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Evaluation of anti-allergic activity of gossypin and suramin in mast cell-mediated allergy model

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The mast cell-mediated allergic reactions are involved in many allergic diseases, such as asthma, allergic rhinitis and sinusitis. Stimulation of mast cells initiates the process of degranulation, resulting in the release of mediators such as histamine and an array of inflammatory cytokines. In this report, we investigated the effect of gossypin (a biflavonoid) and suramin (a synthetic polysulphonated naphtylurea) on the mast cell-mediated allergy model, and studied the possible mechanism of their action. Both gossypin and suramin inhibited (P<0.001) compound 48/80-induced systemic anaphylaxis reactions, antiprurities (P<0.001) and reduced the histamine release in rats. Further, both showed significant (P<0.001) protection against rat peritoneal mast cells activated by compound 48/80. Thus, our findings provide evidence that gossypin and suramin inhibit mast cell-derived allergic reactions.

Keywords: Gosyypin, Suramin, Allergic reactions, Compound 48/80, Histamine, Mast cells.

Allergic rhinitis, asthma and atopic eczema are among the commonest cause of chronic ill health[1]. Approximately 56 million people worldwide (20% of population) suffer from allergic rhinitis[2]. Mast cells, the constituents of virtually all organs and tissue are important mediators of inflammatory responses, such as allergy and anaphylaxis. Anaphylaxis is mediated by histamine released in response to antigen cross-linking of immunoglobulin E (IgE) bound to FCεRI on mast cells. Mast cell activation causes the process of degranulation that results in releasing of mediators, such as histamine and an array of inflammatory cytokines[3,4]. In mast cells, depletion of intracellular Ca^{2+} blocks the IgE-induced TNF-α and IL-6 expression through the NF-kβ signaling pathway[5].

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Abbreviations: BAL, bronchoalveolar lavage fluid; DSCG, disodium cromoglycate; IgE, immunoglobulin E; IL, interleukins; NF-kβ, nuclear factor-kB; NO, nitric oxide; PAF, platelet activating factor; ROS, reactive oxygen species; TNF-α, tissue necrosis factor-α.

Mast cells generate intracellular reactive oxygen species (ROS) in response to antigen challenge and this may be involved in histamine release[6]. Histamine affects the maturation of immune system cells and alters their activation[7]. The natural products have shown significant anti-allerigic activities and are generally devoid of adverse effects observed in long-term use of allopathic medicines, such as caffiec acid phenethyl ester, because they act by suppression of IgE levels through inhibition of NF-kβ activation and release[8,9]. A compound that suppresses NF-kβ activation could be a therapeutic agent for treating allergy. Plant extracts have shown anti-allergic activity and are found to act by decreasing intracellular calcium and membrane stabilization through G-protein[10,11].

Gossypin (3,5,7,3,4-pentahydroxy-8-*o*-glucoside) a bioflavonoids, naturally occurring in various plants belonging to the family of Malvaceae has been reported to exhibit ant-inflammatory action through inhibition of arachidonic acid metabolisium[12] and has also shown anti-hyaluronidase activity[13]. Suramin, a polysulfonated naphtylurea developed originally as atrypanocidal agent has been found to be toxic to many human tumor cell lines and used in clinical

trials as anti-neoplastic drug against different types of cancer[14], and indiscriminately hinders the dissociation of various guanine nucleotides from the G-protein[15].

In this study, we have evaluated the effect of gossypin and suramin on the systemic anaphylaxis, antipruritis, mast cell stabilizing activity and inhibition of histamine release induced by compound 48/80 (a mast cell degranulator) in serum, BAL fluid and blood, while circulation after release from mast cells.

