ANTIALLERGENIC ACTIVITY OF BIRCH BARK DRY EXTRACT WITH AT LEAST 70% BETULIN CONTENT

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Birch bark dry extract (BBDE) containing at least 70% betulin and betulin alone were compared with the reference drugs Suprastin and Claritin for their antiallergenic action. It is established that BBDE and betulin reduce by half the systemic anaphylactic reaction in guinea pigs immunized with ovalbumin from chicken egg white, their action being comparable with that of Suprastin in mild and medium anaphylactic shock. The course of BBDE administration per os or i.p. leads to a significant decrease in the content of IgE antibodies to ovalbumin in mice of various strains immunized in different regimens. The effect of BBDE on anaphylactic contraction of isolated guinea pig ileum samples was evaluated and showed no direct action upon the H1-receptors. Studies on models of pseudoallergenic reaction to concanavalin A in mice and carragenan-induced paw edema in rats showed that the anti-inflammatory action of BBDE is comparable with that of Suprastin and Claritin and is more pronounced than the effect of betulin.

Key words: birch bark dry extract, betulin, antiallergenic action, passive cutaneous anaphylaxis, anaphylactic shock, anti-inflammatory action.

Allergic diseases are some of the most common of human pathologies. The steady growth of allergic diseases, especially in children, has become epidemic during the last 30 – 40 years [1]. Therefore, the discovery of new effective domestic antiallergic preparations without undesirable side effects remains critical.

Birch bark dry extract (BBDE) with betulin content at least 70% that is prepared by an original high-technology method [2] is widely used as a biologically active additive. Lupane-type pentacyclic triterpenoids in the extract possess antioxidant, anti-inflammatory, hepatoprotective, antimutagenic, and several other properties [3 – 6]. Our work on the evaluation of the immunotoxicity and allergenic properties of BBDE using systemic anaphylaxis in guinea pigs revealed antiallergenic activity for BBDE and its ability to suppress pseudoallergic inflammation by concanavalin A [7]. This prompted us to study the antiallergenic activity of BBDE.

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EXPERIMENTAL

BBDE (OOO Birch World, Reg. No. CEZ No. 77.99.03.936.B.000201.02.04) containing at least 70% betulin was prepared by extraction of the outer layer of birch bark with organic solvents and further purification in pure ethanol. BBDE and the main component of BBDE, betulin, were administered *per os* and i.p. at therapeutically effective doses of 50 and 100 mg/kg. BBDE at a dose of 100 mg/kg exhibited antiallergenic activity in our previous investigations [7]. Claritin (loratadine), dexamethasone, and an injectable form of Suprastin (chloropyramine), the daily doses of which for man were extrapolated to mice and rats as before [8], were used as reference preparations. The specific antiallergenic activity of BBDE was evaluated using methodical recommendations approved by the Pharmacological Committee in 2005 [9]. We used male albino guinea pigs from the RAMS Central nursery (250 – 300 g), white mongrel male rats (170 – 200 g), and CBA, Balb/c, and F1 hybrid (CBA × C57BL/6) mice from the RAMS Stolbovaya nursery (18 – 20 g). Animals were kept in the RAMS Pharmacology Institute vivarium under conditions conforming to current sanitary regulations with free access to water and food.

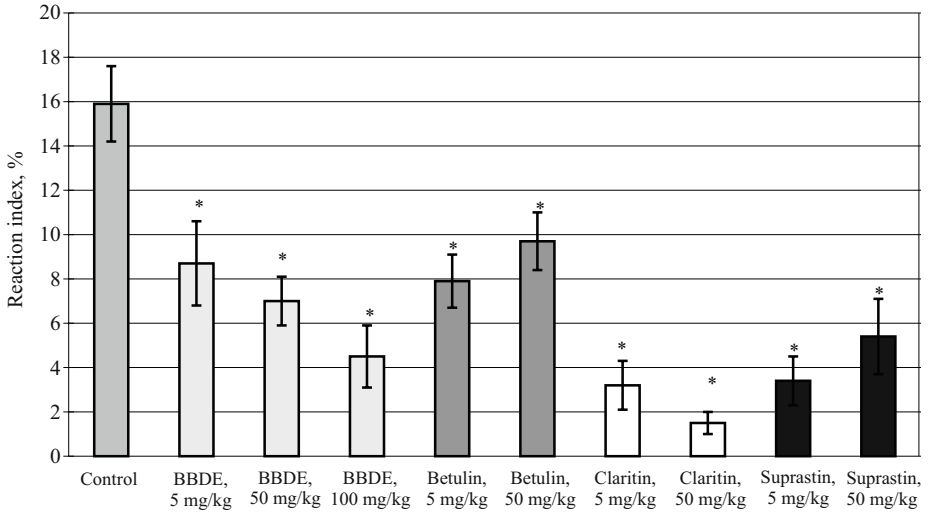

Fig. 1. Effect of single i.p. administration of BBDE compared with betulin, Claritin, and Suprastin on Con A inflammation. Differences from control at *, $p < 0.01$.

