SURVEY OF COTTONSEED EXTRACTS ON GENE EXPRESSION IN HUMAN COLON CANCER CELLS

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Abstract

Cotton plant provides economically important fiber and cottonseed, but cottonseed contributes only 20% of the crop value. Cottonseed value could be increased by providing high value bioactive compounds and polyphenolic extracts aimed at improving nutrition and preventing diseases because plant polyphenol extracts have been used as medicinal remedy for various diseases. The objective of this study was to investigate the effects of cottonseed extracts on cell viability and gene expression in mammalian cells. Human colon cancer cells (COLO 225) were treated with ethanol extracts from glanded and glandless cottonseed followed by MTT, qPCR and immunoblotting assays. Cottonseed extracts showed minor effects on cell viability under the experimental conditions. qPCR assay analyzed dozens of mRNAs involved in several important pathways including glucose transport, lipid biosynthesis and inflammation. Cottonseed extracts showed some effects on the expression of genes coding for DGAT, GLUT, TTP, IL, gossypol-regulated and TTP-mediated genes. Glandless seed kernel extract significantly reduced mRNA levels of many genes involved in glucose transport, lipid biosynthesis and inflammation in colon cancer cells. The inhibitory effects of glandless kernel extract on gene expression may provide a useful opportunity for improving nutrition and healthcare associated with colon cancer. This in turn may provide the potential of increasing cottonseed value by using ethanol extract as a nutrition/health intervention agent.

Introduction

Cotton plant provides important fiber and cottonseed, but cottonseed contributes to about 20% of the crop value. Cottonseed is classified either glanded or glandless depending on its seed with or without gossypol glands (Figure 1A) (Luo et al., 2001; Ma et al., 2016; Wang et al., 2009). Glanded cottonseed contains high concentrations of gossypol (He et al., 2015), which limits its use primarily to feed ruminants due to its toxicity towards humans and most animals (Camara et al., 2015; Coutinho, 2002; Gadelha et al., 2014; Randel et al., 1992; Zeng et al., 2014). Glandless cottonseed has only trace levels of gossypol which may be useful as a food for humans or feed for non-ruminant animals (Cornu et al., 1977; Lusas and Jividen, 1987; Sneed et al., 1980; Thomas et al., 1979). Glanded and glandless cottonseed contains many other bioactive components including quercetin, gallic acid, 3,4-dihydroxybenzoic acid, flavonoids, cyclopropenoid fatty acids, and peptides. Most of these value-added products possess health promotion and disease prevention potentials (Cao, 2019; Cao and Sethumadhavan, 2020a). Since plant bioactive products have been used for disease prevention and treatment since ancient history, cottonseed value could be increased by providing high value bioactive compounds and polyphenolic extracts aimed at improving nutrition and preventing diseases.

Colon cancer is a major disease with approximately 4% men and women developing colorectal cancer during their lifetime (www.cancer.org). According to World Cancer Research Fund International, colorectal cancer is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women. There were over 1.8 million new cases in 2018 (www.wcrf.org).

Plant polyphenols are major bioactive compounds in most diet with beneficial effects on human health (Prior and Gu, 2005). They regulate gene expression in numerous studies. We isolated bioactive extracts from glanded and glandless cottonseed essentially free of gossypol by HPLC-MS analysis (Figure 1B) (Cao et al., 2018). These bioactive cottonseed extracts affect human cancer cell growth (Cao et al., 2018). They also regulate mouse gene expression coding for diacylglycerol acyltransferase (DGAT), tristetraprolin/ZFP36 (TTP) family genes and human antigen R (HuR) (Cao and Sethumadhavan, 2018; Cao and Sethumadhavan, 2019; Cao and Sethumadhavan, 2020b). However, cottonseed extracts on gene expression in cancer cells was unknown.

Our aim was to survey the effects of cottonseed extracts on gene expression in human colon cancer cells. We analyzed the effects of cottonseed extracts on cell viability and expression of 55 genes which were shown to be regulated by cottonseed-derived gossypol in cancer cells (Barba-Barajas et al., 2009; Chang et al., 2004; Dong et al., 2015; Huang et al., 2006; Kitada et al., 2008; Ligueros et al., 1997; Yeow et al., 2006; Yuan et al., 2013) and macrophages (Cao

