size compared to parents. They also differ in number of petals (6) and stamens (6) compared to parents (5). The shape and colour of anther was changed to heart-shaped white compared to rod-shaped purple in the parents.

These four selected callus lines in subsequent culture passage gave rise to two types of regenerants resembling *H. muticus* and *A. belladonna*. The regenerants included 12 asymmetric hybrids resembling *A. belladonna* and 17 resembling *H. muticus*. In a similar finding both *Arabiodopsis* and *Brassica* plants were found to regenerate from the same hybrid callus during the culture passage[12].

Table 2 shows mitotic chromosome analysis of selected calli and regenerated shoots showing intermediate phenotype (symmetric hybrids) or resembling the parental types (asymmetric hybrids). In the hybrid callus the chromosome number ranged from 83 to 140. Few regenerants which possessed intermediate phenotype showed chromosome numbers between 100 and 108. Of the asymmetric hybrid plants, those resembling *A. belladonna* possessed 56 to 100 chromosomes and those resembling *H. muticus* was 24–56. The cytological analysis of the chromosomes for the plants resembling *A. belladonna* showed a few larger-sized chromosomes of *H. muticus* (Figure 2 *c*, *d*). The *H. muticus* type of regenerants showed the presence of smaller-sized chromosomes of *A. belladonna*. This could have resulted in the production of asymmetric hybrids. Cytological anomalies such as elimination of chromosomes, unequal separation of chromosomes with the presence of laggards and multipolar orientation of chromosomes during anaphase were observed when the calli were observed after several subcultures (Figure 2 *e*, *f*).

The chromosome elimination subsequent to somatic hybridization was found to occur in most of the fusion studies. Chromosome elimination was observed in fusion involving *Arabiodopsis thaliana* and *Brassica campestris*[13]. In a study on the fusion between *A. belladonna* and *N. chinensis* the elimination of parental chromosomes has been observed. In most of the cases species-specific elimination takes place[14–16]. Similar findings were also obtained in studies on somatic hybridization between *A. belladonna* and *Datura innoxia*[17]. The phenomenon of chromosome elimination in the present study can be exploited for raising asymmetric hybrids and may be used for limited gene transfer as has been reported earlier with other crops[18]. The present work indicates the possibility of producing an intergeneric somatic hybrid between two medicinally important plants in the absence of a selection system.

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ACKNOWLEDGEMENTS. The work was supported by Council of Scientific and Industrial Research (CSIR), New Delhi. Laboratory facility by Director, CIMAP, Lucknow is acknowledged.

Received 30 May 1994, revised accepted 18 July 1995

Mechanism of wound healing induced by chitosan in streptozotocin diabetic rats

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Diabetes mellitus is a common metabolic disorder associated with many pathological conditions. Prolonged or incomplete wound healing is one among them. This is due to poor blood circulation, alterations in circulating constituents, abnormal platelet aggregation and decreased fibrinolytic activity. The aim of the present study was to envisage the role of chitosan in wound healing and to investigate its effect in normalizing the aggregation pattern of platelets in diabetes. At very low concentrations, chitosan has no effect on platelet aggregation, while high concentrations can aggregate the platelets. Chitosan is not capable of reversing the increased rate of aggregation in diabetic platelets. The number of platelets which may affect the normal clotting process were also significantly lower in diabetic rats. Chitosan was found to be a good wound-healing agent, even though it does not reverse the altered platelet aggregation. Chitosan may be enhancing wound healing by some other mechanism independent of its effect on platelet aggregation.

DIABETES mellitus is caused by decreased secretion of insulin by the β cells of Islets of Langerhans, lack of

glucose-metabolizing enzymes and gluconeogenesis from noncarbohydrate sources. This disease is associated with many pathological conditions. Prolonged or incomplete wound healing is one among them. This is due to poor blood circulation, alterations in circulating constituents, abnormal platelet aggregation and decreased fibrinolytic activity. Platelet aggregation is of primary importance in normal haemostasis. When a blood vessel is cut, a platelet plug is formed by the process of platelet aggregation[1]. So any change in platelet function or in platelet number can finally lead to a difference in the blood-clotting process, which, in turn, causes prolonged wound healing[2]. Poor blood circulation makes diabetics prone to infection of the foot, commonly called diabetic gangrene.