Materials and Methods

Drugs and chemicals

The compound 48/80, suramin sulphate, toluidine blue (02912ED-016), Griess reagent, disodium cromoglycate and toluene di-isocyanate were obtained from the Sigma Chemical Co. (St. Louis, MO, USA). The *o*-phthalaldehyde and RPMI-1640 medium (AT028) were obtained from Hi-Media Laboratories Pvt. Ltd. Mumbai, India. Refrigerated centrifuge (MPW-350R) from MPW Med. Instrument, Warszawa, Poland and UV Spectrophotometer (UV-1601) Shimadzu Corp, Kyoto, Japan were used. All other chemicals and reagents used were of analytical grade.

Extraction and isolation of gossypin

Hibiscus vitifolius Linn (Malvaceae) is a small herb grown abundantly in most of the tropical parts of India. The plant was authenticated by M Venkaiah, Taxonomist, Department of Botany, Andhra University, Viskhapatnam, India. The fresh yellow petals of *H. vitifolius* flowers (1.5 kg) were extracted thrice by refluxing each time with 5 liters of methanol for 3 to 4 h and the extract was concentrated to a small volume under reduced pressure. The concentrate was kept in refrigerator for 48 h, which left a yellow solid. It was filtered and kept for crystallization, which gave a yellow crystalline (9.6 g) substance[16].

Animals

The either sex Balb/c mice (18-25 g) and Sprague-Dawley rats (220-250 g) were purchased from National Institute of Nutrition (NIN), Hyderabad, India. The animals were kept in a room maintained at a temperature of 22 ± 2°C and relative humidity of 55 ± 5% throughout the study. All the experiments and care of animals were as per the guidelines of Institutional Animal Ethics Committee (IAEC/2005/01-09), H S K College of Pharmacy, Bagalkot, Karnataka.

Systemic anaphylaxis reaction

Compound 48/80 (a mast cell degranulator)-induced systemic anaphylaxis reaction was carried out as previously described[17,18]. Briefly, the Balb/C mice were given an intraperitoneally injection of 8 mg/kg body weight of the compound 48/80. Gossypin and suramin were administered intraperitoneally at doses of 10, 20, 40, and 60 mg/kg and disodium cromoglycate (DSCG) as standard (10 mg/kg), 1 h before the injection of compound 48/80 (n = 10/group). Mortality percentage was calculated as the number of dead mice × 100/total number of experimental mice.

Antipruritis activity

Compound 48/80 was administered subcutaneously at 3 mg/kg into dorsal surface of neck of Balb/C mice to induce scratching behavior. Normal group received 50 µl of normal saline instead of compound 48/80. the gosyypin and suramin at doses 10, 20, 40, and 60 mg/kg and DSCG standards (10 mg/kg) were given intraperitoneal 1 h before compound 48/80 was administered. The incidences of scratching behavior on the whole body and at the site injected with compound 48/80 were counted[19].

Mast cell stabilizing activity

Normal saline (10 ml) was injected into the peritoneal cavity of either sex Sprague-Dawely rats. After gentle massage, the peritoneal fluid was collected and transferred into the Eppendorf test tubes containing 7-10 ml of RPMI-1640 (*p*H 7.2-7.4). Mast cells were washed three- times by centrifugation at a low speed (400-500 rpm), the supernatant was discarded and pellet of mast cells was taken into the medium. The gossypin and suramin at doses of 10, 20, 40, and 60 mg/kg and DSCG standard (10 mg/kg) was given to rats daily for 5 days prior to collection of mast cells. Mast cells from control and treated groups were incubated with compound 48/80 (1 µg/ml) at 37°C for 10 min. After incubation, mast cells were stained with toluidine blue (0.1%) and percent protection against degranulation was counted under a high-power microscope (× 45)[20].

Measurement of nitric oxide in serum, BAL and peritoneal fluid

The above rats were anesthetized by ether and then blood was collected from retro-orbital, centrifuged at 500 rpm for 5 min. Serum, peritoneal or BAL fluid supernatant, each 100 µl was reacted with an equal volume of Griess reagent (1% sulfanilamide, 0.1%

napthlethylenediamine dihydrochloride and 2.5% phosphoric acid) at room temprature. The normal group sample was incubated with normal saline, while in control and treated groups, serum and fluid were incubated with compound 48/80 (1 µg/ml). Chromophore absorbance at 450 nm was determined. Nitrite concentration was calculated using sodium nitrite as a standard[21,22].