and Sethumadhavan, 2019) or by ZFP36/TTP in tumor cells (Essafi-Benkhadir et al., 2007; Florkowska et al., 2012; Kim et al., 2012; Lee et al., 2013; Lee et al., 2014; Lee et al., 2011; Milke et al., 2013; Sawaoka et al., 2003; Sharma et al., 2013) and macrophages (Cao et al., 2007; Cao et al., 2008a). These gene products are involved in a variety of biochemical pathways including lipid biosynthesis (DGATs), glucose transport (GLUTs), anti-inflammation (TTP family), pro-inflammation (TNF, COX, CSF, HUA, ILs, VEGFs), cancer development (BCL2, BNIP3, CYP19A1, FAS, HUA, P53, PPARR and TNFSF10), and TTP-mediated mRNA stability (AHRR1, BCL2L1, CSNK2A1, CXCL1, E2F1, ELK1, HIF1a, HMOX1, ICAM1 and ZFAND5). Cottonseed extracts were used to treat human colon cancer cells (COLO 225) followed by MTT assay and quantitative PCR analysis. Our results showed that ethanol extracts from glandless cottonseed kernel significantly reduced the expression of many genes in the colon cancer cells.

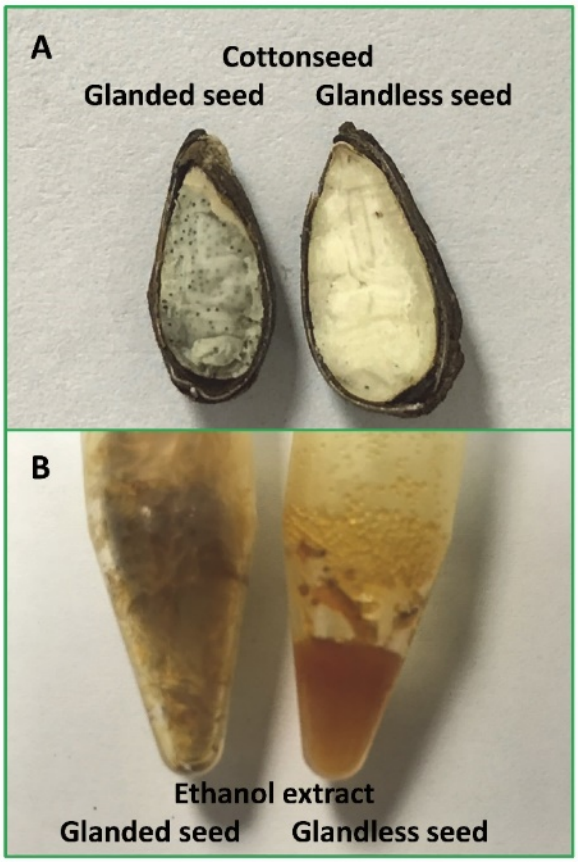

Figure 1. Cottonseed and ethanol extracts. (A) Glanded and glandless cottonseed section. (B) Ethanol extracts from glanded and glandless cottonseed.

Methods

Cottonseed. The cottonseeds were provided by Drs. Michael Dowd and Rick Byler (USDA-ARS) and Tom Wedegaertner (Cotton, Inc.).

Cancer cell line. Human colon cancer cells (COLO 205) were maintained in a humidified incubator at 37°C with 5% CO_2 in RPMI-1640 medium supplemented with 10% (v:v) fetal bovine serum, 0.1 million units/L penicillin, 100 mg/L streptomycin, and 2 mmol/L L-glutamine.

Cottonseed extracts. Seed kernel extract was isolated by fractionation, defatting, and ethanol extraction, and seed coat extract was isolated by fractionation, defatting, acetic acid extraction, and ethanol extraction (Cao et al., 2018) (Figure 1). HPLC-MS estimated the ethanol extracts contained trace amount of gossypol (0.82 ng gossypol/mg extract in glanded seed coat, 0.03 ng gossypol/mg extract in glanded seed kernel, 0.37 ng gossypol/mg extract in glandless seed coat and 0 ng gossypol/mg extract in glandless seed kernel) (Cao et al., 2018).

Cell culture and chemical treatment. Cell culture was according to previous procedures (Cao, 2004; Cao and Anderson, 2011; Cao et al., 2008a). Cells were treated with 0, 5, 10, 20, 30, 40, 50 and 100 µg/mL of ethanol extracts ("0" treatment as the vehicle control corresponding to 1% DMSO present in all of the culture medium).

Cell viability assay. MTT based-In Vitro Toxicology Assay Kit was used to determine cell cytotoxicity (Cao et al., 2018).

Real-time qPCR primers and probes. Fifty-five genes were selected for qPCR analysis of their expression in the colon cancer cells as described previously (Cao et al., 2021). These genes were shown to be regulated by cottonseed-derived gossypol in cancer cells and macrophages or regulated by ZFP36/TTP in tumor cells and macrophages (Table 1).