In some recent studies, agonists like ADP, hydrogen peroxide, thrombin, etc., were used to understand the process of platelet aggregation. Many workers reported the effect of chitosan as an enhancer in wound healing. Chitosan in solution coagulates red cell membranes. It is this property of chitosan which finds wide applications in the field of neurosurgery and wound healing[3].

In the present study our aim is to envisage the role of chitosan in wound healing in diabetes and to investigate the effect of chitosan in normalizing the altered platelet aggregation in diabetes mellitus.

Animals used were Sprague Dawley strains of rats with ~ 200 gm body weight. They were maintained on gram (*Cicer arietinum*) and tap water supplied *ad libitum*.

For wound-healing studies, female rats were used. They were divided into group I (control rats), group II (control rats with chitosan powder applied to artificial wounds), group III (diabetic rats), group IV (diabetic rats with chitosan powder applied to artificial wounds).

Animals used for platelet aggregation studies were classified into group I (control animals) and group II (diabetic animals).

Diabetes was induced using streptozotocin (STZ)[4]. Body weight and blood glucose levels were measured throughout the experimental period. Glucose level from tail vein blood was estimated with GOD-PAP method by using a glucose kit (Merck).

In order to examine the process of wound healing, two artificial wounds, one on the back of the neck and the other on the leg, were made in the above four groups of animals by removing the cutis and subcutis (2–3 cm long). Then these artificial wounds in group II and group IV animals were treated with chitosan powder and the other two groups were left as such. Wound healing was assessed visually.

Platelets were prepared by collecting blood from tail vein of control and diabetic rats using the method of Domino *et al.*[5]. Platelets were counted using haemocytometer under phase contrast microscope[6]. Platelet aggregation was assayed using the method of Jamaluddin and Krishnan[7]. The PRP was diluted to make it 7×10^7 cells/ml of the experimental solution. The agonist used was 1 mg/ml chitosan solution. The agonist concentration used varied from 15 to 60 μg.

For finding out the initial rate of platelet aggregation, the number of single platelets was counted by withdrawing the sample at zero time and at various intervals after adding the agonist from a cuvette kept outside the spectrophotometer and counted immediately for finding out the percentage of single platelets[6].

Streptozotocin administration caused an elevation in the blood glucose level, decrease in body weight and increase in water uptake in all experimental animals (group II) (Table 1).

At very low concentrations of the agonist (3–15 μg/ml), the turbidity was found to increase as a function of time up to a particular point, which then stabilized in both control and diabetic samples (Figure 1).

At high concentrations of chitosan (50–60 μg/ml), the turbidity increased first and then decreased rapidly and stabilized. The normal agonist epinephrine also showed the same pattern of aggregation (data not shown). In diabetic samples the aggregation started within 10 s,

Table 1. General characteristics of experimental rats

	Blood glucose level (mg/dl)	Body weight (g)	Water uptake (ml)
Control	96.4 ± 3.0	170 0 ± 3 5	17 7 ± 1.1
Diabetic	256.9 ± 26.0*	147 5 ± 7.3*	61.0 ± 6.7*

Values are mean ± SEM of 5–6 separate experiments.
*$P < 0.05$ compared to control.