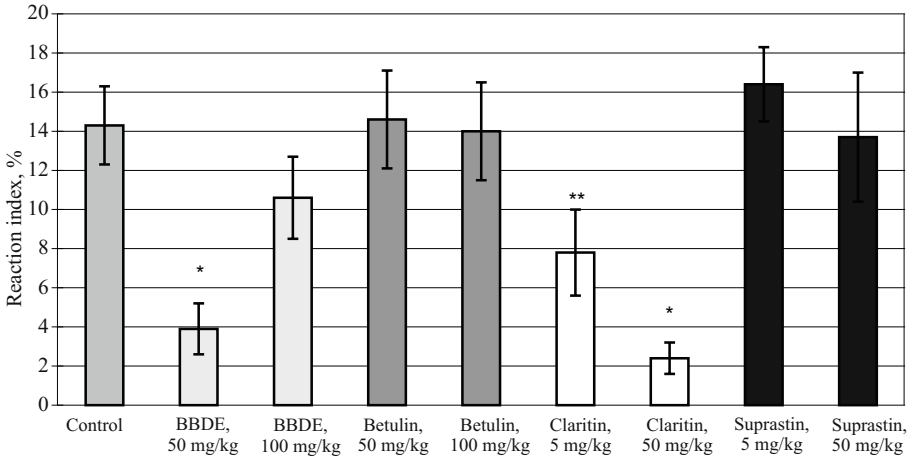

Fig. 2. Effect of single *per os* administration of BBDE compared with betulin, Claritin, and Suprastin on Con A inflammation. Differences from control at *, $p < 0.05$; **, at $p < 0.01$.

General anaphylaxis [10] was induced using a sensitization model of intact guinea pigs with chicken egg-white solution (0.6%, CEW, main allergenic component ovalbumin) [11]. The effect of BBDE on the production of allergen-specific IgE antibodies to ovalbumin (OVA) was studied using a model of passive cutaneous anaphylaxis (PCA) [9]. Mice of experimental groups F_1 (CBA × C57BL/6) were administered BBDE over 10 d *per os*. Mice of the Balb/c line were immunized with OVA by the literature method [12].

The effect of BBDE on anaphylaxis of isolated guinea pig ileum smooth muscle was studied according to the methodical recommendations [9]. Contraction of the ileum was induced by histamine solution (10^{-7} M) [13]. In the first series of experiments, BBDE, Suprastin, and loratadine were injected after a stable reaction to histamine was obtained and 5 – 60 min before the next injection of the agonist. The final concentration of BBDE dissolved in ethanol (96.6%) was 2×10^{-3} mg/mL for 5 – 15 min exposure of the extract and 4×10^{-2} mg/mL for 60 min exposure. In the second series of experiments, contraction of guinea pig ileum induced by histamine was evaluated after three BBDE injections to guinea pigs immunized with OVA.

The effects of BBDE and betulin on the pseudoallergic reaction to the non-immunologic activator concanavalin A (Con A) were studied in CBA mice at doses of 5, 50, and 100 mg/kg with Claritin and Suprastin as references [9]. The effects of BBDE and betulin on carragenan-induced rat paw edema [9] were compared with that of dexamethasone. The

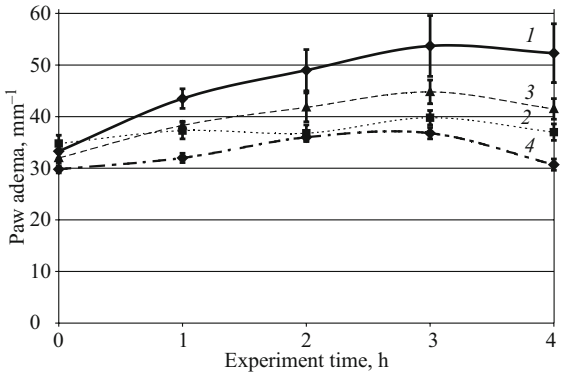

Fig. 3. Effect of BBDE compared with betulin and dexamethasone on rat paw edema developing after carragenan administration: control (*1*), BBDE, 50 mg/kg (*2*), betulin, 50 mg/kg (*3*), dexamethasone, 1.4 mg/kg (*4*).

dynamics of edema development after carragenan injection were recorded every hour for 4 d using a micrometer.