RNA isolation and cDNA synthesis. RNA isolation and cDNA synthesis were essentially as described (Cao and Sethumadhavan, 2018).

Quantitative real-time PCR analysis. The qPCR assays were described (Cao et al., 2013a; Cao et al., 2013b; Cao and Shockey, 2012; Cao et al., 2013c) and performed according to the MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments (Bustin et al., 2009). BCL2 mRNA was used as the internal reference because it had the minimal variation of gene expression among the 55 genes tested.

Data analysis and statistics. The relative expression in fold was determined with $2^{-"CT}$ or $2^{-""CT}$ equations (Livak and Schmittgen, 2001). The data in the figures and tables represent the mean and standard deviation of three and 24 independent samples, respectively. These data were subjected to statistical analysis using ANOVA with SigmaStat 3.1 software (Systat Software). Student-Newman-Keuls method and Tukey test were used to perform multiple comparisons among the treatments with different concentrations of cottonseed extracts (Cao and Anderson, 2011).

Table 1. Human mRNA targets analyzed by qPCR; whose levels are regulated by cinnamon extract, gossypol or TTP as indicated.

ID	mRNA	Name	Reference
H1	Ahrr1	Aryl hydrocarbon receptor repressor	TTP (Lee et al., 2013)
H2	Bcl2	B-cell lymphoma 2	Gossypol (Kitada et al., 2008)
H3	Bcl2l1	B-cell lymphoma 2 like 1	TTP (Frevel et al., 2003)
H4	Bnip3	BCL2 protein-interacting protein 3	Gossypol (Yuan et al., 2013)
H5	Cd36	Cluster of differentiation 36/fatty acid translocase	TTP (Xu et al., 2015)
H6	Claudin1	Maintain tissue integrity and water retention	TTP (Sharma et al., 2013)
H7	Cox1	Cyclooxygenase 1	TTP (Warzych et al., 2012)
H8	Cox2	Cyclooxygenase 2	TTP (Sawaoka et al., 2003)
H9	Csnk2a1	Casein kinase 2 alpha 1	TTP (Lee et al., 2011)
H10	Ctsb	Cathepsin B	TTP (Fuhrmann et al., 2015)
H11	Cxcl1	Chemokine (C-X-C motif) ligand 1	TTP (Datta et al., 2008)
H12	Cyclind1	Cyclin D1	Gossypol (Ligueros et al., 1997)
H13	Cyp19a1	Cytochrome P450 family 19 subfamily A member 1	Gossypol (Dong et al., 2015)
H14	Dgat1	Diacylglycerol O-acyltransferase 1	Cinnamon (Cao et al., 2019; Ludwig et al., 2002)
H15	Dgat2a	Diacylglycerol O-acyltransferase 2a	Cinnamon (Cao et al., 2019; Dey et al., 2014)
H16	Dgat2b	Diacylglycerol O-acyltransferase 2b	Cinnamon (Cao et al., 2019; Dey et al., 2014)
H17	E2f1	E2F transcription factor 1	TTP (Lee et al., 2014)
H18	Elk1	ETS transcription factor	TTP (Florkowska et al., 2012)
H19	Fas	Fas cell surface death receptor	Gossypol (Chang et al., 2004)
H20	Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	Reference (Chen et al., 2011)
H21	Glut1	Glucose transporter 1	Cinnamon (Cao et al., 2008a)
H22	Glut2	Glucose transporter 2	Cinnamon (Cao et al., 2008a)
H23	Glut3	Glucose transporter 3	Cinnamon (Cao et al., 2008a)
H24	Glut4	Glucose transporter 4	Cinnamon (Cao et al., 2008a)
H25	Hif1a	Hypoxia inducible factor 1 subunit alpha	TTP (Fahling et al., 2012)
H26	Hmgr	3-Hydroxy-3-methylglutaryl-CoA reductase	(Kagami et al., 2008)
H27	Hmox1	Heme oxygenase 1	TTP (Jamal et al., 2013)
H28	Hua	Human antigen a	Gossypol (Cao and Sethumadhavan, 2019)
H29	Icam1	Intercellular adhesion molecule 1/CD54	(Shi et al., 2014)
H30	Inos	Inducible nitric oxide synthase	(Su et al., 2008)
H31	Insr	Insulin receptor	(Cao et al., 2007)

