

GLYCAEMIC RESPONSES TO THREE DIFFERENT HONEYS GIVEN TO NORMAL AND ALLOXAN-DIABETIC RABBITS

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Abstract

Blood glucose levels of normal and diabetic rabbits were determined after oral administration of graded doses of three different types of honeys; namely honeys of *Apis florea* (Small-Bee) and *Apis dorsata* (Large-Bee) and an adulterated commercial honey. The chemical analysis showed that commercial honey was adulterated with a saturated sucrose solution as it contained lower ash but higher nonreducing sugar levels than the natural ones. Oral administration of pure small or large-bee honeys in 5ml/kg/ doses could not produce a significant ($P > 0.05$) increase in glucose levels in normal and alloxan-diabetic rabbits whereas the adulterated honey significantly raised the blood glucose levels in normal and hyperglycaemic rabbits even at this low dosage. In higher doses of 10 ml/kg and 15 ml/kg body weight, all the three honeys produced a significant ($P < 0.05$ or $P < 0.001$) rise in blood glucose levels of normal as well as alloxandiabetic rabbits. It may, therefore, be suggested that pure natural honeys in low doses may be recommended as a source of carbohydrates and even as a sweetening agent in place of sucrose to the human patients suffering from diabetes mellitus (JPMA 39:107, 1989).

INTRODUCTION

To lessen the risk of cardiovascular disease American, Canadian and British diabetes associations have recently recommended increasing the carbohydrate intake by the diabetic patients. They have agreed on the point that foods which raise the blood glucose level least for a given carbohydrate content are most suitable¹. These considerations have raised the question as to what particular carbohydrate rich foods should be recommended to the patients suffering from diabetes. Natural honey from hilt stations rich with certain specific plant species has been empirically considered in the folklore to be not only non-injurious but also a cure for diabetic patients² -It is frequently used even today as a sweetening agent in place of sucrose in eastern/unani herbal antidiabetic preparations. Moreover, it has been regarded empirically even as a hypoglycaemic agent in the indigenous medicine^{3,4}. The present investigation were, therefore, carried out to determine the chemical analysis of three different honeys and to study their effects on the blood glucose levels in normal and diabetic albino rabbits.

MATERIAL AND METHODS

Chemicals Used:

Alloxan-monohydrate, alpha-D-glucose (anhydrous) methanol and potassium sodium tartrate were obtained from B.D.H. Laboratories (Chemical Division), Poole, England. Glacial acetic acid, benzoic acid, O'toluidine, thiourea, DNS (3,5 dinitro salicylic acid) and trichloroacetic acid were obtained from E. Merck Darmstadt, West Germany. All other chemicals and reagents used were of analytical grade prepared by E. Merck or B.D.H. Laboratories. Tolbutamide was obtained from Hoechst (Pakistan) Ltd., Karachi. Animals Used: Adult, healthy rabbits of a local strain weighing between 1000-1500 g were used in these experiment. The animals were kept in an air-conditioned animal room. They were offered

a balanced rabbit feed prepared by the Nutrition department of the University and allowed tap water ad libitum. The effects of honeys were studied on blood glucose levels on the normally fed (non-fasted) rabbits. In addition, separate experiments were performed to study the effects on blood glucose levels of the non-fasted alloxan-treated diabetic rabbits.

Preparation of Diabetic Rabbits:

A group of rabbits were made diabetic by injecting them intravenously with 150 mg/kg body weight of alloxan monohydrate. Eight days after injection of the alloxan monohydrate, blood glucose levels of all the surviving rabbits were determined by the O-toluidine method⁵. Rabbits with blood glucose levels of 350-550 mg/100 ml were considered as diabetic and were employed for further study as already reported by Sharma et al.⁶.

Honeys used and determination of their Chemical

Composition:

Honeys from small bee (*Apis florea*) and large bee (*Apis dorsata*) were obtained from a vifrage of Punjab. The samples were collected in pure form directly from the honey combs and were preserved in glass jars after proper processing. Similarly, a low priced honey sample was purchased from the market of Faisalabad. All the samples were analysed chemically for their mineral contents (Ash), moisture, total reducing sugar and non-reducing sugar contents by the procedures described in AOAC⁷.

Grouping of Rabbits:

The rabbits were randomly divided into different groups of 6 animals each. Animals of group I to IV were normal and healthy (Nondiabetic), while the animals of the group V to VIII were made diabetic by administering alloxan monohydrate. Group I served as untreated control and they received 20 ml of water⁸ orally. The animals of groups II to IV were treated orally with 5, 10 and 15 ml/kg body weight of honey diluted upto 20 ml/kg with distilled water. The animals of group V and VI were treated with tolbutamide (250 mg and 500 mg) a standard hyperglycaemic agent. Similar grouping was followed for testing all the 3 types of honeys. To test the effect of honeys on hyperglycaemic animals the alloxan-diabetic rabbits were similarly grouped. Animals of group V were kept as diabetic control and were administered 20 ml of water only. The group VI to VIII were treated orally with 5, 10 and 15 ml/kg body weight of honey diluted upto 20 ml with distilled water. Similar grouping was followed for testing all the 3 types of honey in diabetic animals. Preparation and Administration of Honeys and

Tolbutamide:

The amount of honey required for each animal was calculated on body weight basis and the required amount of honey was weighed by using an electronic balance. This was well mixed with water and the final volume was always made upto 20 ml. The honey solution obtained was then administered orally to each animal by using stainless steel feeding needle connected with 30 ml (B.D.) record syringe. Similarly the amount of tolbutamide required by each rabbit was calculated and the amount was drawn from the tolbutamide injection (Rastinon)R and diluted to 20 ml. This solution was then administered orally by the method described above.