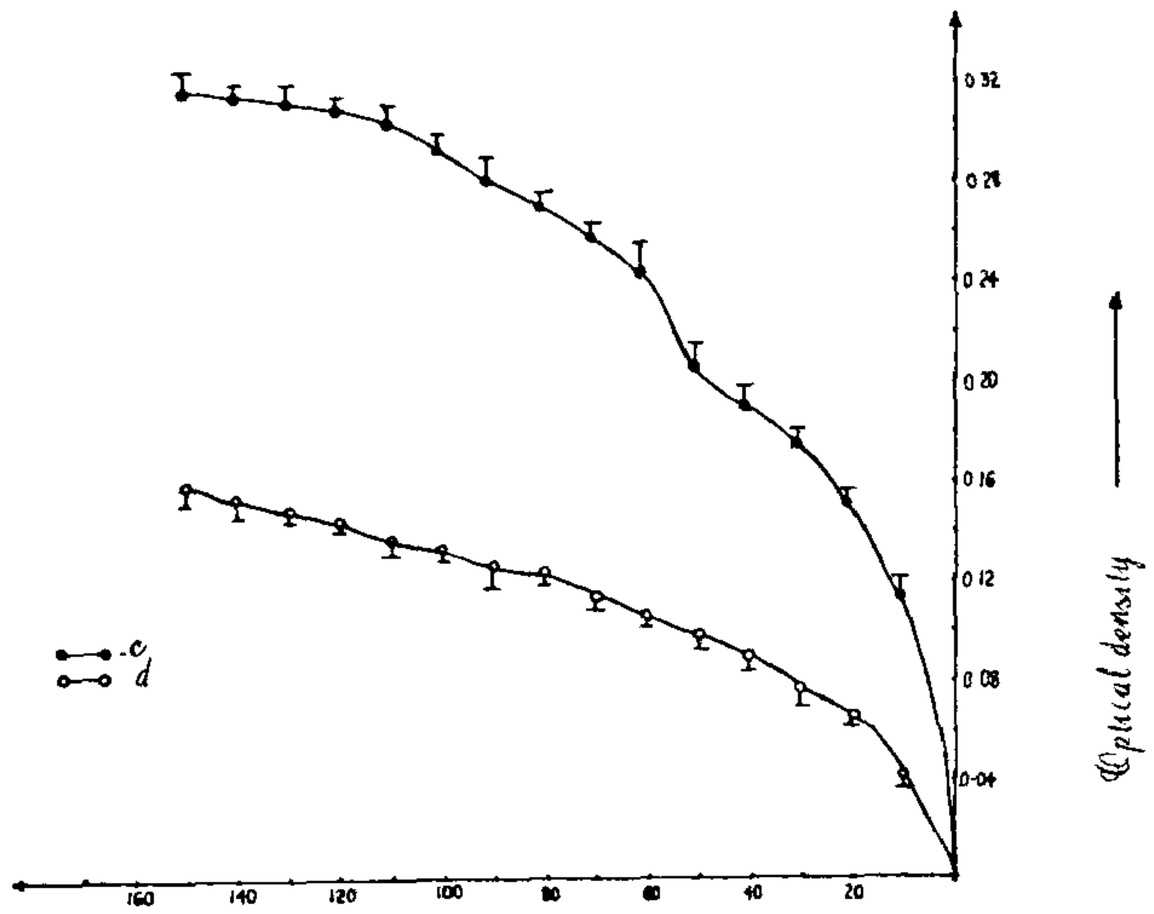

Figure 1. Platelet aggregation pattern induced by low concentrations of chitosan in control (●) and diabetic (O) rat platelet-rich plasma Values are mean ± SEM of 4–6 separate experiments.

while in control it started within 20 s (Figure 2). The decrease in turbidity was associated with a decrease in the percentage of single platelets. The initial rate of aggregation (r_0) obtained showed significant increase ($P < 0.05$) in diabetic samples compared to controls (Figure 3).

