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Anti-Inflammatory Activity of Select Sorghum (*Sorghum bicolor*) Brans

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ABSTRACT The bran fractions of certain varieties of sorghum (*Sorghum bicolor*) grain are rich sources of phytochemicals and antioxidants. In this article, the anti-inflammatory actions of extracts of select sorghum brans were evaluated in two experimental inflammatory systems: (1) the release of cytokines by lipopolysaccharide-activated peripheral blood mononuclear cells and (2) 12-*O*-tetradecanoylphorbol acetate (TPA)-induced ear edema in mice. A 1:200 dilution of a 10% (wt/vol) ethanol extract of black sorghum bran significantly inhibited the secretion of the pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α. Ethanolic extracts of both black and sumac varieties of sorghum bran significantly reduced edema in inflamed ears as measured by ear thickness and ear punch weight 6 hours following TPA application. The degree of inhibition was similar to that observed with indomethacin. Black sorghum bran significantly diminished the increase in myeloperoxidase activity 24 hours following the application of TPA. No anti-inflammatory activity was observed with white and Mycogen sorghum bran varieties or with oat, wheat, or rice brans in the mouse ear model. The anti-inflammatory activity observed with these brans correlated with their phenolic content and antioxidant activity. These results demonstrate that select sorghum bran varieties possess significant anti-inflammatory activity.

KEY WORDS: • *cytokines* • *edema* • *flavonoids* • *proanthocyanidins* • *12-O-tetradecanoylphorbol acetate*

INTRODUCTION

SORGHUM GRAIN, one of the world's five most important grains along with maize, wheat, rice, and barley, has long been a staple in human diets.[1] Some sorghum varieties have extremely high contents of phenolic compounds that aid in the natural defense of plants against pests and diseases. These phytochemicals, primarily located in the bran fraction, result in the plant having significant antioxidant properties.[2,3] These antioxidant compounds fall into three major categories: phenolic acids, flavonoids, and tannins. The phenolic acids are divided among two classes—benzoic and cinnamic acid derivatives—whereas the tannins are largely condensed tannins or proanthocyanidins. The major flavonoids in sorghum grain are 3-deoxyanthocyanins, including apigenidin and luteolinidin. The phytochemical content of specific varieties of sorghum bran is much higher than most widely cultivated grains.[4–6] Flavonoids have been shown to reduce inflammation in many experimental models.[7–9] However, the effect of select sorghum brans in experimental models of inflammation has not been examined.

Tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β are two pro-inflammatory cytokines implicated in the pathogenesis of many inflammatory diseases.[10–12] The progression of rheumatoid arthritis, inflammatory bowel disease, and sepsis syndrome has been linked to the dysfunction of cytokine regulation, resulting in the alteration in the balance of pro- and anti-inflammatory cytokines.[10] In addition, pro-inflammatory cytokines play a major role in mediating other inflammatory events such as the induction of cyclooxygenase (COX)-2, an enzyme that is responsible for the edema and vasodilation associated with inflammation.[13] The dermal administration of 12-*O*-tetradecanoylphorbol acetate (TPA) to mouse ears is a well-characterized model that has been shown to elicit an inflammatory response mediated via the up-regulation of COX-2 expression, resulting in increased vascular permeability and edema with infiltration of neutrophils.[14,15] The purpose of this study was to examine the effect of select sorghum brans on both the release of cytokines in activated mononuclear cells and the extent of inflammation in the TPA mouse ear model.

MATERIALS AND METHODS

Materials

TPA, indomethacin, hexadecyltrimethyl-ammonium bromide, 3,3′,5,5′-tetramethylbenzidine dihydrochloride, gallic acid, 2,4,6-tri(2-pyridyl])-*s*-triazine (TPTZ), and vanillin were purchased from Sigma Chemical Co. (St.

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Louis, MO, USA). RPMI 1640 culture medium was purchased from GIBCO (Grand Island, NY, USA), Ficoll-Hypaque was obtained from ICN Biomedicals, Inc. (Aurora, OH, USA), and enzyme-linked immunosorbent assay (ELISA) kits for IL-1β and TNF-α were products of Biosource International (Camarillo, CA, USA).

Animals

Male Swiss Webster mice (Charles River Laboratory, Wilmington, MA) weighing 20–24 g were housed in polycarbonate cages with Tek-Fresh bedding (Harlan Laboratories, Indianapolis, IN). The mice were kept in an environmentally controlled room maintained at 21°C with a 12:12-hour light:dark cycle. The animals were allowed free access to rodent chow (Purina Lab Diet 5001, Ralston Purina, St. Louis) and water throughout the duration of the experiment. All experimental procedures were approved by the Animal Care and Use Committee at the University of Georgia, Athens, GA, USA.