Collection of Blood:

Just after drug administration, the animal was held in a wooden rabbit-holder and immediately 0.1 ml of blood was collected from the saphenous vein. Similarly, samples for blood glucose were collected at 4, 10 and 24 hours after drug administration. Blood samples were collected after pricking the vein with a needle, the blood was collected with a 0.1 ml blood sugar pipette. After collection of blood, the pricked site of the vein was pressed with a cotton swab soaked with 70% ethyl alcohol to protect the rabbit against infection.

Determination of Blood Glucose Levels:

Blood glucose was determined by the method of Fings et al using the O-toluidine reagent. This method gives results very close to the glucose oxidase method and is one of the most widely used manual methods.

Statistical Analysis:

Mean blood glucose levels were expressed as mg/100 ml \pm SEM in all the experiments and Student's "t" test was used to check their significance.

RESULTS

Chemical Analysis of Honeys:

TABLE. Chemical compositions of Honeys of *Apis florea*, *Apis dorsata* and a low-priced commercial Honey used in these Studies.

S. No.	Contents	Types of Honey		
		Honey of <i>Apis florea</i> (Small-bee Honey) (g%)	Honey of <i>Apis dorsata</i> (Large-bee Honey) (g%)	Low-priced Commercial Honey (g%)
1.	Ash	0.611	0.505	0.303
2.	Moisture	20.16	19.05	19.10
3.	Total Sugars	70.05	70.30	76.72
4.	Reducing Sugars	64.89	64.80	67.12
5.	Non-Reducing Sugars	5.16	5.50	9.60

Each value is the mean of at least 3 estimations.

Table shows that ash contents were 0.611 g%, 0.505 g%, 0.303 g% in *Apis florea*, *Apis dorsata* and low-priced commercial honey, respectively. Since the proposed Codex Alimentarius standard's requirement of ash contents is 0.6 — 1.0 %, the commercial honey tested was found to possess lower ash contents as compared to the natural honeys indicating that in commercial honey, there might be mixing of artificial honey. By doing this though the volume of honey have increased but the total content of a naturally occurring substance such as ash have decreased. Moisture contents of the honeys did not exceed the limits and were not liable to fermentation. The principal constituent of honeys was found to be the reducing sugars which were within the recommended limits. By the addition of super saturated sucrose solution to a natural honey would increase its volume but will not significantly affect its contents of reducing sugars. The non-reducing sugars contents were 5.16 g%, 5.50 g% and 9.60 g%

in Apis florea, Apis dorsata and commercial honey, respectively. It is well established that the non-reducing sugar in the honey is maximally sucrose. Its value in commercial honey was found to be quite high which clearly indicated that sucrose was added to some natural honey. Nevertheless, the non-reducing sugars of natural honeys from Apis florea and Apis dorsata used in this study were according to the standard.

Effect of Graded Doses of Honeys on Blood Glucose levels in Normal Rabbits:

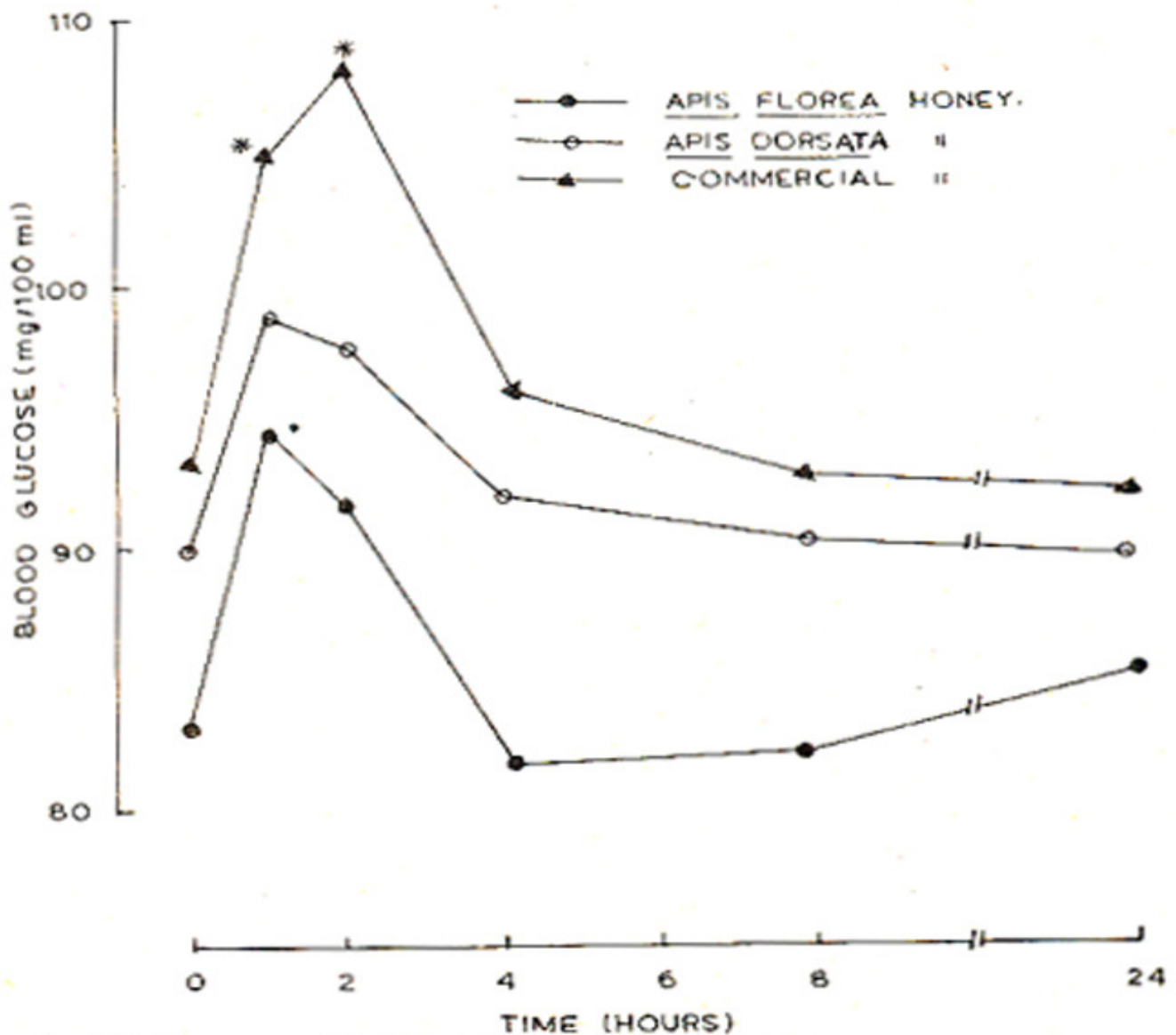


Figure I Blood Glucose levels of normal Rabbits (mg/100 ml) after oral administration of 5 ml/kg of Natural Honeys of *Apis florea*, *Apis dorsata* and low-priced commercial Honey.

* Significant difference from zero level ($P < 0.05$). All others values are non-significantly different ($P > 0.05$) from the zero hour.

Number of animals in each group = 6.

Figure 1 shows that blood glucose levels of rabbits treated with 20 ml water was found to be statistically the same ($P > 0.05$) at all intervals. The blood glucose levels of animals treated with 5 ml/kg of small-bee honey slightly increased at 1 and 2 hours but this increase was statistically insignificant ($P > 0.05$). Glucose level at 1, 4, 8 and 24 hour intervals did not differ from the zero hour

level. Similarly, administration of 5 ml/kg of large-bee honey did not significantly ($P > 0.05$) affect the blood glucose levels at all intervals checked. However, the commercial honey at this dosage raised ($P < 0.05$) the blood glucose levels from 82 ± 2.1 mg /100 ml to 100 ± 3.03 and 102 ± 2.6 mg/100 ml (Figure 1) at 2 and 24 hours.