Determination of histamine release from blood

The gossypin and suramin administered intraperitoneally at doses 10, 20, 40 and 60 mg/kg and DSCG standard (10 mg/kg,) were administered to rats daily 5 days prior to collection of blood. The rats were anesthetized by ether and blood was collected from retro-orbital plexus. The same blood was treated with compound 48/80 (1 µg/ml), shaken thoroughly and centrifuged at 400 rpm for 10 min. The supernatant was used for measuring the histamine content by *o*-phthaldialdehyde spectrofluorometric method. The fluorescence intensity was measured at emission 438 nm and excitation 353 nm using Spectrofluorometer[23].

Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analyses were performed using Prism software (PRISM Pvts Ltd. USA) by using One-way-Analysis-of-Variance (ANOVA), followed by Dunett's multiple comparison tests and Chi-square test was used for anti-anaphylactic evaluation. *P* value less than 0.05 was considered as significant.

Results

Compound 48/80-induced systemic anaphylaxis

To determine the effect of gossypin and suramin on allergic reaction, compound 48/80 was used as a model for induction of a systemic fatal allergic reactions as shown in Table 1. Administration of compound 48/80 into mice of control showed 100% mortality by fatal shock, but in gossypin and surmin-treated animals showed dose-dependent protection. The standard DSCG exhibited 80% protection as compared to control group. In addition, ED_{50} of gossypin and suramin was found to be 27 and 32 mg/kg, respectively

Antiprutitis activity

Percentage of scratching behavior was significantly (P<0.001) reduced in mice treated with gossypin and suramin, as compared to control group. The results are summarized in Table 2. These two test compounds showed dose-dependent protection on par with reference standard DSCG. Their ED_{50} value was found to be 25 and 56 mg/kg, respectively.

Mast cell stabilizing activity

The results of mast cell stabilizing activity of gossypin and suramin are summarized in Table 3.

Table 1—Effect of gossypin and suramin on compound 48/80-induced anaphylaxis reaction in Balb/c mice

[Values expressed as mean ± SEM, n = 10]

Treatment groups	Dose (mg/kg, i.p)	Mortality (%)	Protection mast cell degranulation (%)
Control	-----	100	----
DSCG standard	10	30	80**
Gossypin	10	80	20
	20	60	40*[a]
	40	30	70***[b]
	60	20	80***[b]
Suramin	10	90	10
	20	80	20
	40	30	70***[b]
	60	40	60***[b]

Levels of significance: *P<0.05, **P<0.01, ***P<0.001 compared to control group; [a]P<0.01, [b]P<0.001 compared to standard DSCG.

Table 2—Effect of gossypin and suramin on compound 48/80-induced pruritis in Balb/c mice

[Values expressed as mean ± SEM, n = 6]

Treatment groups	Dose (mg/kg, i.p)	Scratching numbers	Inhibition of scratching (%)
Normal (normal saline)	1 ml/kg (p.o)	40.83 ± 1.49	----
Control	----	321.7 ± 12.9[a,c]	----
DSCG standard	10	151.5 ± 5.45***	52.90
Gossypin	10	235.7 ± 6.16	26.73
	20	167.3 ± 7.96**	47.99
	40	136.2 ± 7.70***[b]	57.66
	60	127.2 ± 7.32***[c]	60.46
Suramin	10	243.2 ±2.34	60.46
	20	188.2 ± 5.34*	24.40
	40	178.7 ± 3.21**	41.49
	60	156.4 ± 6.32***	51.38

Statistical analyses were performed using One-way-Analysis-of-Variation (ANVOA), followed by Dunnett's multiple comparison test. Levels of significance: *P<0.05, **P<0.01, ***P<0.001 compared to control group; [a]P<0.001 compared to normal; [b]P<0.01, [c]P<0.001 compared to standard DSCG.