H32	Il2	Interleukin 2	TTP (Ogilvie et al., 2005)
H33	IL6	Interleukin 6	TTP (Hochdorfer et al., 2013)
H34	IL8	Interleukin 8	TTP (Balakathiresan et al., 2009)
H35	Il10	Interleukin 10	TTP (Gaba et al., 2012)
H36	Il12	Interleukin 12	TTP (Gu et al., 2013)
H37	Il16	Interleukin 16	TTP (Milke et al., 2013)
H38	Il17	Interleukin 17	TTP (Datta et al., 2010)
H39	Leptin	Body fat and obesity hormone	(Xu et al., 2010)
H40	Map1lc3a	Microtubule-associated proteins 1 light chain 3A	(Voss et al., 1998)
H41	Map1lc3b	Microtubule-associated proteins 1 light chain 3B	(Voss et al., 1998)
H42	Nfkb	Nuclear factor kappa B	(Jiang et al., 2012)
H43	P53	Tumor suppressor	Gossypol (Barba-Barajas et al., 2009)
H44	Pim1	Proto-oncogene serine/threonine-protein kinase	TTP (Kim et al., 2012)
H45	Pparr	Peroxisome proliferator-activated receptor gamma	Gossypol (Huang et al., 2006)
H46	Rab24	Ras-related oncogene 24	(Militello et al., 2013)
H47	Rpl32	Ribosomal protein L32 (60S ribosomal unit)	Reference (Brattelid et al., 2010)
H48	Tnf	Tumor necrosis factor	TTP (Hochdorfer et al., 2013)
H49	Tnfsf10	Tumor necrosis factor superfamily, member 10	Gossypol (Yeow et al., 2006)
H50	Ulk2	Unc-51 like autophagy activating kinase 2	(Gao et al., 2011)
H51	Vegf	Vascular endothelial growth factor	TTP (Essafi-Benkhadir et al., 2007)
H52	Zfand5	Zinc finger AN1-type containing 5	TTP (He et al., 2012)
H53	Zfp36	Zinc finger protein 36	TTP (Cao et al., 2007)
H54	Zfp36l1	Zinc finger protein 36 like 1	TTP (Cao et al., 2007)
H55	Zfp36l2	Zinc finger protein 36 like 2	TTP (Cao et al., 2007)

Results

Effect of cottonseed extracts on colon cancer cell viability. Before cottonseed extracts on gene expression were analyzed, we evaluated the effect of the ethanol extracts on colon cancer cell growth. Human colon cancer cells (COLO 225) were treated with 10-100 µg/mL of cottonseed extracts for 2 and 24 h. MTT assay was used to estimate the effect of cottonseed extracts on cell viability. MTT assay did not show significant changes in the viability of colon cancer cells under treatments with various concentrations for 2 or 24 h (data not shown).

Effect of glanded coat extract on gene expression. We first analyzed the effect of glanded coat extract on gene expression in human colon cancer cells. SYBR Green qPCR analyzed the expression of all 55 genes with BCL2 mRNA as the internal reference and 1% DMSO treatment as the sample control. It appeared that the expression of COX2, GLUT1, LEPTIN, TNF, and TNFSF10 was increased by the glanded coat extract. Other gene expression was reduced by the glanded coat extract, including BCL22L2, CLUDIN1, CSNK2A1, CTSB, CXC1, DGAT1, GLUT1, HIF1, ZFAND5 and ZFP36. The expression of the rest genes not mentioned above was not affected by the glanded kernel extract (data not shown).

Effect of glanded kernel extract on gene expression. We then analyzed the effect of glanded kernel extract on gene expression in human colon cancer cells. The expression of ELK1, FAS, and GAPDH genes was increased by the glanded kernel extract. The expression of other genes was not affected by the ethanol extract (data not shown).

Effect of glandless coat extract on gene expression. Thirdly, we analyzed the effect of glandless coat extract on gene expression in human colon cancer cells by qPCR using BCL2 mRNA as the internal reference and 1% DMSO treatment as the sample control. The expression of FAS, GAPDH, GLUT1, and ZFP36 was increased by the glandless coat extract, but only CXC1 expression was reduced by the coat extract. The expression of the rest genes not mentioned above was not affected by the ethanol extract (data not shown).

Effect of glandless kernel extract on gene expression. Finally, we analyzed the effect of glandless kernel extract on gene expression. Similarly, glanded cottonseed kernel extract treated human colon cancer cells and SYBR Green qPCR analyzed mRNA levels of 55 genes with BCL2 mRNA as an internal reference and 1% DMSO treatment as the sample control. qPCR data indicated that much more genes were affected by the glandless kernel extracts. The effect of the glandless kernel extract on gene expression were analyzed in detail according to gene families as described below (Figures 2-3).