Preparation of sorghum extracts

Sorghum brans were gifts from Dr. Scott Bean (U.S. Department of Agriculture, Manhattan, KS, USA) and Dr. Lloyd Rooney (Texas A&M University, College Station, TX, USA). Sumac, Mycogen 627 (red and bronze pericarp, respectively), and black and white sorghums were used in these studies. Sorghum varieties were decorticated by abrasive dehulling to obtain the bran fractions.[2,16] To extract phytochemicals, 50% ethanol was added to the sorghum brans (in a range of concentrations from 8.3% to 25% [wt/vol]). The mixture was agitated for 1 hour at room temperature. The extract was then centrifuged at 1,000 *g* for 20 minutes to remove the precipitate and filter-sterilized to obtain the final extract.

Total phenolic content

Total phenolic content of each sample was assayed in triplicate using the Folin-Ciocalteu method.[17] In a polystyrene cuvette, 20 μL of each diluted sample, 1.58 mL of distilled water, and 100 μL of Folin-Ciocalteu reagent were added and mixed well. After 10 minutes, 300 μL of sodium carbonate solution was added. After the 2-hour incubation at room temperature, the absorbance was measured at 765 nm in a Beckman (Palo Alto, CA, USA) DU 600 series spectrophotometer. Gallic acid was used as the standard. Results are expressed as mg of gallic acid equivalent (GAE)/g of bran.

Ferric reducing antioxidant power (FRAP)

FRAP values were determined in triplicate using a method modified from that of Benzie and Strain.[18] Ten microliters of each sample, 30 μL of distilled water, and 300 μL of FRAP reagent were added to a cuvette. The FRAP reagent was prepared with 25 mL of 300 m*M* acetate buffer (pH 3.6), 2.5 mL of 10 m*M* TPTZ dissolved in 40 m*M* HCl, and 2.5 mL of 20 m*M* ferric chloride solution. After 6 minutes at 37°C, 340 μL of distilled water was added to the cuvette, and absorbance was measured at 593 nm in a Beckman DU 600 series spectrophotometer. The results are expressed as mmol of ferrous sulfate formed/100 g of dry bran.

Vanillin-HCl assay for tannins

The tannin concentration was determined for each sorghum bran extract variety in triplicate following a modified version of the vanillin-HCl method.[19] Two hundred microliters of each sample was added to 2 mL of the vanillin reagent. The vanillin reagent was prepared by mixing 1% vanillin in methanol to 8% concentrated HCl, diluted in methanol (1:1, vol/vol) and warmed to 30°C prior to use. After 20 minutes, the absorbance of the samples was measured at 500 nm in a Beckman DU 600 series spectrophotometer. Blanks were made up with 4% HCl in the absence of vanillin. Catechin was used as the standard, and the results are expressed as mg of catechin/g of bran.

Isolation of human blood monocytes

Human blood was collected in heparinized tubes, diluted twofold with RPMI 1640 culture medium, and layered over Ficoll-Hypaque (2.5:7.0 [vol/vol]). The mononuclear cells were collected by centrifugation for 20 minutes at room temperature at 400 *g*. The cells were washed and cultured with RPMI 1640 culture medium containing 10% fetal calf serum, 50 units/mL penicillin, 50 μg/mL streptomycin, and 2 m*M* L-glutamine.

Cellular release of pro-inflammatory cytokines

Human peripheral blood mononuclear cells (1×10^5) were cultured in round-bottom microtiter plates in 0.2 mL of RPMI 1640 culture medium containing 1 μg/mL *Escherichia coli* O111:B4 lipopolysaccharide (LPS) and various dilutions (1:100–1:400) of black sorghum bran extract (10% [wt/vol] in 50% ethanol). For each milliliter of culture medium, this corresponds to amount of extractable phenols from 1 mg to 0.25 mg of black sorghum bran. Control cells were treated with the same volume of 50% ethanol. After 24 hours, the culture supernatants were collected by centrifugation and stored at −70°C. The culture supernatants were thawed and assayed for IL-1β and TNF-α using commercial ELISA kits. A standard curve using recombinant cytokines was included in each assay.

