A CONSTITUENT OF *PTEROCARPUS MARSUPIUM*, (−)-EPICATECHIN, AS A POTENTIAL ANTIDIABETIC AGENT

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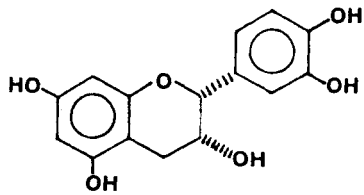
ABSTRACT.—An active constituent of *Pterocarpus marsupium*, (−)-epicetechin (**1**), has been reported to reverse hyperglycemia in alloxan diabetic rats when given before or within 24 hr after the dose of alloxan. However, when doses of (−)-epicatechin (30 mg/kg, i.p., twice daily for 3 days) are begun 92 hr after alloxan, there is no significant difference in blood glucose levels between control and (−)-epicatechin treated rats. These data suggest that, although (−)-epicatechin may protect against alloxan toxicity under certain conditions, the usefulness of (−)-epicatechin appears minimal in the treatment of already established diabetic states.

Extracts of *Pterocarpus marsupium* Roxb. (Leguminosae) have been reported to be potentially useful as hypoglycemic agents in the treatment of diabetes mellitus. Five hours after oral administration of extracts of *Pterocarpus marsupium* heartwood to fasted rabbits, blood glucose levels dropped approximately 25% (1). When the ethyl acetate soluble fraction of an ethanol extract of *Pterocarpus marsupium* bark was given (i.p.) to rats, blood glucose levels in alloxan diabetic animals returned to normal. The same dose of extract had little effect on blood glucose levels in normal rats (2). It has recently been reported that the active hypoglycemic principal of the bark is (−)-epicatechin (**1**) (3). Furthermore, it has been reported that the hypoglycemic effect of the bark extract and isolated (−)-epicatechin is due to regeneration of beta cells in the pancreatic islets of alloxan diabetic rats (2,3).



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The dosing protocol in the aforementioned studies involved giving several i.p. doses of *Pterocarpus marsupium* extract or (−)-epicatechin prior to or beginning within 24 hours after administration of the diabetogenic chemical, alloxan. Under these conditions, both the bark extract and (−)-epicatechin normalized blood glucose levels in the alloxan-treated animals by 4 days after alloxan administration. These results prompted the investigators (2,3) to suggest, "(−)-epicatechin should prove to be a most important antidiabetic drug, provided it is taken for detailed clinical trials on diabetic patients." However, the diabetogenic effects of alloxan are notoriously variable during the first 48 hours after administration. Changes in blood glucose levels are often triphasic, consisting of initial hyperglycemia, hypoglycemia for 24–48 hours, followed by more or less permanent hyperglycemia (4). If a drug is to be useful in the treatment of type I diabetes mellitus, it may be more valid to study its effects on blood glucose levels in rats after they have entered the third phase of alloxan's action, *i.e.*, they are stable and "permanently" diabetic.

The following study was done to test whether (−)-epicatechin can reverse diabetic symptoms in alloxan-treated rats after they have stabilized at a specific level of hyperglycemia. We feel that an effect, or lack of an effect, of (−)-epicatechin on such a population of animals would be a more valid indication of the potential clinical usefulness of (−)-epicatechin.

EXPERIMENTAL

ANIMALS.—Adult male rats of Wistar descent with an average weight of 218±7 g were used. All animals were maintained with free access to Purina Lab Chow and tap water. Prior to alloxan administration, Lab Chow was withheld for 24 hours and only water remained available. Lab chow was returned one hour after alloxan administration and remained freely available for the remainder of the study.

CHEMICALS.—Alloxan (Lot #11F3905) was purchased from Sigma Chemical Co., St. Louis MO: (−)-epicatechin (Lot #0115BHBH) was purchased from Aldrich Chemical Co., Milwaukee, WI. Due to the instability of alloxan in aqueous systems (4), solutions for dosing were prepared (in approximately 2 ml of normal saline) individually for each animal just prior to injection. (−)-Epicatechin solutions (3 mg/ml) were prepared daily in normal saline solution.

DOSING PROTOCOL.—Baseline blood glucose levels were determined 24 hr prior to alloxan administration, after which the animals were fasted overnight. Alloxan (125 mg/kg, i.p.) was given 24 hours later (designated time zero, table 1). The dose of alloxan was determined from the dose-response data of Chawalit *et al.* (5), so that a minimum number of animals died and a fairly wide range of hyperglycemia was produced. Seventy-two hours after alloxan, blood glucose levels were measured. Based on these determinations, the rats were ranked from highest to lowest blood glucose level. Subsequently, the animals were selected by pairing those with the two highest levels, the next two highest levels, *etc.* One member of each pair was given (−)-epicatechin (30 mg/kg, i.p.); the other member, serving as a control, was given an equivalent volume of saline solution at 92, 100, 116, 124, 140 and 148 hr after alloxan. During this administration period, blood glucose levels were measured at 120, 168 and 288 hr after alloxan.