Glandless kernel extract on reference gene expression. The expression of the two well-known reference genes GAPDH and RPL32 was analyzed in the colon cancer cells after treatment with various concentration of glandless kernel extract. The qPCR data showed that glandless kernel extract treatment resulted in a large reduction of both GAPDH and RPL32 mRNA levels in the cells (Figure 2A).

Glandless kernel extract on gossypol-related gene expression. Expression of several genes were regulated by gossypol in cancer cells (Barba-Barajas et al., 2009; Chang et al., 2004; Dong et al., 2015; Huang et al., 2006; Kitada et al., 2008; Ligueros et al., 1997; Yeow et al., 2006; Yuan et al., 2013) and macrophages (Cao and Sethumadhavan, 2019). BNIP3, CYP19A1, FAS, HUA, P53, PPARR and TNFSF10 gene expression was analyzed in the colon cancer cells after being treated with glandless kernel extract with various concentrations. The expression of FAS, HUA, P53 and PPARR genes was inhibited to a large extent by the glandless kernel extract (Figure 2B).

Glandless kernel extract on DGAT gene expression. Diacylglycerol acyltransferases (DGATs) catalyze the rate-limiting step of triacylglycerol biosynthesis in eukaryotes (Cao, 2011; Liu et al., 2012). DGATs are classified with DGAT1 and DGAT2 subfamilies in animals and additional DGAT3 subfamily in plants (Cao, 2011; Cao et al., 2013c; Liu et al., 2012; Shockey et al., 2006) with DGAT2 mRNA being the major form of DGAT mRNAs in mouse adipocytes and macrophages (Cao, 2018; Cao and Sethumadhavan, 2018) but DGAT1 as the major one in the colon cancer cells (Cao et al., 2021). The qPCR data showed that glandless kernel extract inhibited DGAT1, 2a and 2b expression in the human colon cancer cells (Figure 2C).

Glandless kernel extract on GLUT gene expression. Glucose transporter (GLUT) family proteins are responsible for glucose uptake in mammalian cells. Four forms of GLUTs are present in mammalian cells (Cao et al., 2008a). The glandless kernel extract treatment only decreased GLUT1 mRNA levels without much effect on the other GLUT isoforms (Figure 2D). GLUT4 mRNA levels were very low so that it was difficult to be measured with sufficient confidence.