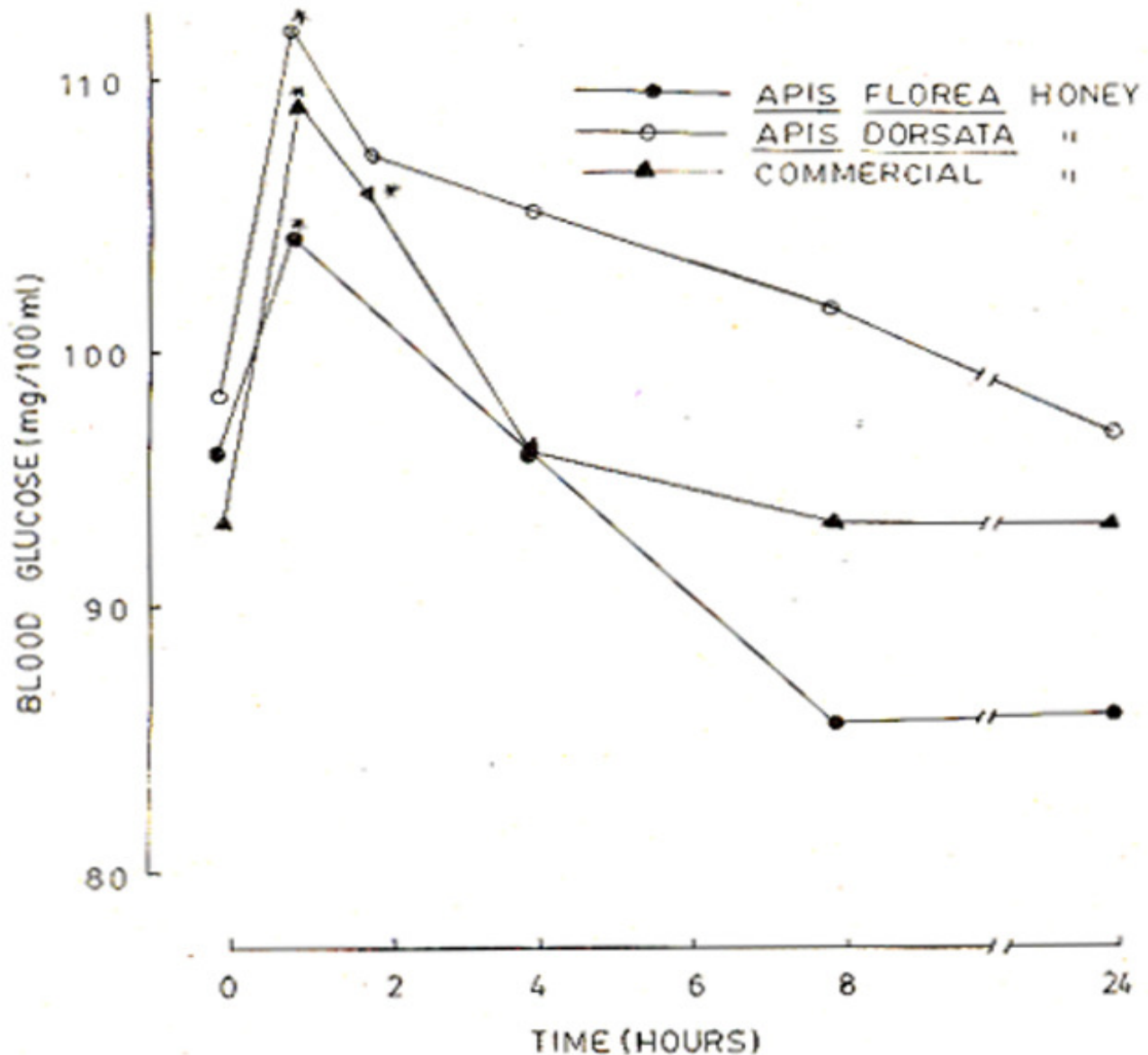


Figure 2. Blood Glucose levels of normal Rabbits (mg/100 ml) after oral administration of 10 ml/kg of natural Honeys of *Apis florea*, *Apis dorsata* and a low-priced commercial Honey. Significant difference from zero level ($P < 0.05$). All the other values are non-significantly different ($P > 0.05$) from the zero hour. Number of animals in each group = 6.

Figure 2 shows that blood glucose level of rabbits treated with 10 ml/kg of small-bee honey increased significantly at 1 hour but the increase at 2, 4, 8 and 24 hours was not significantly higher than at zero hour. Similarly, with 10 ml/kg of large-bee honey the blood glucose increased at 1 hour significantly. However, increases at 2, 4, 8 and 24 hours were not significantly high. The blood glucose level of rabbits treated with 10 ml/kg body weight of commercial honey increased significantly at 1 and 2 hours when the levels were 109 ± 3.1 and 106 ± 2.5 mg%. However, increase at 4, 8 and 24 hours was not significantly ($P > 0.05$) higher than zero hour interval(Figure 2).

