.10 to 42.92 ± 0.57 after 14 days garlic therapy showing significant reduction the p value being <0.001 . When $2.5 \mu\text{mol}$ of ADP were used the value of intensity of aggregation in diabetics showed significant reduction from 60.98 ± 4.67 to 55.8 ± 2.73 the p value being <0.001 .while with $5 \mu\text{mol}$ of ADP intensity of aggregation in diabetics following 14 days garlic therapy showed similar significant reduction from 72.27 ± 3.35 to 67.8 ± 2.97 the p value being <0.001 (figure4).



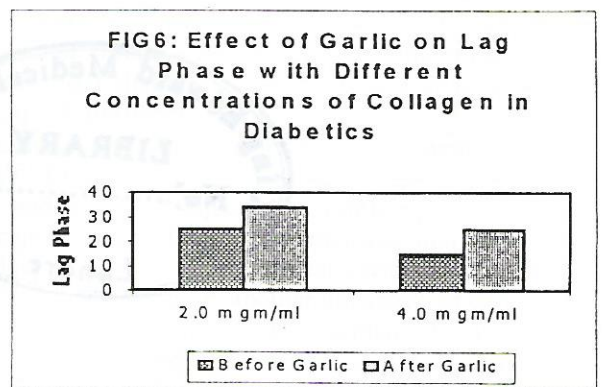
When collagen was used as the aggregating agent the

value of intensity of aggregation with $2 \mu\text{g}/\text{ml}$ was reduced significantly from 71.25 ± 2.74 to 65.94 ± 2.94 p value being <0.001 .with $4 \mu\text{g}/\text{ml}$ of collagen in diabetics the value of intensity of aggregation was reduced significantly from 81.84 ± 2.98 to 76.06 ± 2.76 (figure5).



Lag phase

With collagen concentrations of $2 \mu\text{g}/\text{ml}$ and $4 \mu\text{g}/\text{ml}$ in controls the value of lag phase was 57.52 ± 1.51 and 39.06 ± 1.63 respectively. When $2 \mu\text{g}/\text{ml}$ of collagen was used in diabetics the value of lag phase before garlic therapy of 24.82 ± 2.9 was changed significantly to 34.50 ± 2.6 14 days after garlic therapy p value being <0.001 . Similarly, with $4 \mu\text{g}/\text{ml}$ of collagen the value of lag phase in diabetics following 14 days garlic therapy was 24.60 ± 2.5 which when compared with pretreatment value of 14.73 ± 2.4 showed that platelet aggregation had been significantly reduced p value being <0.001 (figure6).



Discussion

This study revealed that ADP-induced platelet aggregation was enhanced in diabetics as compared to control and with garlic therapy for 14 days a highly significant reduction in

rate and intensity of aggregation was observed in diabetics^{11,12,13}

Similarly, with collagen the enhanced pre-garlic therapy value of rate and intensity of aggregation in diabetics was significantly reduced after 14 days garlic therapy. Moreover, the value of lag phase with different concentrations of collagen was increased significantly following 14 days of garlic administration. All these comparisons showing that garlic therapy inhibits platelet aggregation in diabetics.

Conclusion

The results of the study show that garlic therapy inhibits hyperaggregation of platelets in diabetics as it significantly affects the various aggregation parameters. Hence, garlic due to its platelet aggregation inhibiting effect can be utilized prophylactically to reduce vascular complications which are frequently encountered in diabetics especially in cases of acetylsalicylic acid (aspirin) intolerance.

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