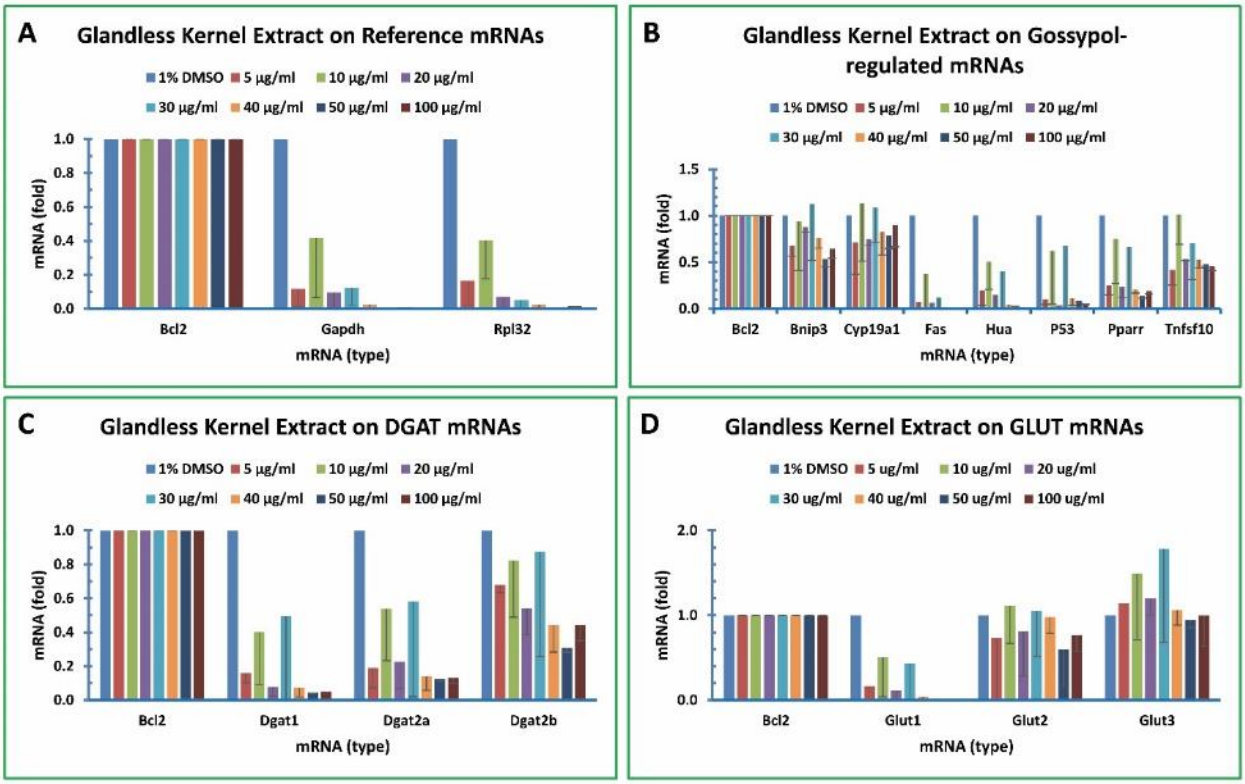

Figure 2. Glandless kernel extract regulated the expression of genes coded for qPCR reference mRNAs, genes reported to be regulated by gossypol, and genes coded for DGAT and GLUT mRNAs in human colon cancer cells. Human colon cancer cells (COLO 225) were treated with gossypol for 8 h. The data represent the mean and standard

deviation of three independent samples. A, Genes coded for qPCR reference mRNAs, B, Genes reported to be regulated by gossypol, C, Genes coded for DGAT mRNAs, D, Genes coded for GLUT mRNAs.

Glandless kernel extract on TTP gene expression. Tristetraprolin (TTP/ZFP36) family proteins regulate mRNA stability (Blackshear, 2002). TTP family genes have anti-inflammatory properties with therapeutic potential for inflammation-related diseases (Carballo et al., 1998; Lai et al., 1990). TTP family proteins consist of three members in mammals (ZFP36 or TTP, ZFP36L1 and ZFP36L2) and the fourth member in mouse and rat but not in humans (ZFP36L3) (Blackshear, 2002; Blackshear et al., 2005). SYBR Green qPCR showed that ZFP36 and ZFP36L1 mRNAs were reduced by the glandless kernel extract (Figure 3A). ZFP36L2 mRNA levels were too low to be assessed reliably.

Glandless Kernel Extract on IL Gene Expression. Several interleukins (ILs) are regulated by TTP family proteins which bind to AU-rich elements (ARE) of IL mRNAs and destabilizes the transcripts. TTP-regulated ILs include IL2 (Ogilvie et al., 2005), IL6 (Hochdorfer et al., 2013), IL8 (Balakathiresan et al., 2009), IL10 (Gaba et al., 2012), IL12 (Gu et al., 2013), IL16 (Milke et al., 2013) and IL17 (Datta et al., 2010). SYBR Green qPCR showed that glandless kernel extract increased IL12 mRNA levels but decreased IL16 mRNAs (Figure 3B). IL8 and IL10 mRNA levels were difficult to compare due to their low levels in the colon cells.

Glandless Kernel Extract on Proinflammatory Gene Expression. Several proinflammatory cytokine mRNAs are destabilized by TTP family proteins, including tumor necrosis factor-alpha (TNF±) (Cao, 2004; Cao et al., 2003; Carballo et al., 1998; Lai et al., 1999), granulocyte-macrophage colony-stimulating factor/colony-stimulating factor 2 (GM-CSF/CSF2) (Carballo et al., 2001; Carballo et al., 2000) and cyclooxygenase 2/prostaglandin-endoperoxide synthase 2 (COX2/PTGS2) (Sawaoka et al., 2003). TNFα and GM-CSF mRNAs are stabilized in TTP knockout mice and in cells derived from them (Carballo et al., 1998; Carballo et al., 2000), resulting in excessive levels of these cytokines causing a severe systemic inflammatory syndrome including arthritis, autoimmunity, and myeloid hyperplasia (Phillips et al., 2004; Taylor et al., 1996). Elevated levels of TTP reduce inflammatory responses in macrophages (Sauer et al., 2006). These previous studies suggest that TTP is an anti-inflammatory protein. Glandless kernel extract decreased COX1, LEPTIN and TNF mRNAs in the colon cancer cells (Figure 3C).