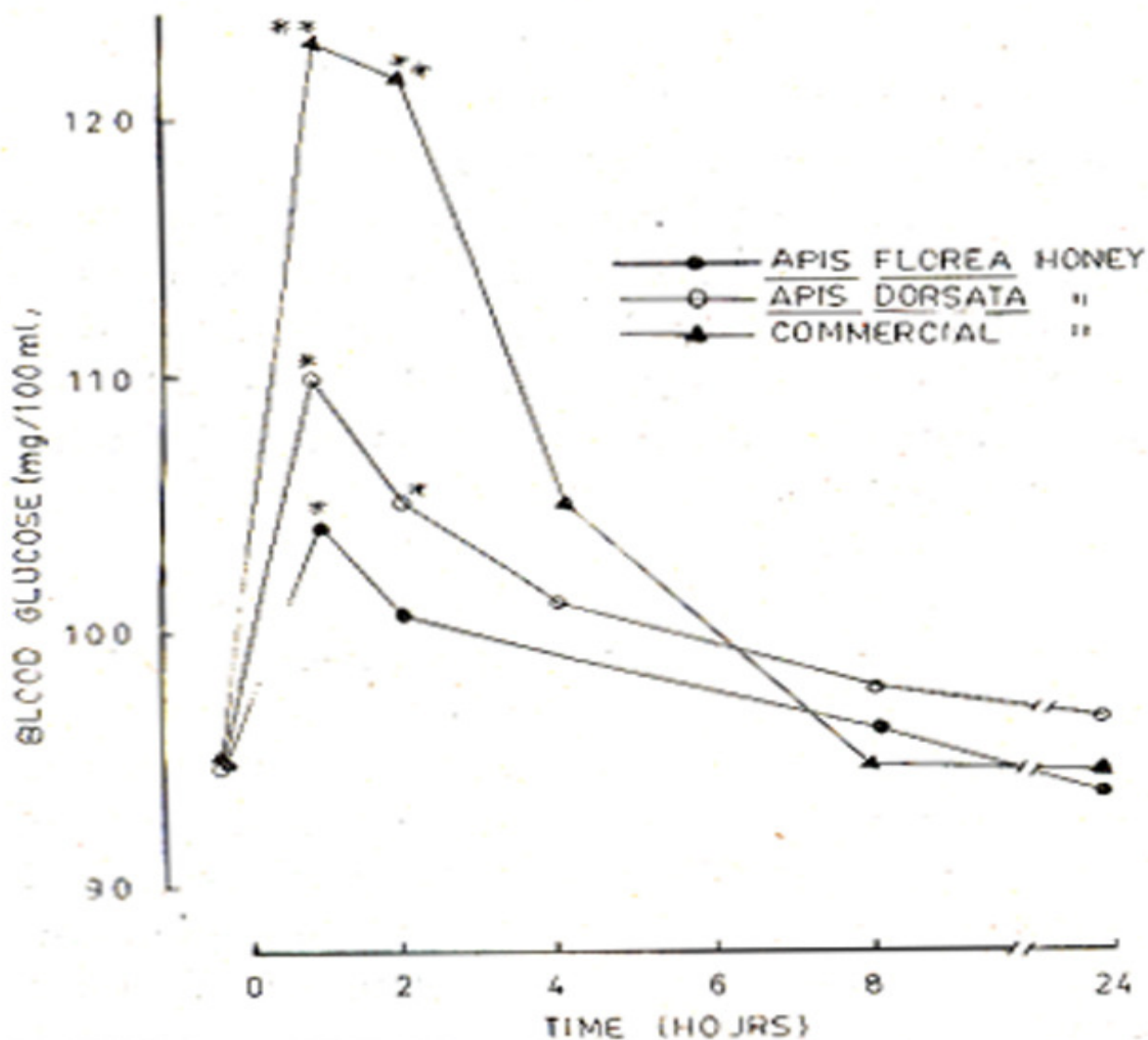


Figure 3. Blood Glucose levels of normal Rabbits (mg/100 ml) after oral administration of 15 ml/kg of natural Honey of *Apis florea*, *Apis dorsata* and a low-priced commercial Honey.

* Significant difference from zero level ($P < 0.05$).

** Highly significant difference from zero level ($P < 0.001$). All other values are non-significantly different ($P > 0.05$) from the zero hour.

Number of animals in each group = 6.

As shown in Figure 3, the blood glucose of animals treated with 15 ml/kg of small-bee honey at 1 hour interval was also significantly higher than at zero level. The values at 2, 4, 8 and 24 hour intervals were found to be not significantly higher than at zero hour. The blood glucose of animals treated with 15 ml/kg of large-bee honey at 1 and 2 hours were significantly higher than at zero hour but values at 4, 8

and 24 hour intervals were not significantly higher. The levels with 15 ml/kg body weight of commercial honey at 1 and 2 hours were highly significantly ($P < 0.001$) higher than at zero level. However, values at 4, 8 and 24 hour intervals were found to be non-significant.

Effect of Honeys on Blood Glucose in Diabetic Rabbits:

The results of alloxan administration were in agreement with others^{8,10,11}. Mean blood glucose of diabetic rabbits treated with 20 ml of water alone did not alter but treatment with 5 ml/kg of small-bee honey produced no significant increase in blood at 1, 2, 4, 8 and 24 hour intervals. The blood glucose levels remained unaffected with 5 ml/kg of large-bee honey. However, treatment with 5 ml/kg of commercial honey produced a significant increase in blood glucose at 1 hour while the increase was highly significant at 2 hours but the levels at 4, 8 and 24 hours were not significantly different from the zero hour level (Figure 4).