Cellular toxicity of sorghum bran extracts

Human peripheral blood mononuclear cells were cultured for 24 hours in RPMI 1640 medium containing 10% fetal calf serum with same concentrations of black sorghum bran extract (10% [wt/vol] in 50% ethanol) used in the cytokine release experiments. At the end of the incubation period, propidium iodide was added (final concentration, 25 μg/mL). A FACScan™ flow cytometer (Becton Dickinson [BD], San Jose, CA, USA) and was used to determine

the percentage of dead cells using WinMDI version 2.8 software (http://facs.scripps.edu/software.html).[20]

TPA ear edema model

TPA (2 μg/20 μL of acetone) was applied to the inner and outer surfaces of the mouse ear for inducing acute-type skin inflammation. At 20 minutes following the TPA-induced injury, the bran extracts in 50% ethanol were applied (20 μL per ear) to the same site. Except where indicated, a 25% (wt/vol) extract in 50% ethanol was used in these experiments; this amounts to the extractable phenols from 5 mg of bran. The effects observed with the sorghum brans were compared to those of indomethacin (0.5 mg/20 μL of acetone), a nonsteroidal anti-inflammatory drug. Each experiment included a group of animals treated with acetone and then 50% ethanol, referred to as the control group. Another group of animals was treated with TPA and then with 50% ethanol and is referred as the vehicle-treated TPA group. Mice were euthanized 6 hours after TPA treatment. Sections 7 mm in diameter of the right and left ear were weighed, and the thickness was recorded. Ear edema was expressed as the increase in ear thickness due to the inflammatory challenge. Ear thickness was measured before and after the inflammatory response by using a micrometer (series IP65, Mitutoya America Corp., Aurora, IL, USA). To minimize variation due to technique, a single investigator performed the measurements throughout any one experiment.

Myeloperoxidase (MPO) activity

MPO activity was used to assess neutrophil infiltration into the ear. Mice were euthanized 24 hours after TPA treatment, and 7-mm-diameter sections of the right and left ear were removed for determination of MPO.[21,22] The tissue punch was homogenized with a small sample laboratory Tissue Tearor homogenizer (model 985-370, Biospec Products, Bartlesville, OK, USA) in 0.75 mL of 80 m*M* phosphate-buffered saline (PBS) (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide for 45 seconds on ice. The homogenate was transferred to a microfuge tube, and the vessel was washed with 0.75 mL of hexadecyltrimethylammonium bromide in PBS buffer and added to the microfuge tube. The sample was centrifuged at 12,000 *g* at 4°C for 15 minutes. Thirty microliters of sample was added to a 96-well microtiter plate in triplicate. Two hundred microliters of a mixture containing 100 μL of 80 m*M* PBS (pH 5.4), 85 μL of 0.22 *M* PBS (pH 5.4), and 15 μL of 0.017% hydrogen peroxide was added to each sample well. The reaction was started with the addition of 20 μL of 18.4 m*M* tetramethylbenzidine HCl in 8% aqueous dimethylformamide followed by incubation of the plates at 37°C. After 3 minutes, the plates were placed on ice, and 30 μL of 1.46 *M* sodium acetate (pH 3.0) was added to stop the reaction. MPO enzyme activity was assessed colorimetrically using a Bio-Tek (Winooski, VT, USA) microplate reader (ELx808) at an absorbance of 630 nm and expressed as optical density per biopsy.

Statistical analysis

All results are reported as mean ± SEM values. Student's *t* test or one-way analysis of variance was used to statistically analyze data subsequently followed by Tukey's test to detect significant differences among treatment groups ($P < .05$). SigmaStat (SPSS Science, Chicago, IL, USA) was used to perform all statistical analyses.

RESULTS

Phenolic content of sorghum brans

The contents of phenols and FRAP values of sumac, black, white, and Mycogen sorghum bran varieties were initially determined. Total phenols of sumac and black sorghum bran varieties were 20- and 7.5-fold greater than that of white sorghum bran extract and 8.9- and 3.3-fold greater than that of Mycogen sorghum bran extract, respectively (Table 1). The FRAP values of sumac and black sorghum bran extracts (48.4 ± 0.6 mmol/100 g and 15.6 ± 0.4 mmol/100 g, respectively) were greater than those of white and Mycogen sorghum bran extracts. The total phenols and FRAP values were also measured for oat, rice, and wheat brans (Table 1). These brans were found to be low in phenolic content and antioxidant capacity compared to sumac and black sorghum bran varieties. The tannin content was determined for each ethanolic extract of sorghum bran. Sumac sorghum bran had a high concentration of tannins (113 mg/g); black, white, and Mycogen sorghum bran varieties contained no detectable amount of tannins. These results indicate that sumac and black sorghum bran have a higher content of total phenols and antioxidant capacity compared to other types of sorghum or non-sorghum brans.