Glandless Kernel Extract on TTP-targeted Other Gene Expression. Other TTP-regulated mRNAs have been reported in the literature (Table 1). SYBR Green qPCR analyzed the mRNA levels of AHRR, BCL22L1, CD36, CLAUDIN1, CSNK2A1, CTSB, CXD1, E2F1, ELK1, HIF1A, HOMX1, ICAMI, PIM1, and ZFAND5 genes. Glandless kernel extract decreased all of these TTP-targeted mRNAs except CD36 and E2F1 mRNAs (Figure 3D).

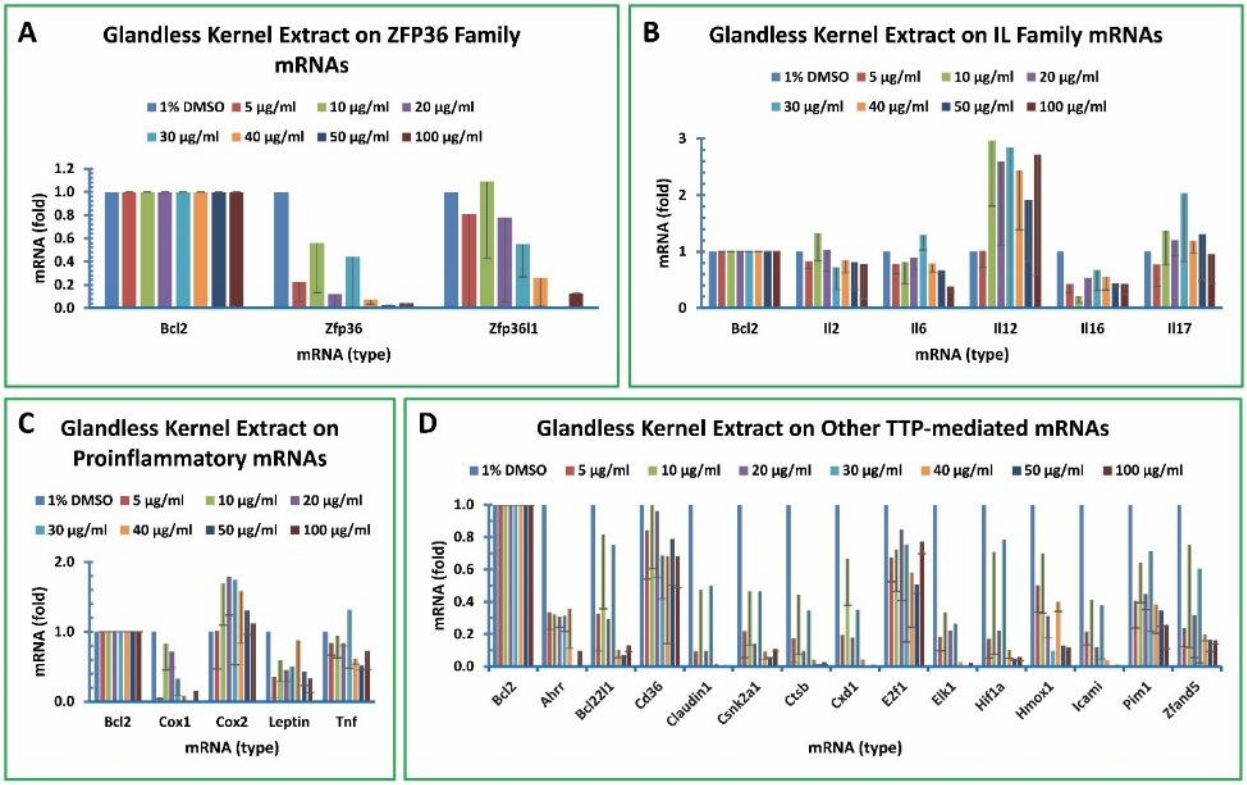

Figure 3. Glandless kernel extract regulated the expression of genes coded for TTP family, IL family, TTP-mediated proinflammatory cytokine and other mRNAs in human colon cancer cells. Human colon cancer cells (COLO 225) were treated with gossypol for 8 h. The data represent the mean and standard deviation of three independent samples. A, Genes coded for TTP family mRNAs. B, Genes coded for IL family mRNAs. C, Genes coded for TTP-mediated proinflammatory cytokine mRNAs. D, Genes coded for other TTP-mediated mRNAs.

Glandless Kernel Extract on Other Gene Expression. A few other gene targets were selected for the analysis of gene expression. The qPCR assays showed that glandless kernel extract decreased the expression of HMGR, INSR, MAPL1C3A, MAPL1C3B, and NFKB mRNA levels. The effect of glandless kernel extract on ULK2 mRNA levels was not much and the effect on CYCLIND1 mRNA levels was difficult to assess due to large variation of the results (data not shown).