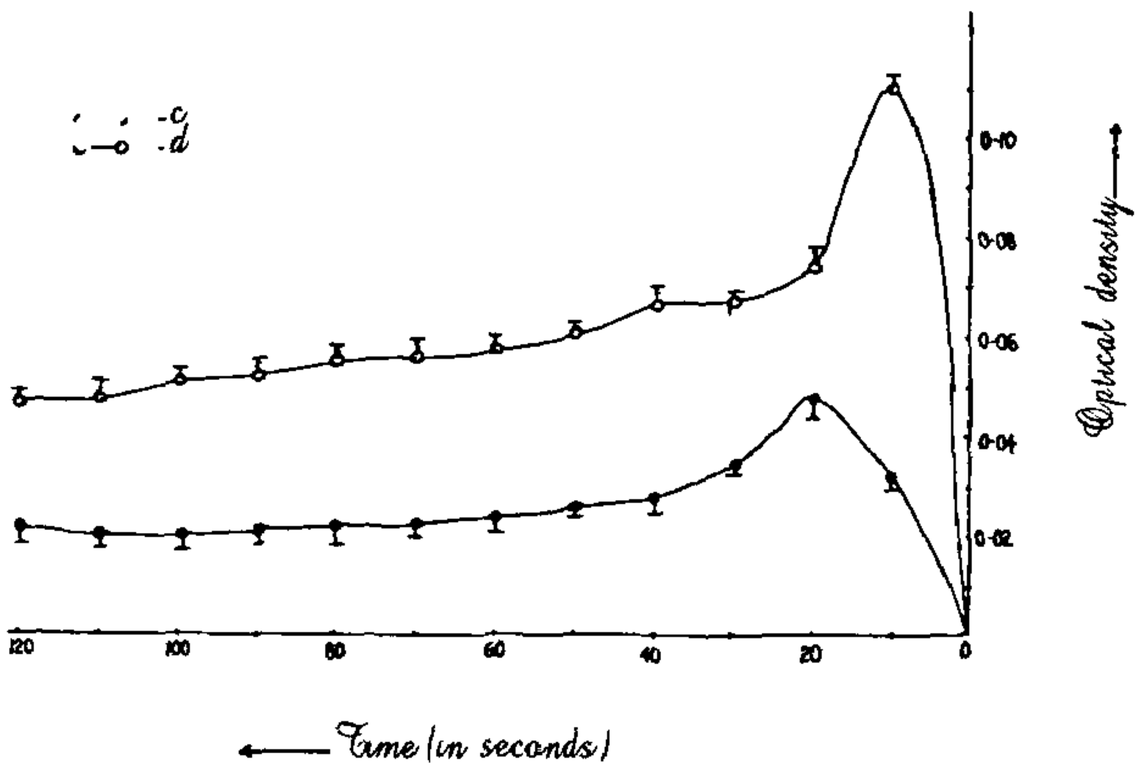

Figure 2. Platelet aggregation pattern induced by high concentrations of chitosan in control (●) and diabetic (O) rat platelet-rich plasma. Values are mean ± SEM of 4–6 separate experiments.