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Cytokine release from mononuclear cells

The anti-inflammatory effects of high phenolic black sorghum bran extract (10% [wt/vol] in 50% ethanol) were evaluated using LPS-stimulated peripheral blood mononuclear cells. As illustrated in Figure 1A, black sorghum bran extract inhibited in a dose-dependent manner the release of the pro-inflammatory cytokine, TNF-α. A 1:200 dilution significantly inhibited TNF-α release by 52% and a 1:100

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Table 1. Total Phenolic Content and Antioxidant (FRAP) Values of Various Brans

Bran	*Phenolic acid (mg of GAE/g)*	*FRAP value (mmol/100 g)*
Sumac sorghum	62.5 ± 0.6	48.4 ± 0.6
Black sorghum	23.4 ± 0.9	15.6 ± 0.4
Mycogen sorghum	7.0 ± 0.4	4.4 ± 0.2
White sorghum	3.1 ± 0.2	1.7 ± 0.6
Rice	6.0 ± 0.3	5.3 ± 0.1
Wheat	3.9 ± 1.2	1.2 ± 0.1
Oat	0.8 ± 0.1	0.6 ± 0.1

Data are mean ± SEM values of triplicate determinations.

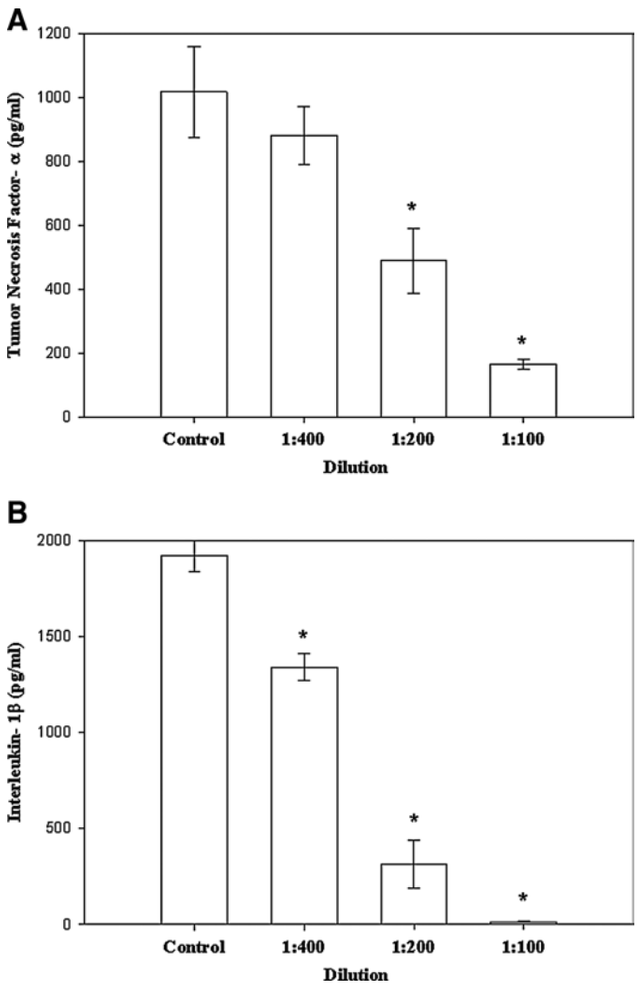

FIG. 1. Inhibition of (**A**) TNF-α and (**B**) IL-1β release from human mononuclear cells by a 10% (wt/vol) ethanol extract of black sorghum bran. Cells were incubated with 1 μg/mL *E. coli* O111:B4 LPS and various dilutions of the black sorghum bran extract. After 24 hours, the contents of TNF-α and IL-1β were determined by ELISA. Data are mean ± SEM values of two separate experiments. *$P < .05$ compared to control cultures.