There was significant (P<0.001) protection of mast cell degranulation and the observed mast cell stabilizing activity of both compounds was dose-dependent. They ED_{50} value was found to be 26 and 52 mg/kg, respectively.

Serum, peritoneal fluid and BAL fluid nitric oxide

The gossypin showed significant (P<0.001) decrease in nitric oxide level in serum, rat peritoneal fluid (P<0.001) and BAL fluid (P<0.001) in comparison with control group, but suramin did not show significant activity. Treatment with DSCG significantly (P<0.001) reduced nitrate levels in serum, peritoneal fluid and BAL fluid induced by compound 48/80. The results are summarized in Table 4.

Effect on histamine release from blood

Inhibitory effect of gossypin, suramin and standard DSCG on compound 48/80-induced histamine release from mast cells is shown in Table 5. These compounds inhibited histamine release from blood in dose-dependent (P<0.001) manner.

Table 3—Effect of gossypin and suramin on compound 48/80-induced mast cell activation in rats

[Values expressed as mean ± SEM; n = 10]

Treatment groups	Dose (mg/kg, i.p)	Activated mast cells (%)
Normal (normal saline)	1 ml/kg (p.o)	49.67 ± 2.445
Control	-----	82.17 ± 2.664[a,c]
DSCG standard	10	31.83 ±1.985***
Gossypin	10	61.67 ± 3.144*
	20	55.17 ± 2.105**
	40	39.50 ± 2.217***[b]
	60	30.20 ± 3.285***[c]
Suramin	10	77.23 ± 2.341
	20	61.21 ± 3.212*
	40	52.21 ± 3.987**
	60	48.31 ± 4.352***[b]

Statistical analyses were performed using One-way-Analysis-of-Variation (ANVOA), followed by Dunnett's multiple comparison test. Levels of significance: *P<0.05, **P<0.01, ***P<0.001 compared to control group, [a]P<0.01 compared to normal; [b]P<0.01, [c]P<0.001 compared to standard (DSCG).

Discussion

Allergy includes allergic rhinitis, anaphylaxis, purities and asthma — the diseases associated with inflammatory conditions. Murine systemic anaphylaxis reactions are important parameters for evaluating anti-allergic property[24]. In our study, gossypin, suramin and DSCG (a reference standard) showed significant (P<0.001) dose-dependent protection in compound 48/80-induced murine systemic anaphylactic reactions. NF-kβ transcription factor is reported to be crucial to the initiation and maintenance of inflammatory reactions by the modulation of several pro-inflammatory mediators, including TNF-α and IL-6. Gossypin and suramin (a G-protein inhibitor) might be involved in the downregulation of NF-kβ, TNF-α and IL-6, The above nuclear factors activation and overexpression are response to release of inflammatory mediator histamine by degranulation of mast cell and leading to allergic reactions. In mast cells, TNF-α has been

Table 4—Effect of gossypin and suramin on nitrate levels in rats

[Values expressed as mean ± SEM, n = 6]

Treatment groups	Dose (mg/kg, i.p)	Serum nitric oxide (nM/ml)	RPMC nitric oxide (nM/ml)	BAL nitric oxide (nM/ml)
Normal (Normal saline)	1 ml /kg (p.o)	11.78 ± 0.444	6.017 ± 0.341	11.70 ± 0.651
Control	-----	31.62 ± 3.762[a]	21.15 ± 1.326[b]	41.08 ± 0.870[b]
DSCG standard		17.48 ± 1.052**	13.23 ± 0.765**	26.08 ±1.920***
Gossypin	10	27.62 ± 1.421*	20.33 ± 1.127	41.20 ± 1.268
	20	21.37 ± 0.618**	18.53 ± 0.912	37.53 ± 1.700*
	40	18.97 ± 1.632***	16.65 ± 0.880*	33.27 ± 1.125**
	60	12.68 ± 2.659***	10.67 ± 0.826**	28.72 ± 1.075***
Suramin	10	29.54 ± 1.232	20.21 ± 0.342	38.76 ± 3.231
	20	27.65 ± 0.432	19.87 ± 2.341	37.45 ± 2.341
	40	26.32 ± 0.347	17.32 ± 2.321	36.43 ± 1.214
	60	24.21 ± 2.543*	14.12 ± 3.210*	37.42 ± 0.321