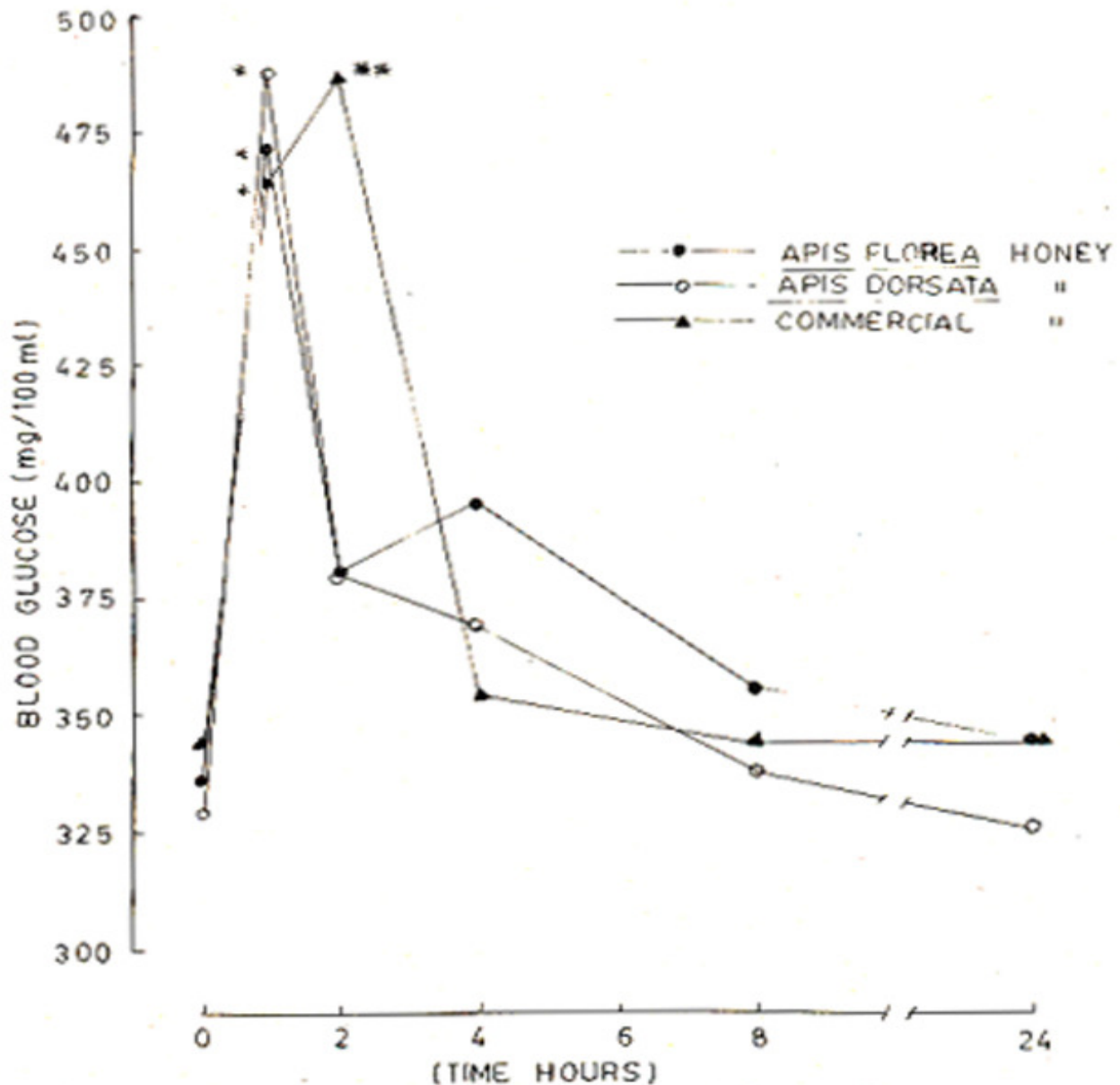


Figure 4. Blood Glucose levels of Alloxan-Diabetic Rabbits (mg/100 ml) after oral administration of 5 ml/kg of natural Honeys of *Apis florea*, *Apis dorsata* and a low-priced commercial Honey.

* Significant difference from zero level ($P < 0.05$).
 All other values are non-significantly different ($P > 0.05$) from the zero hour.
 Number of animals in each group = 6.

The blood glucose levels of rabbits treated with 10 ml/kg of small-bee honey after 1 hour was significantly higher from the zero hour. However, at 2, 4, 8 and 24 hours the levels were statistically not significant. Treatment with 10 ml/kg of large-bee honey was significantly higher after 1 hour from the level at zero hour. Again at 2, 4, 8 and 24 hours the levels were not significant. The levels of

animals treated with 10 ml/kg of commercial honey after 1 hour was significantly higher while at 2 hour interval, there was a highly significant increase. At 4, 8 and 24 hours, levels were found to be statistically not significantly different from zero level (Figure 5).