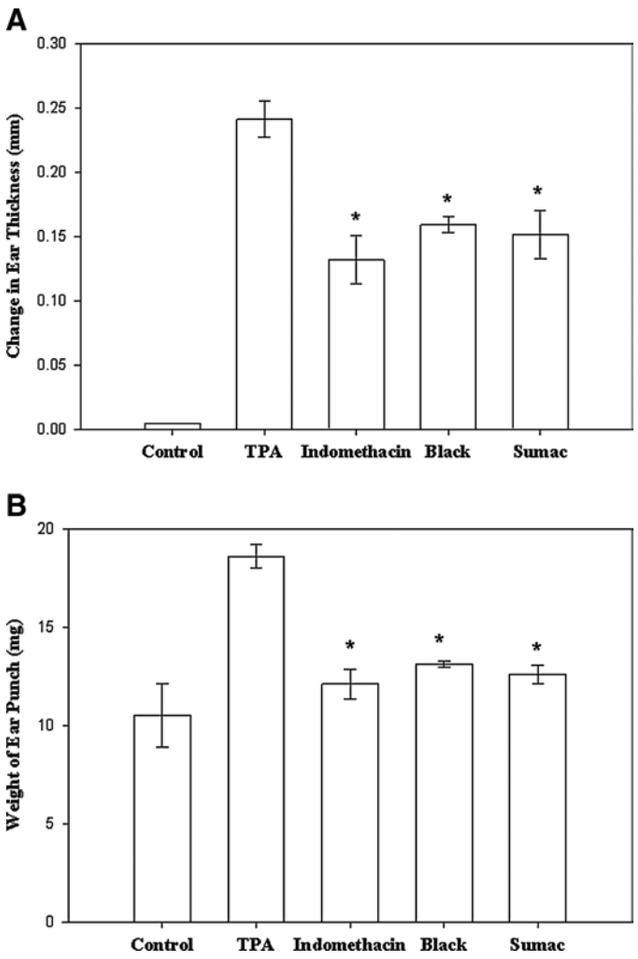

FIG. 2. Anti-inflammatory effect of sorghum bran extracts and indomethacin applied following TPA administration. Sumac and black sorghum bran extracts or indomethacin was applied to mouse ears following the application of 2 μg of TPA. The changes in (**A**) ear thickness and (**B**) the weight of ear punches were determined after 6 hours. Data are mean ± SEM values of eight animals per group. *$P < .05$ compared to the vehicle-treated TPA group.

dilution by 84%. A 1:400 dilution did not produce a decrease in cytokine release. Furthermore, black sorghum bran extract had a more profound effect on the release of cytokine IL-1β (Fig. 1B). At a 1:400 dilution, IL-1β release was significantly inhibited by 30%, whereas a 1:100 dilution produced a near total inhibition of release.

The effect of black sorghum bran extracts on cell viability was also measured to determine if the effects of black sorghum bran on mononuclear cell cytokine secretion were related to cell death. The proportion of dead cells in the untreated and ethanol-treated cells was similar to the cells treated with black sorghum bran extract at a dilution of 1:400 (1.7%) and 1:200 (2.4%). A dose of 1:100 of sorghum bran extract was associated with a 3.3% cell death. These results indicate that the reduction of TNF-α and IL-1β was not a result of toxicity of black sorghum bran.

Effect of ethanol extracts of sorghum brans on TPA-induced mouse ear inflammation

The change in ear thickness mediated by TPA after 6 hours was from 0.005 ± 0.002 mm to 0.24 ± 0.01 mm, and ear punch weight increased from 10.5 ± 1.6 mg to 18.6 ± 0.6 mg. As illustrated in Figure 2A, black and sumac bran extracts (20 μL per ear of a 25% [wt/vol] extract in 50% ethanol) applied 20 minutes following the induction of inflammation with TPA significantly decreased ear thickness by 33% and 36%, respectively. Furthermore, the weights of the ear punches were reduced by 30% and 32% with black and sumac bran extracts, respectively (Fig. 2B). Similar results were observed when ears were pretreated with black and sumac

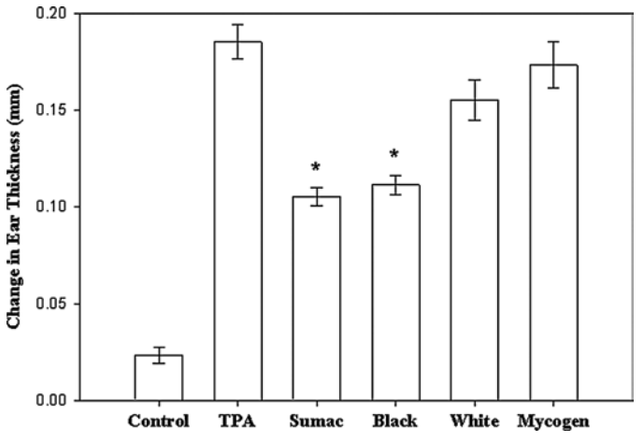

FIG. 3. Effect of various sorghum bran extracts applied following TPA administration. Sorghum bran extracts were applied to mouse ears following the application of 2 μg of TPA. The change in ear thickness was determined after 6 hours. Data are mean ± SEM values of eight animals per group. *$P < .05$ compared to the vehicle-treated TPA group.