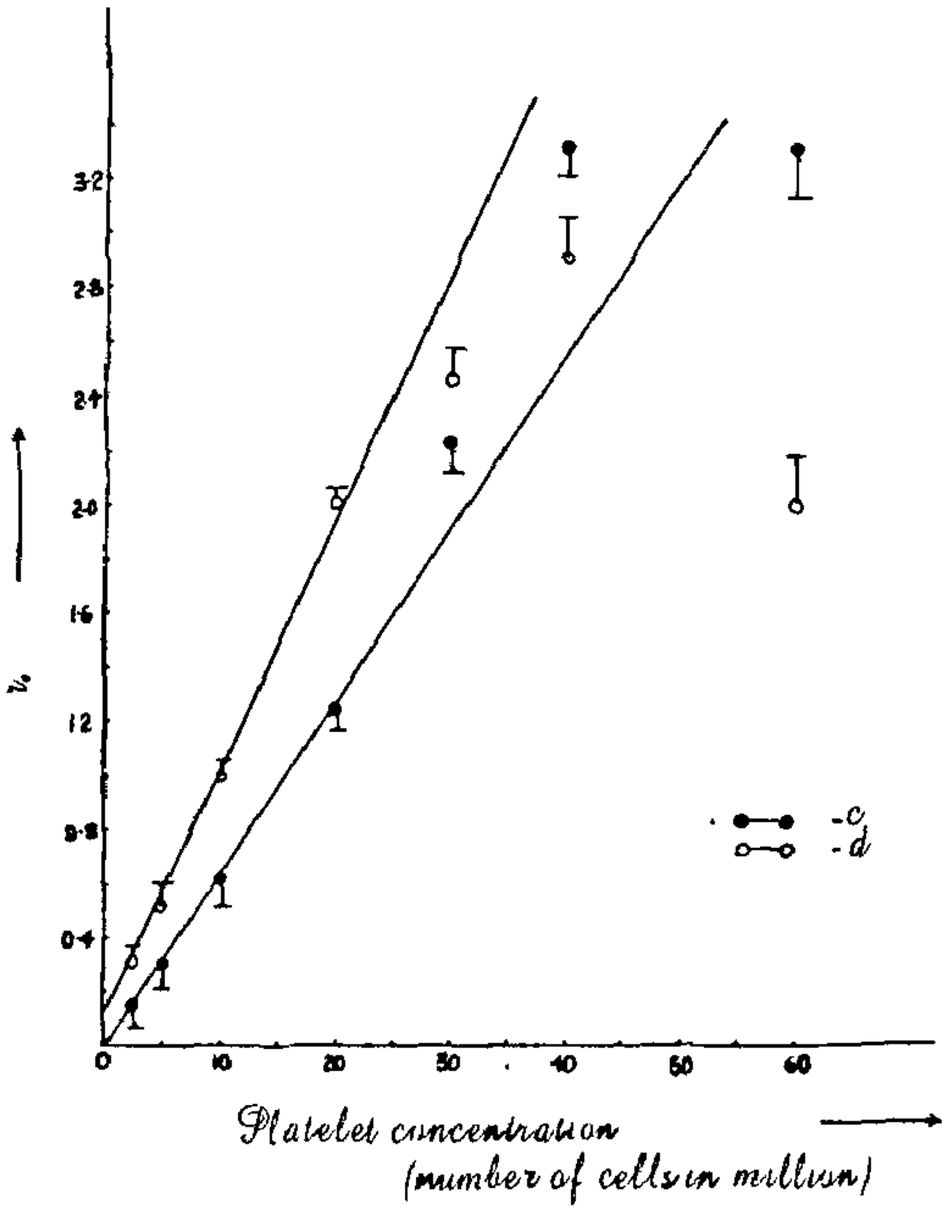

Figure 3. Changes in spectrophotometrically determined initial rates (r_0) of chitosan-induced aggregation of control (●) and diabetic (O) rat platelet-rich plasma as function of platelet concentration The lines were drawn to show deviation of data from linearity. r_0 was calculated from the graph of percentage of single platelet (on Y axis) against time (on X axis) The slope of the graph is expressed as r_0.

The number of platelets in diabetic rats was significantly less ($P < 0.05$) compared to control rats (Table 2).

Visual observation of chitosan applied on artificial wounds in experimental animals showed considerable difference in wound-healing capability compared to controls without chitosan treatment. The chitosan-applied wounds in diabetic and control rats healed much earlier than the wounds without chitosan treatment (Table 3).

The significant elevation of blood glucose level, decrease in body weight and increase in water uptake in STZ-induced diabetes mellitus is due to altered carbohydrate metabolism brought about by selective destruction of Islet β cells by the toxin[8].

From our findings it was observed that very low concentrations of chitosan did not enhance platelet aggregation. From their study Olsen *et al.*[3] reported the mechanism by which chitosan induces gel formation with RBC, shown to be due to interaction of the positively charged chitosan polymers with receptors containing neuraminic acid residues on the cell membrane. The mechanism behind the interaction between platelets and chitosan may be in the same pattern. At very low concentrations there may be inadequate binding sites for chitosan. This may be the reason why low concentrations of chitosan did not enhance platelet aggregation.

We observed enhanced platelet aggregation with high concentrations of chitosan. The optical density, which

Table 2. Platelet counts in diabetic and control rats

	Animal status (Numbers/ml of blood × 10^8)	
	Male	Female
Control	2.45 ± 0 16	3.16 ± 0.26
Diabetic	1 69 ± 0.04*	1 87 ± 0.10*

Values are mean ± SEM of 4–5 separate experiments.
*$P < 0$ 05 compared to control.