RESULTS AND DISCUSSION

The study of the effect of i.p. injection of BBDE and Suprastin at doses of 50 mg/kg on systemic anaphylaxis showed that injection of BBDE and Suprastin suppressed by 52.4 and 71.4%, respectively, the strength of the systemic anaphylactic reaction (Table 1). In the next experiments, i.p. injection of BBDE and betulin at doses of 50 mg/kg suppressed by 57.9 and 52.6%, respectively, the strength of the systemic anaphylactic reaction in guinea pigs compared with the control (Table 1). Administration of BBDE *per os* for 10 d to F_1 (CBA × C57BL/6) mice immunized with OVA reduced significantly the titres of IgE antibodies to OVA by 75.9% in the PCA reaction in rats (Table 2). Injecting BBDE or betulin i.p. three times at doses of 50 mg/kg to Balb/c mice starting on the day of the sensitizing injection also decreased titres of allergen-specific IgE antibodies to OVA in the PCA reaction by 81.6%. Thus, BBDE and betulin at doses of 50 mg/kg, like in the previous experiments [7], halved the systemic anaphylactic reaction in guinea pigs immunized by CEW. Their activity was comparable to that of Suprastin for mild and medium anaphylactic shock. The decreased titres of allergen-specific IgE antibodies to OVA confirmed that the results on suppression by BBDE upon administration at doses of 50 and 100 mg/kg of the systemic anaphylactic reaction in guinea pigs.

Reliable changes of ileum contractile activity due to histamine was not observed in guinea pigs upon exposure to BBDE in various concentrations with specimens of longitudinal ileum for 5 min, 1 h, and 1 d (Table 3). The H_1-receptor blockers Suprastin and loratadine at various concentrations induced pronounced suppression of histamine spasmogenic activity on guinea pig ileum. In another series of experiments, it was shown that i.p. injection of BBDE three times at a dose of 50 mg/kg to guinea pigs caused significant suppression by 1.5 times ($p < 0.05$) compared to the control (100%) in contractile activity of ileum specimens (67.7 ± 3.6%). Results from a study of BBDE activity toward

TABLE 1. Effect of BBDE, Betulin, and Suprastin on Strength of Anaphylactic Shock in Guinea Pigs

Index	BBDE compared with Suprastin			BBDE and Betulin		
	Control, $n = 10$	BBDE, 50 mg/kg, $n = 10$	Suprastin, 50 mg/kg, $n = 10$	Control, $n = 10$	BBDE, 50 mg/kg, $n = 10$	Betulin, 50 mg/kg, $n = 9$
Index of WE-IGL E reaction	2.1	1.0	0.6	1.9	0.8	0.9

Note: Here and in Table 2, n = number of animals in group.

TABLE 2. Effect of BBDE and Betulin on Passive Cutaneous Anaphylaxis (PCA)

Administration pathway	Animal group	PCA (mean titre log E of antibodies to OVA in $\log_2$)
Per os BBDE to hybrid mice F_1 (CBA × C57BL/6) for 10 d	Control (OVA), $n = 9$	2.9 ± 0.2
	BBDE, 50 mg/kg, $n = 9$	0.7 ± 0.3 ($p < 0.01$)
3 × i.p. BBDE and betulin to Balb/c mice	Control (OVA), $n = 10$	3.8 ± 0.1
	BBDE, 50 mg/kg, $n = 12$	0.7 ± 0.3 ($p < 0.01$)
	Betulin, 50 mg/kg, $n = 10$	0.8 ± 0.3 ($p < 0.01$)

TABLE 3. Effect of BBDE, Suprastin, and Loratadine on Contractile Activity of Guinea Pig Ileum Longitudinal Sections Induced by Histamine at Various Exposure Times

Group	Exposure time	Contractile activity of ileum longitudinal section, %
Control		100
BBDE	5 min	89.1 ± 3.8
Suprastin, 1×10^{-10} mg/mL	5 min	46.5 ± 4.1
Suprastin, 2×10^{-3} mg/mL	5 min	26.5 ± 1.5
Suprastin, 0.2 mg/mL	5 min	Complete suppression
Loratadine, 0.01 mg/mL	15 min	28.4 ± 3.2
Control (alcohol)	1 h	100
BBDE	1 h	90.1 ± 17.1
Control (alcohol)	24 h	100
BBDE	24 h	108.3 ± 11.2

anaphylactic contraction of guinea pig ileum smooth muscle induced by histamine showed a lack of direct action of BBDE on histamine H_1-receptors. The pronounced suppression of contractile activity of ileum specimens by three i.p. injections of BBDE to guinea pigs may indicate a mediated effect of this preparation on H_1-receptors.