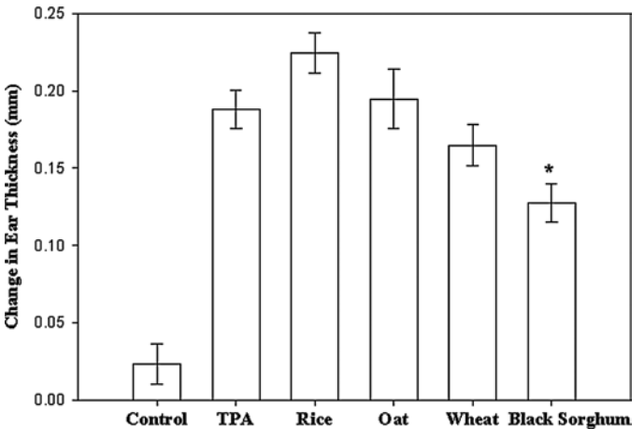

FIG. 5. Effect of various non-sorghum bran extracts applied following TPA administration. Various bran extracts were applied to the mouse ears following the application of 2 μg of TPA. The change in ear thickness was measured after 6 hours. Data are mean ± SEM values of eight animals per group. *$P < .05$ compared to the vehicle-treated TPA group.

sorghum bran extracts 20 minutes prior to TPA application (data not shown). The anti-inflammatory effect of sorghum extracts was compared to that of indomethacin, a nonsteroidal anti-inflammatory drug. Indomethacin (0.5 mg per ear) administered 20 minutes following TPA administration decreased ear edema and the weight of the ear punches by 45% and 35%, respectively. This decrease was not significantly different from the anti-inflammatory effect produced by black and sumac sorghum bran extracts.

The effect of various sorghum bran varieties on ear inflammation was examined. White and mycogen sorghum bran extracts (25% [wt/vol] in 50% ethanol) applied 20 minutes following TPA treatment did not effectively decrease ear edema, as measured by ear thickness (Fig. 3). ◀F3 In the same experiment, sumac and black sorghum bran extracts significantly reduced ear thickness by 43% and 40%, respectively. These results demonstrate that anti-inflammatory activity of sorghum bran varieties was observed in only the sorghum brans with high phenolic levels.

To determine the dose–response effect of black sorghum bran extract, 20 μL of the extracts was applied to mouse ears at concentrations ranging from 8.3% to 25% (wt/vol in 50% ethanol) following TPA treatment. An 8.3% (wt/vol) black bran extract did not alter ear thickness (Fig. 4). ◀F4 Black sorghum bran extract at 12.5% (wt/vol) significantly reduced ear edema by 31%, whereas the 25% (wt/vol) extract produced a still greater anti-inflammatory effect, decreasing ear edema to 48% of that observed in the TPA-treated animals.

The anti-inflammatory activity of non-sorghum brans was also examined. In this experiment, 12.5% (wt/vol) extracts in 50% ethanol were used. Rice, oat, and wheat bran extracts were ineffective in reducing mouse ear edema (Fig. 5). ◀F5 Black sorghum bran significantly reduced the change in ear edema by 32%.

An additional experiment was undertaken to determine the MPO activity in mouse ear homogenates biopsied 24 hours after the application of TPA. A 25% (wt/vol) black bran extract applied 20 minutes after TPA treatment significantly reduced MPO activity by 70% (Fig. 6); this reduction was similar to the inhibition observed with indomethacin treatment (85%). ◀F6

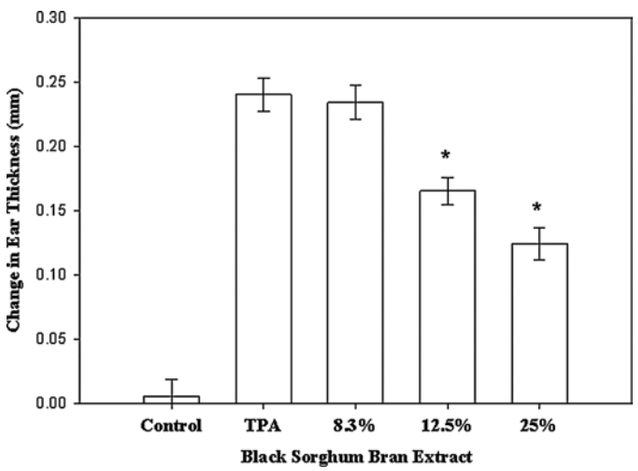

FIG. 4. Anti-inflammatory effect of black sorghum bran extract applied following TPA administration. Various concentrations of black sorghum bran extract were applied to the mouse ears following the application of 2 μg of TPA. The change in ear thickness was measured after 6 hours. Data are mean ± SEM values of eight animals per group. *$P < .05$ compared to the vehicle-treated TPA group.