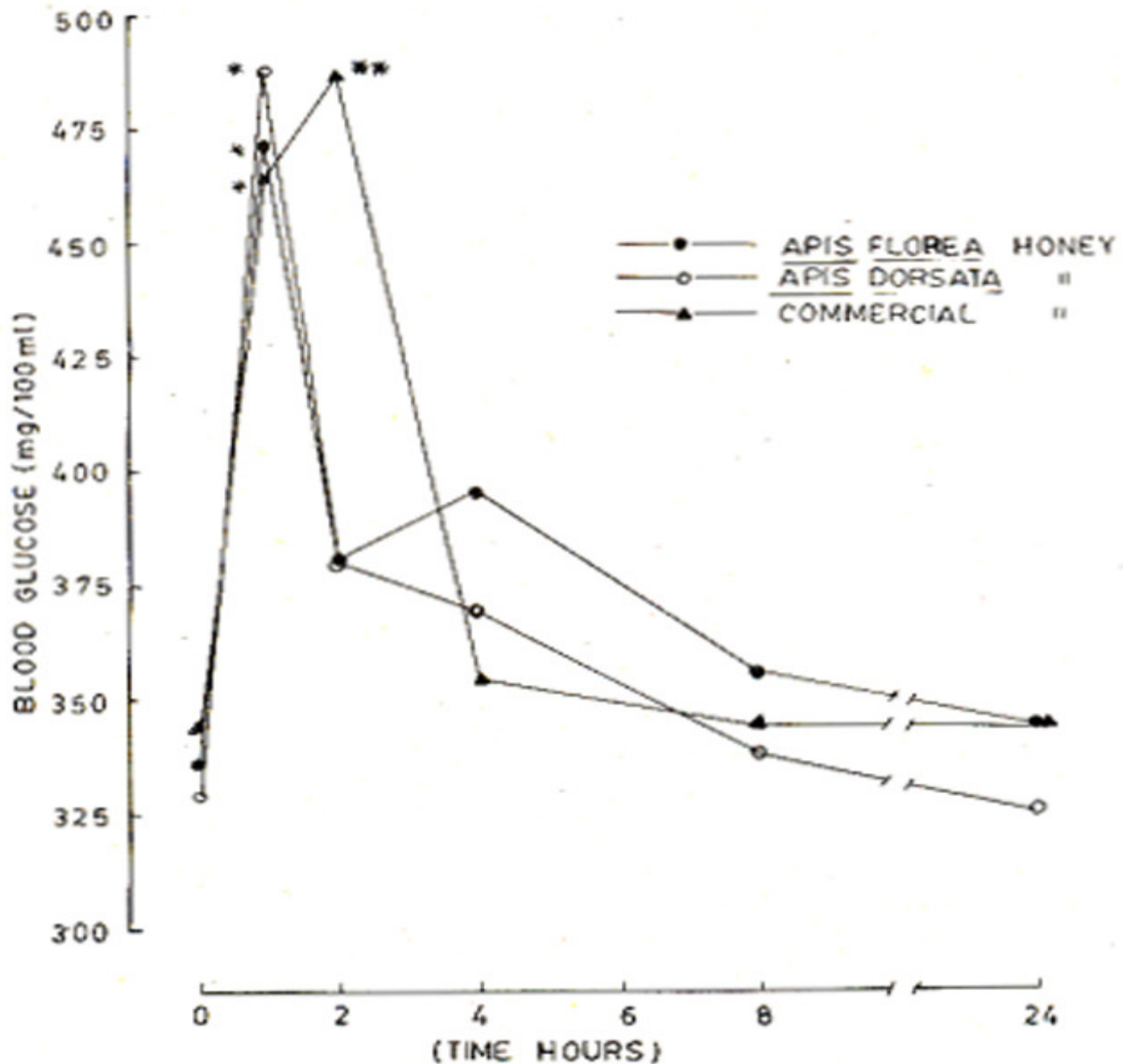


Figure 5. Blood Glucose levels of Alloxan Diabetic Rabbits (mg/100 ml) after oral administration of 10 ml/kg of natural Honeys of *Apis florea*, *Apis dorsata* and a low-priced commercial Honey. * Significant difference from zero level ($P < 0.05$). ** Highly significant difference from zero level ($P < 0.001$). All other values are non-significantly different ($P > 0.05$) from the zero hour. Number of animals in each group = 6.

The blood glucose of animal treated with 15 ml/kg of small-bee honey after 1 hour was significantly

higher than the blood glucose at zero hour. At 2, 4, 8 and 24 hours, the levels were, however, not significant from zero level. Similarly, treatment with 15 ml/kg of large-bee honey caused a highly significant increase in glucose level at 1 hour interval only. However, administration of 15 ml/kg of commercial honey produced a highly significant increase in glucose levels at 1 and 2 hour interval. At 24 hours interval, glucose level was found to be similar to the zero hour level (Figure 6).