Discussion

One way to increase cottonseed value is to isolate bioactive materials for improving nutrition and preventing diseases. In this study, we observed that the expression of the majority of genes was significantly reduced by glandless cottonseed kernel extract, although their expression was less affected by three other cottonseed ethanol extracts (glanded cottonseed coat, glanded cottonseed kernel, and glandless cottonseed coat extracts).

Cottonseed extracts exhibited only minor effect on the viability of human colon cancer cells under the experimental conditions. Our previous study showed that gossypol strongly inhibited human cancer cell viability (Cao et al., 2018). The current data confirm our HPLC-MS analyses that the cottonseed extracts are essentially free of the toxic compound gossypol (Cao et al., 2018). Our study showed that expression of many genes in human colon cancer cells was somewhat affected by cottonseed ethanol extracts. Although extracts isolated from glanded seed coat and kernel as well as glandless seed coat showed less effects on gene regulation, the expression of the majority of genes was significantly reduced by glandless seed kernel extracts (Figures 2-3).

The most important observation of this study was that glandless kernel extract decreased the mRNA levels of the great majority of the 55 genes tested, including GAPDH involved in the sixth step of breakdown of glucose in glycolysis (Chen et al., 2011) and RPL32, a component of the large 60S submit of ribosomes involved in protein synthesis

(Brattelid et al., 2010) (Figure 2A), the genes known to be involved in cancer development, such as BNIP3 involved in the permeability of outer mitochondrial membrane (Yuan et al., 2013), CYP19A1 localized to the endoplasmic reticulum and catalyzed the last steps of estrogen biosynthesis (Dong et al., 2015), FAS, a member of TNF-receptor superfamily playing a key role in programmed cell death (Chang et al., 2004), P53 involved in preventing genome mutation (Barba-Barajas et al., 2009), PPARR, a nuclear receptor involved in gene expression regulation (Huang et al., 2006) and TNFSF10, a TNF super family member functioning as a ligand that induces apoptosis (Yeow et al., 2006) (Figure 2B), the DGAT family members DGAT1, DGAT2a and 2b responsible for the last and rate-limiting step of triacylglycerol biosynthesis (Cao, 2011; Cao, 2018) (Figure 2C), and GLUT1 responsible for glucose transport across the plasma membranes (Cao et al., 2008b) (Figure 2D). In addition, glandless kernel extract reduced ZFP36 mRNA levels in the TTP family which bind to the AU-rich elements of some mRNAs and cause destabilization (Cao, 2004; Carballo et al., 1998) (Figure 3A). It increased IL12, a T-cell stimulating factor (Gu et al., 2013) but decreased IL16 functions as a chemoattractant, a modulator of T cell activation, and an inhibitor of HIV replication (Milke et al., 2013) mRNAs levels in the IL family members (Figure 3B), decreased LEPTIN involved in energy balance (Xu et al., 2010) and TNF, a cytokine promoting inflammation (Hochdorfer et al., 2013) mRNA levels (Figure 3C), and appeared to decrease all of the TTP-targeted mRNAs including AHRR1 (Lee et al., 2013), BCL2L1 (Frevel et al., 2003), CSNK2A1 (Lee et al., 2011), CXCL1 (Datta et al., 2008), HIF1a (Fahling et al., 2012), E2F1 (Lee et al., 2014), ELK1 (Florkowska et al., 2012), HMOX1 (Jamal et al., 2013), ICAM1 (Shi et al., 2014) and ZFAND5 (He et al., 2012) (Figure 3D). Finally, glandless kernel extract appeared to decrease the expression of HMGR (Kagami et al., 2008), INSR (Cao et al., 2007), MAPL1C3A (Voss et al., 1998), MAPL1C3B (Voss et al., 1998), and NFKB (Jiang et al., 2012) mRNA levels.

Conclusions

This study showed that most of the gene expression in human colon cancer cells was not affected by ethanol extracts isolated from glanded cottonseed coat and kernel as well as glandless cottonseed coat, but the expression of the majority of genes was significantly reduced by glandless cottonseed kernel extracts. The inhibitory effects of glandless kernel extract on gene expression in the colon cancer cells may provide a useful opportunity for improving the healthcare associated with colon cancer since it is safe without toxic gossypol contamination and effective in decreasing the expression of so many genes related to cancer development. This in turn may provide the potential of increasing the value of cottonseed by using cottonseed-derived ethanol extracts as a health intervention agent.

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