DISCUSSION

Important processes related to the inflammatory response were significantly reduced by ethanolic extracts of sumac

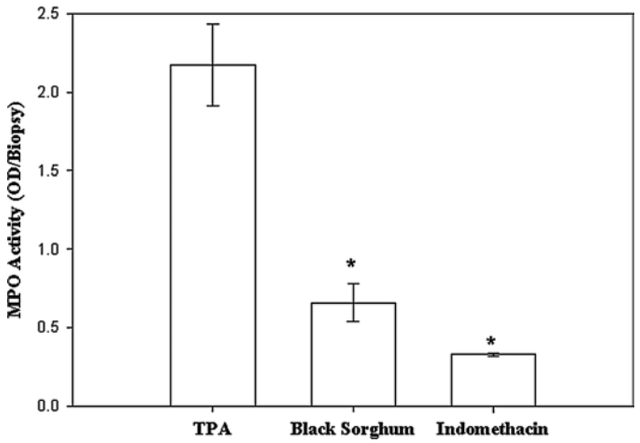

FIG. 6. Effect of black sorghum bran extract and indomethacin on ear tissue MPO activity. MPO activity was measured in ear punches 24 hours after TPA administration. Mice were treated with black sorghum bran extract, indomethacin, or a vehicle control following TPA application. Data are mean ± SEM values of eight animals per group. $*P < .05$ compared to the vehicle-treated TPA group. OD, optical density.

and black varieties of sorghum brans. An ethanolic extract of black sorghum bran inhibited the release of TNF-α and IL-1β in LPS-stimulated peripheral blood mononuclear cells at dilutions ranging from 1:100 to 1:200 and 1:100 to 1:400, respectively. In the mouse ear model, decreases were observed in MPO activity (an index of migration of polymorphonuclear leukocytes to the dermis) (at 24 hours) and in acute edema (at 6 hours) when black sorghum bran extract was applied following TPA-induced injury. The ear thickness and ear punch weight in the groups treated with black and sumac bran extracts were statistically similar to the ear thickness and ear punch weights of the mouse ears receiving indomethacin treatment. Nonsignificant reductions in ear edema were observed in the treatment groups receiving bran extracts of white and Mycogen sorghum bran varieties and oat, wheat, and rice brans.

LPS is a major cell wall component of Gram-negative bacteria that orchestrates macrophage pro-inflammatory gene expression.[13] This response begins with LPS forming a complex with LPS-binding protein and binding to the molecule CD14 on the surface of macrophages.[23] This results in the activation of Toll-like receptors. The nuclear factor κB (NF-κB) inflammatory cascade[24] is initiated following the activation of the Toll-like receptors.[25,26] NF-κB appears to be responsible for the transcription, production, and secretion of immunoregulators such as TNF-α and IL-1β, nitric oxide (NO), a pro-inflammatory molecule produced by inducible NO synthase, and prostaglandins, mediators involved in acute inflammation.[27,28] TNF-α and IL-1β participate in the pathogenesis of many inflammatory diseases, including arthritis, colitis, and heart disease.[10] Flavonol,[29] luteolin,[30] vanillin,[31] and ferulic acid[32] have been shown to decrease inflammation by either inhibiting gene expression or inhibiting the activity of mediators involved in the inflammatory process, including adhesion molecules, chemokines, cytokines, and enzymes that control prostaglandin synthesis (COX).[12,24,33–35] Antioxidants such as quercetin[36] and resveratrol[37] block the release of NO by inhibiting NF-κB activation and expression of inducible NO synthase. Black sorghum bran extract inhibited the release of TNF-α and IL-1β in LPS-stimulated peripheral blood mononuclear cells. These results are similar to those observed with muscadine extracts[33] and garlic powder extracts.[38]

The application of TPA to the ear initiates a series of inflammatory cascades. It is well established that TPA exerts its effects through the activation of protein kinase C with the subsequent activation of NF-κB. This event is then followed by the stimulation of cytosolic phospholipase A_2, resulting in arachidonic acid mobilization via COX and lipoxygenase (LOX) enzymes and the synthesis of prostaglandins and leukotrienes.[39] These mediators are responsible for the recruitment of macrophages, neutrophils, and other leukocytes that release histamine and bradykinins, thereby promoting inflammation.[14] For example, prostaglandin E_2 release from the keratinocytes amplifies vascular permeability changes produced by histamine and bradykinin,[14] increases the infiltration of activated neutrophils into the dermis, and increases the secretion of cytokines IL-1 and TNF-α from keratinocytes.[34] It is tempting to hypothesize that select sorghum bran extracts are modulating inflammation by decreasing the release of TNF-α and IL-1 from keratinocytes in the inflamed ear. However, the role of TNF-α in this model of inflammation has not received much attention.[40] At present, there is insufficient evidence supporting the hypothesis that antioxidants inhibit the release of TNF-α from keratinocytes in the acute phase of TPA-induced inflammation. However, using a 4-day treatment schedule of TPA, Huang *et al.*[41] demonstrated that black tea theaflavin derivatives did inhibit both edema and the increase in cytokine IL-1β and IL-6 levels in mouse ears, supporting the possibility that select sorghum brans may inhibit the release of cytokines in ear inflammation, similar to that observed in LPS-stimulated monocytes. It is interesting to note that IL-1 can stimulate prostaglandin synthesis via up-regulation of COX-2 and phospholipase A_2[42] and that inhibition of prostaglandin synthesis can lead to the attenuation of the TPA-induced ear inflammation.[8,43–47] Chi *et al.*[48] reported that wogonin, a flavone, significantly alleviated inflammation in a subchronic TPA ear model by reducing inflammatory mRNA expression for COX-2 and TNF-α. The eukaryotic transcription factor NF-κB has been known to regulate COX-2 expression and is a critical target for anti-inflammatory agents. Several natural products block the phosphorylation and subsequent degradation of IκBα, thereby inhibiting NF-κB translocation to the nucleus and activation of gene expression.[49–51] In addition to gene expression, COX or LOX activity has been shown to be inhibited by certain phenolic acids, resulting in reduction in ear edema.[52–55] However, the efficacy of a phenolic depends directly on its structure.[53] For example, quercetin significantly reduced LOX activity, whereas caffeic acid had little effect on this enzyme and inhibited COX instead.[53] Some