Table 3. Visual observation of wound healing in control and experimental rats

Days	Control	Diabetic	Control + chitosan	Diabetic + chitosan
1	–	–	–	–
2	–	–	–	–
3	–	–	–	–
4	–	–	–	–
5	–	–	–	–
6	–	–	+	–
7	–	–	+	+
8	–	–	+	+
9	–	–	+	+
10	–	–	+	+
11	–	–	+	+
12	–	–	+	+
13	+	–	+	+
14	+	–	+	+

–, No healing.
+, Complete healing

is a direct measure of turbidity, increased first and then decreased. Many workers have reported the pattern of ADP-induced platelet aggregation[9]. The pattern of chitosan-induced aggregation resembles the ADP-induced pattern. Thus, chitosan can act as a normal agonist like ADP. The initial increase in optical density was reported to be due to shape change of platelets[10].

In the present study we observed increased platelet aggregation in diabetic condition. This can be explained by the findings of many workers. In 1972, Kwaan *et al.*[11] found out that there is significant increase in ADP-induced platelet aggregation in diabetic samples compared to controls. Aggregation in washed platelets from rats with diabetes was enhanced; this results in prolongation of thrombogenesis[12]. Platelet aggregation in response to ADP in washed platelets was increased in diabetic animals compared to controls. This is due to increased platelet size in diabetic animals[13]. *In vitro* platelet hyperaggregation was found induced with epinephrine in diabetic condition[14].

The observed significant decrease in the number of platelets in diabetic rats can be explained as follows. Zucker explains in his study that in severe atherosclerosis and in diseases such as thrombosis and diabetes, in addition to the growth factor, two other substances, platelet factor-4 and β thromboglobulin are released in large amounts, resulting in their rapid depletion from the circulation[15]. So the observed decrease in platelet number in both sexes of animals during diabetes may be related to altered secretory function of platelets. The decreased platelet number directly affects the platelet plug formation, which is an essential step involved in blood clotting. But from a visual observation we found that chitosan enhances wound healing in diabetic condition. Our result also showed that chitosan cannot normalize platelet aggregation in diabetic conditions. These two results point out that there is no relation between chitosan-induced platelet aggregation and wound healing. Biagini *et al.*[16]. reported that in patients undergoing plastic surgery, the donor sites were treated with *N*-carboxybutyl chitosan, which enhanced wound healing. This *N*-carboxybutyl chitosan leads to formation of regularly organized cutaneous tissue and reduces anomalous healing. Although chitosan was not found to help in normalizing the altered platelet aggregation, it helps in wound healing. Thus, chitosan-induced enhanced wound healing in diabetes observed in the present study may be due to organization of newly formed tissue and thus help in damage repair.

In conclusion this study presents the effect of the bioactive polymer chitosan in wound healing in diabetes mellitus.

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ACKNOWLEDGEMENTS This work was supported by a grant from Department of Atomic Energy, BARC, India. PSP thanks CSIR for a fellowship.

Received 19 June 1995; revised accepted 15 July 1995

Permeability of cuticle of *Helicoverpa armigera* (Hübner) larvae to deltamethrin

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Permeability of the cuticle of *H. armigera* larvae to deltamethrin has been studied in three populations originating from Delhi, Sirsa and ICRISAT. Delayed penetration of topically applied ^{14}C-deltamethrin was observed in the Sirsa population. This suggests that the permeability of the cuticle plays an important role in imparting resistance in the Sirsa population, whereas in Delhi and ICRISAT populations it is not very important.

REDUCED permeability of the cuticle may be a factor conferring resistance in insect pests to insecticides[1–6]. *Helicoverpa armigera* (Hübner) has acquired resistance to synthetic pyrethroids (SPs) in major parts of India[7–10]. Recently, by a very indirect method some evidence was presented to suggest that the cuticle may, perhaps, play some role in manifestation of resistance to SPs in *H.*