BLOOD GLUCOSE ASSAY.—To obtain blood samples for measurement of glucose levels, the tip of the rat's tail was snipped, and blood was collected into a 25 microliter heparinized capillary tube. The sample was placed in 1 ml of ice cold 0.33M perchloric acid, mixed well, and centrifuged at 2000 g for 10 min. Aliquots of the supernatant were analyzed for glucose by the glucose oxidase-peroxidase method (6).

EFFECT OF (−)-EPICATECHIN ON ALLOXAN INDUCED DIABETES.—The results of this study are summarized in table 1. The dose of alloxan selected (125 mg/kg) was lethal to only one animal (#11) and produced a range of blood glucose levels from 138 to 612 mg/dl after 72 hr. The blood glucose levels gradually decreased from 72 to 288 hr. after alloxan in both control and

TABLE 1. Blood glucose levels (mg/dl) in alloxan-treated[a] male rats with and without subsequent (−)-epicatechin administration.

Animal Number	Treatment[b] Group	Time from Alloxan Injection (hr)				
		−24	72	120	168	288
11	Control	128	612	—[c]	—	—
14	Epicatechin	141	612	413	387	382
4	Control	126	503	438	302	387
15	Epicatechin	117	462	385	496	367
13	Control	137	410	300	332	232
16	Epicatechin	130	410	380	351	263
12	Control	128	388	359	328	324
8	Epicatechin	132	369	309	290	281
3	Control	134	366	305	334	222
2	Epicatechi	134	362	318	266	278
5	Control	130	227	131	147	133
1	Epicatechin	111	167	147	128	126
7	Control	119	160	131	145	135
9	Epicatechin	122	152	120	130	132
6	Control	136	141	124	149	120
10	Epicatechin	124	138	178	132	117

[a]All rats received alloxan (125 mg/kg, i.p.) at time zero.
[b]Rats in the epicatechin groups received (−)-epicatechin (30 mg/kg, i.p.) at times 92, 110, 116, 124, 140 and 148 hours; control rats were given equivalent volumes of saline (i.p.).
[c]Rat number 11 died 96 hours after alloxan.

(−)-epicatechin treated rats. For the overtly hyperglycemic animals (72 hr blood glucose >200 mg/dl), the mean decrease in blood glucose levels from 72 to 288 hours was 129 mg/dl in the (−)-epicatechin treated group and 119 mg/dl in the control group. By comparing each pair of animals in table 1, it is obvious that (−)-epicatechin is virtually without effect on hyperglycemia when doses are initiated several days (92 hr) after alloxan administration. Moreover, analysis of the pooled data for each time point (mean±SEM), with the Student's t test, indicated that there was no statistically significant difference in blood glucose levels between control and treated groups at any time point. However, due to the wide range of blood glucose levels after alloxan (range 138–612 mg/dl), mean values are not reported since they do not accurately represent the overall design and results of the study.

DISCUSSION

Although extracts of *Pterocarpus marsupium* heartwood have been reported to decrease blood glucose levels in normoglycemic rabbits (1), previous reports (2) and preliminary results from our laboratory (data not shown) indicate that such extracts are without effect in normoglycemic rats. However, if twice daily injections of *Pterocarpus marsupium* extract or (−)-epicatechin (30 mg/kg) are initiated 24 hr after a diabetogenic dose of alloxan, blood glucose levels remain normal when compared to rats treated with alloxan alone (2,3). These data suggest that (−)-epicatechin (or possibly another component of *Pterocarpus marsupium* extract) does indeed protect against alloxan toxicity to some extent. However, protection against alloxan toxicity is a property shared by many other chemicals and certain endogenous compounds (4) which are not useful therapeutic agents in the treatment of diabetes mellitus.

In our current studies, doses of (−)-epicatechin were administered to alloxan-treated rats starting 92 hr after alloxan administration. Essentially, the study tests whether (−)-epicatechin can reverse pancreatic damage and the related hyperglycemia of a well established diabetic state. Although blood glucose levels do decrease from 72 to 288 hr after alloxan in (−)-epicatechin treated rats, this decrease if not significantly different from the decrease observed in control animals. Therefore, our study suggests, at least in alloxan-treated rats, that (−)-epicatechin does not effectively stimulate islet *beta* cell regeneration or decrease hyperglycemia once the diabetic state has been firmly established. Further studies, possibly with other animal models for human diabetes, will be required before (−)-epicatechin can be considered a viable antidiabetic agent for use in human clinical studies.

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