Anti-inflammatory properties of BBDE were studied using models of acute edema to Con A and carragenan. Injection (i.p.) of BBDE, betulin, and reference preparations Suprastin and Claritin to CBA mice at doses of 5 and 50 mg/kg (BBDE at 100 mg/kg also) caused pronounced suppression of an inflammatory reaction to Con A (Fig. 1). Administration (*per os*) of BBDE once at a dose of 5 mg/kg did not reliably suppress the inflammatory reaction index to Con A (12.4 ± 2.2) compared to the control (13.8 ± 1.6). Single administration (*per os*) of BBDE at a dose of 50 mg/kg suppressed significantly the inflammatory reaction to Con A compared with the control (Fig. 2). A single administration (*per os*) of betulin at doses of 50 and 100 mg/kg and Suprastin at doses of 5 and 50 mg/kg did not affect the inflammatory reaction to Con A. Administration (*per os*) of Claritin at doses of 5 and 50 mg/kg produced pronounced dose-dependent suppression of the inflammatory reaction to Con A (by 45.5 and 83.2%, respectively).

Single injection (i.p.) of BBDE at a dose of 50 mg/kg to white mongrel male rats suppressed significantly edema due to carragenan for the whole duration of the experiment (Fig. 3). Local inflammatory reaction in control rats reached a maximum 3 d after carragenan injection. By this time edema had decreased by 25.9% in animals that were administered BBDE. Injection (i.p.) of dexamethasone decreased edema by 31.5% compared with the control group 3 d after carragenan injection. A significant decrease of edema (by 12.0%) was observed only 2 d after carragenan injection for injection of betulin. It was shown at the end of the experiment (4 d after carragenan injection) (Table 4) that injection of BBDE suppressed reliably (by 34.6%) the mass increase of test-group paws (by 55.4%) compared to the control. Experimental results for pseudoallergy models of Con A in mice and rat-paw edema due to carragenan confirmed that BBDE has anti-inflammatory activity (over a broad range of doses for various methods of administration) that is comparable with that of a hydrocortisone homolog, dexamethasone, and more pronounced than that of betulin.

According to the literature, lupane-type pentacyclic triterpenoids in BBDE such as betulin and several minor components, in particular lupeol and betulinic acid, are pharmacologically active compounds. The activity of the triterpenoid phytocomplex is greater than that of the separate components. Some of the triterpenoids in BBDE are present as caffeates. The caffeoyl group is known to be capable of intensifying biological activity [14, 15]. In particular, the antiradical properties of birch-bark extracts have been explained by the presence in them of betulin caffeates and oleic and betulinic acids [16]. The suppression of the systemic anaphylactic reaction may also be related to the ability of BBDE to induce selectively γ-interferon and inhibit production of Th_2 helpers and IgE antibody synthesis, thereby converting the allergic response to an immune one [17].

TABLE 4. Effect of BBDE Compared with Betulin and Dexamethasone on Acute Paw Edema 4 h After Carragenan Administration

Dose of studied compound	Number of animals in group	Reaction index: $I_r = \frac{(P_{ex} - P_c)}{P_c} \times 100\%$
Control	8	57.8 ± 7.2
BBDE, 50 mg/kg	8	37.8 ± 4.0 ($p < 0.05$)
Betulin, 50 mg/kg	8	54.0 ± 2.9
Dexamethasone, 1.4 mg/kg	8	25.8 ± 4.0 ($p < 0.01$)

The mechanism of action and chemical structure of the lupane-type triterpenoids are similar to those for polyhydroxylated sterols found in certain plant species [18, 19]. According to the literature, betulin and betulinic acid have an affinity for glucocorticosteroid receptors and possess anti-inflammatory activity comparable to that of dexamethasone [5]. The anti-inflammatory activity of BBDE is probably due to the fact that betulin, like corticosteroids, inhibits phospholipase A_2 [20]. Steroid-like compounds capable of suppressing phospholipase A_2 activity and, therefore, delaying the initial stage of various forms of inflammation, are currently being sought. Such compounds are also less toxic compared to classical corticosteroids. Prolonged use of steroid-like compounds of plant origin does not produce such distinct side effects as the use of corticosteroid anti-allergenic preparations does [18].

Thus, a comparison showed that the triterpenoid phytocomplex BBDE possesses antiallergenic and anti-inflammatory activity that is stronger than betulin and indicated that the discovery of antiallergenic drugs based on BBDE is likely.

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