phytochemicals have reduced ear edema via the inhibition of prostaglandin synthesis but failed to have an effect on COX and LOX activity and gene expression. Instead, these natural products are believed to inhibit phospholipase A_2 activity, resulting in decreased concentrations of inflammatory mediators.[46,56] In the TPA mouse ear model, ear thickness reaches its maximum at 6 hours after TPA treatment and moderately decreases at 24 hours.[44] The increased ear thickness is characterized by edema, increased vascular permeability, and increased swelling in the dermis.[44] Twenty-four hours following TPA-induced injury, a maximum influx of neutrophils is observed in the dermis[34] and is quantified by the MPO assay. Neutrophil infiltration is associated with the generation of oxidative stress in the epidermis by releasing hydrogen peroxide and reactive oxygen species. In time, activity of superoxide dismutase, an enzyme involved in destroying free radicals and reactive oxygen species, is decreased, and gluthathione levels are depleted, thereby propagating the oxidative state.[57] Flavonoids with high antioxidant capacity have been reported to potently reduce inflammation and the production of hydrogen peroxide in this animal model.[58] Select sorghum bran varieties, such as sumac and black, contain high amounts of phytochemicals and antioxidants.[4] This property is not observed in white and Mycogen sorghum brans and in other common brans consumed in the diet such as wheat, oat, and rice brans.

Sumac sorghum bran contains is a rich source of condensed tannins also known as proanthocyanidins, specifically containing epicatechin and catechin as constituent units.[3,59] Sumac sorghum bran has a very high content of tannins (113 mg of tannins/g of bran). Black sorghum, low in tannin content, contains anthocyanins, a class of flavonoids.[5,60] The anthocyanins present in black sorghum are unique as they do not contain a hydroxyl group on the C-ring and are referred to as 3-deoxyanthocyanins, specifically apigeninidin and luteolinidin.[3,4] Awika *et al*.[5] have identified and quantified these two anthocyanins using high-performance liquid chromatography. Apigeninidin and luteolinidin represented 36–50% of the total anthocyanin content in black sorghum bran. The antioxidant activity of black sorghum bran is higher than that of some fruits such as strawberries, plums, and blueberries.[3] Although sumac sorghum bran had a much greater phenolic content and antioxidant capacity than black sorghum bran, their anti-inflammatory effects were found to be quite similar, suggesting that differences exist in the efficacy of the individual phenolic constituents.

Poor penetration of drugs through the skin can be a major drawback for the development of percutaneous drug delivery. However, *in vivo* skin penetration studies have shown that topically administered proanthocyanidins can be absorbed through the skin surface and can penetrate into the epidermis and dermis layers of the skin.[61,62] The absorption of proanthocyanidins is dependent upon the structure of the individual molecule, specifically the location of carboxyl groups.[61] Phenolic acids that are present in sorghum brans such as gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, catechin, and vanillin all are reported to rapidly penetrate human skin. Absorption occurred in as quickly as 30 minutes,[63] indicating that phytochemicals such as flavonoids and proanthocyanidins can inhibit TPA-induced inflammation by precutaneous absorption.

Many nonsteroidal anti-inflammatory drugs on the market suffer from the side effects that can occur from the usage of these pharmaceuticals. Therefore, it is desirable to develop agents that posses anti-inflammatory properties devoid of the negative aspects of conventional drug therapy. In this regard, select varieties of sorghum bran may be found to be of value in the treatment of certain inflammatory conditions.

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