Statistical analyses were performed using One-way-Analysis-of-Variation (ANVOA), followed by Dunnett's multiple comparison test. Levels of significance:*P<0.05, **P<0.01, ***P<0.001 compared to control group; [a]p<0.01, [b]p<0.001 compared to normal.

Table 5—Effect of gossypin and suramin histamine released from blood in rats

[Values expressed as mean ± SEM, n = 6]

Treatment groups	Dose (mg/kg, i.p)	Blood histamine (µg/ml)
Normal (normal saline)	1 ml/kg (p.o)	0.041 ± 0.073
Control	----	0.140 ± 0.085[a]
DSCG standard	10	0.055 ± 0.012**
Gossypin	10	0.086 ± 0.010*
	20	0.074 ± 0.0143*
	40	0.039 ± 0.012**
	60	0.025 ± 0.324***
Suramin	10	0.110 ± 0.1120
	20	0.106 ± 0.040*
	40	0.084 ± 0.064*
	60	0.059 ± 0.072**

Statistical analyses were performed using One-way-Analysis-of-Variation (ANVOA), followed by Dennett's multiple comparison test. Levels of significance: *P<0.05, **P<0.01,***P<0.001 compared to control group; [a]P<0.01 compared to normal.

found to be prestored in cytoplasmic granules and is known to be released with histamine and thereby suggesting its potential role in early inflammation[24].

Mast cells play a key role in the immediate type of allergic reactions through the release of numerous mediators and cytokines. Mast cell degranulation is also elicited by the synthetic compound 48/80, and it has been used as a direct and convenient model to study the mechanism of anaphylaxis[25]. Numerous reports have established[26] that stimulation with compound 48/80 or IgE initiates the activation of signal transduction pathway, which leads to histamine release. Recent studies have shown that compound 48/80 and other polybasic compounds directly activate G-proteins[26]. Compound 48/80 increases the permeability of lipid bilayer membrane by causing the perturbation in the membrane.

The intracellular calcium pathways are crucial to the degranulation of mast cells. Agents that stimulate an intracellular calcium level have been shown to induce mast cell degranulation[27]. Calcium movements in mast cells represent a major target for effective anti-allergic drugs, as this is an essential event linking stimulation to secretion. Earlier study in mice has shown that plant active constituents exert anti-allergic activity against compound 48/80-induced mast cell degranulation mainly through the inhibitory effect of histamine release and mast cell membrane stabilization property[9].

The present study suggested that gossypin might stabilize the lipid bilayer membrane, thus preventing compound 48/80-induced perturbation through G-protein activation and also inhibiting histamine release, because excessive histamine release stimulates the ROS production, thus leading to lipid peroxidation and oxidative stress. Earlier, we have reported that gossypin exhibits potential antioxidant property[28], thus substantiates the observed anti-allergic activity. Suramin acts directly on G-protein α-subunits and blocks their activation by inhibiting the exchange of GDP for GTP[29]. The anti-allergic activity exhibited by suramin was probably through the inhibition of calcium and consecutive release of histamine by modulating the G-protein function. The gosyypin showed significant inhibition of NO in serum and BAL fluid, because the NO might act as a source of free radicals, leading to the damage of tissues and infiltration of lymphocytes and inflammatory reactions. Further studies are required to elucidate anti-allergic effect of gossypin and suramin at molecular level to develop their therapeutic application for treating inflammatory allergic diseases.

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