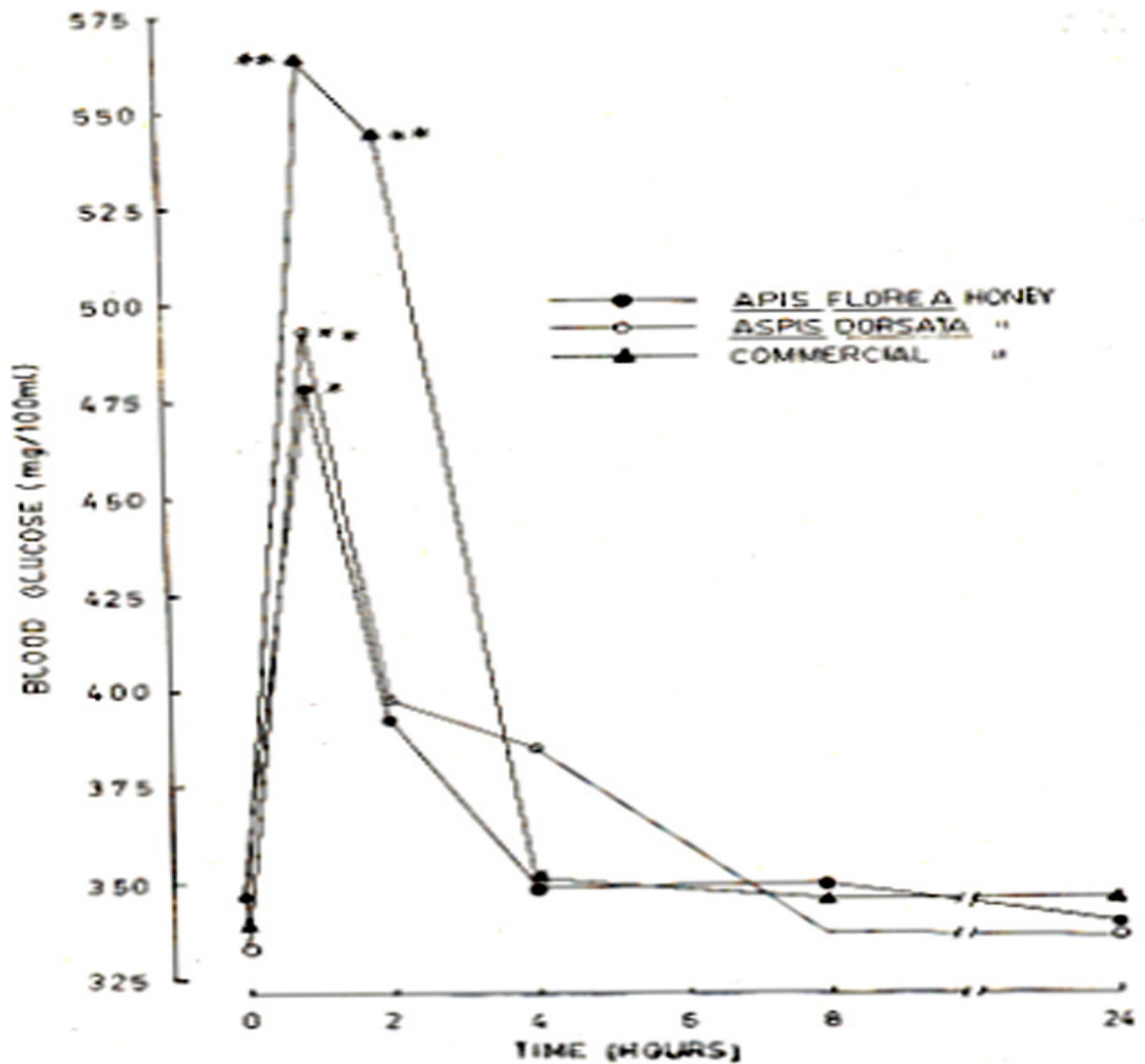


Figure 6. Blood Glucose levels of Alloxan Diabetic Rabbits (mg/100 ml) after oral administration of 15 ml/kg of natural Honeys of *Apis florea*, *Apis dorsata* and a low-priced commercial Honey.

* Significant difference from zero level ($P < 0.05$).

** Highly significant difference from zero level ($P < 0.001$).

All other values are non-significantly different ($P > 0.05$) from the zero hour.

Number of animals in each group = 6.

DISCUSSION

Honey is produced naturally by several types of bees from the nectar of various plants. Although it is

sweet in taste and is known to contain certain sugars but has been claimed in the eastern medicine to exert antidiabetic properties⁴. Many practitioners of indigenous medicine (Hakims) are still using it in various formulations to treat human diabetic patients and is also commonly used to sweeten the diabetic foods and drinks. Beekeepers have also claimed that numerous diabetic patients have recovered from diabetes by using honey as their source of carbohydrates². Several international diabetes associations have agreed on the point that foods which raise blood glucose level least for a given carbohydrate content are most suitable¹. However, it was not known as to whether or not honey could be prescribed to the diabetic patients. The chemical analysis of the honeys showed that the concentrations of non-reducing sugars were 5.16 g%, 5.50 g% and 9.60 g% in *Apis florea* (small-bee), *Apis dorsata* (large-bee) and commercial honeys, respectively. The major non-reducing sugar in the honey is sucrose. Its highest limit in the honey is 5 per cent.^{12,13} Thus, sucrose in the commercial honey was higher, indicating that sucrose syrup has been added to some natural honey. However, non-reducing sugars in both the natural honeys tested were near to the standard. Administration of 10 and 15 ml/kg body weight of small-bee honey significantly raised the blood glucose levels of treated rabbits at one hour interval thus suggesting that natural small-bee honey raises the blood glucose levels. Similarly, blood glucose levels of normal rabbits were also significantly elevated by the large-bee honey at one and even 2 hours at 15 ml/kg dose. However, the rise in blood glucose levels at various doses were also acute. These data also suggest that the commercial honey was adulterated with sucrose syrup. The effects of all the three types of honeys were also studied in the alloxan-diabetic rabbits. Alloxan exerts highly selective cytotoxic action on the beta cells of the islets of Langerhans producing the classical signs of human diabetes i.e.) hyperglycaemic, glycosuria, polydipsia and polyuria, loss in the body weight and acidosis¹⁴. A single injection of 150 mg/kg of alloxan in rabbits was found to kill the beta cells¹⁵. The blood glucose levels of the alloxan-induced diabetic rabbits treated with 10 and 15 ml/kg of small-bee honey were also found to be significantly raised. Similar results were obtained with the large-bee honey and the commercial honey which also produced acute hyperglycaemia in diabetic rabbits as they did in normal rabbits. In the light of the data discussed it was concluded that, in contrast to the common belief, pure natural honeys do not exert hypoglycaemic effect in the rabbits. Evidently, this is due to their high reducing and non-reducing sugar contents. Moreover, in 10 and 15 ml/kg doses all three honeys tested produced a significant rise in blood glucose levels in both normal and diabetic rabbits. However, as has already been presumed only those honey-bees which are fed on the nectar of some specific plants could produce hypoglycaemic honey. Therefore, it is possible that honeys used in this study were not those of honey-bees fed on plants of that nature. However, natural honeys from small and large honey-bees at low dosage of 5 ml/kg only could not produce significant hyperglycaemic effect in normal as well as diabetic rabbits and the honey adulterated with a saturated solution of sucrose could only produce a significant rise in blood glucose levels even at 5 ml/kg dose level in both normal and diabetic rabbits. Therefore, it is conceivable that pure honey from either small or large bees may be prescribed in low doses to the moderately diabetic patients and may even be employed in small amounts as a sweetening agent. In large doses, however, the honey in principle seems to be contraindicated as all other sugars or carbohydrate-rich drinks or foods for the diabetics.

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