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REAL TIME-QUANTITATIVE POLYMERASE CHAIN REACTION ANALYSIS OF THE ANTI-INFLAMMATORY EFFECT OF AQUEOUS ROOIBOS (Aspalathus linearis) EXTRACT ON DIESEL EXHAUST PARTICLES-INDUCED HEPATIC INFLAMMATION

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Air pollution has been implicated in the etiology of different pathological conditions in human. Though air pollution is heterogeneous in composition, most of its deleterious effects have been linked to the particulate matter (PM) component. In this study we used the real time-quantitative polymerase chain reaction (RT-qPCR) method to evaluate the protective effect of aqueous rooibos (Aspalathus linearis) extract (RE) from the inflammation induced by diesel exhaust particles (DEP) in the liver of exposed Wistar rats. One group of wistar rats was exposed to DMSO and saline vehicles (control), another group was given RE (50 mg/kg) orally for 4 weeks. The third group was pre-treated with oral dose of 50 mg/kg RE for 2 weeks before concomitant exposure to 50 mg/kg RE orally and 0.7 mg/kg DEP subcutaneously for further 2 weeks. The fourth group was given 0.7 mg/kg DEP subcutaneously for 2 weeks. Rats were then sacrificed and liver tissue collected and stored at -80 ^{o}C for further analysis. Real time-quantitative polymerase chain reaction was used to analyse IL-8, IL-10, TNFα, NF-κB, IκB and IκKB gene expressions as indices of inflammation. Rooibos extract pre-treatment significantly ($p<0.001$) decreases the mRNA levels of IL-8 and TNFα induced by diesel exhaust particles exposure, but increased IL-10 mRNA level significantly ($p<0.05$). While rooibos extract pre-treatment caused significant ($p<0.001$) increase in IκB gene expression compared to diesel exhaust particles only, it did cause significant ($p<0.05$) decrease in NF-κB and IκKB mRNA levels. In conclusion, the results suggest that rooibos extract has the ability to mitigate against diesel exhaust particles-induced inflammation in hepatic tissue by a mechanism that may involve the suppression of the NF-κB signalling pathway.

Keywords: Inflammation; Diesel exhaust particles; Liver; Air pollution; Rooibos; Gene expression

INTRODUCTION

Rooibos (Aspalathus linearis (Burm.f.) R.Dahlgren) tea is the major plant species, accounting for over 80% of the total plant species, found in the Cape Floral Kingdom (Joubert et al., 2008). The leaves and stem of this plant had been used in herbal tea preparation for health benefit effect since the 1700s. Two forms of rooibos are produced during manufacturing-the traditional fermented and green unfermented rooibos. Both of these products contain antioxidants mostly flavonoids as well as aspalathin, aspalalinin, dihydrochalcone and dihydrochalcone glucoside. Several studies have highlighted the antioxidant and anti-inflammatory activities of rooibos (Joubert et al., 2004; Marnewick et al., 2009). The tea has also been shown to exhibit anti-mutagenic and cancer modulating abilities (Marnewick et al., 2009).

Diesel exhaust particles (DEP) generated from the incomplete combustion of diesel fuel from diesel engines of motor vehicles, industries and generators, are the major particulate matter (PM) released into the environment and thus contribute significant proportion to air pollution especially in the urban settings (Lloyd and Cackette, 2001). Several in vitro and in vivo studies have shown the induction of oxidative stress and inflammation as major mechanisms in the adverse health effects of DEP (Yin et al., 2013; Lawal et al., 2015, 2016; Lawal, 2017; 2018). Diesel exhaust particles and particulate matter have been shown to induce inflammatory cytokines production via nuclear factor kappa B (NF-κB) pathway activation (Lawal, 2017) and this may serve as a therapeutic target for ameliorating the adverse effects of diesel exhaust particles on cells.

NF-κB (nuclear factor kappa-light-chain-

enhancer of activated B cells) is a transcription factor that regulates pro-inflammatory signalling pathway and the production of pro-inflammatory cytokine and chemokines in disease tissues (Ghosh et al., 1998; Monaco et al., 2004). It can be activated by at least two separate pathways: the "canonical" pathway, which can be triggered by pro-inflammatory cytokines such as IL-1, tumor necrosis factor α (TNFα) and Toll-like microbial pattern recognition receptors (TLRs) (Karin and Ben-Neriah, 2000) and an "alternative" pathway activated by TNF-family cytokines (but not TNFα) - CD40 ligand, B cell activating factor and receptor activator of NF-κB ligand (Bonizzi et al., 2004). The phosphorylation of inhibitory kappa B (IκB) by inhibitory kappa B kinase (IκKB) regulates the canonical pathway and NF-κB activation (Lawrence, 2009).

In this study, we examined the effects of aqueous rooibos extract on the canonical pathway of NF-κB activation in the liver of rats exposed to diesel exhaust particles using real time-quantitative polymerase chain reaction method in order to identify a therapeutic target for the treatment of diesel exhaust particles-induced hepatic inflammation.

MATERIALS AND METHODS

Materials

TRI Reagent® was purchased from Zymo Research (USA). ProtoScript II First Strand cDNA Synthesis Kit, quick-load purple 100 bp DNA ladder and Luna Universal qPCR Master Mix were bought from BioLabs (New England). Primers were obtained from Inqaba biotec (Hatfield, South Africa). All other chemicals and reagents were purchased from Sigma-Aldrich (Germany).

Methods

Diesel Exhaust Particles Collection and Extract Preparation

DEP was collected on a high capacity glass-fibre filters as previously described (Li et al., 2004). The particles were collected from a 1996 model six-cylinder MAN tipper truck engine (engine #G0967, 146 hp) from Akure tipper garage in Ondo State, Nigeria. The diesel exhaust particles methanol extract was prepared as previously described (Lawal et al., 2015) and the extract was reconstituted in DMSO (100 μg of dried extract/ μl DMSO). The polycyclic aromatic hydrocarbons (PAHs) content and the redox potential capacity of the extract have been characterised in our previous study (unpublished). GC-MS analysis shows that fluoranthene and fluorene are the most and least abundant of the PAHs respectively, detected in the extract.

Preparation of Aqueous Rooibos Extract

Freshpak® Rooibos Tea (Batch # 6001156905236; a product of Entryce Beverage, South Africa) containing twenty tagless tea bags, was obtained from the Shoprite Shopping Mall in Akure, Ondo State, Nigeria. Aqueous extract of the tea was prepared as previously described (Marnewick et al., 2011) and the lyophilized extract was reconstituted in 0.9% saline at a concentration of 10 mg/ml. The reconstituted extract was kept at -20 °C for further use.

Animal Treatment

Adult male Wistar rats weighing 80-100 g were obtained from local animal vendor in Akure, Ondo State, Nigeria. The animals were treated in compliance with approved protocols of the Animal Ethics Committee of School of Sciences, Federal University of Technology Akure (FUTA) and Scientific Procedures (Act 1986, UK House Office). The animals were divided into 4 experimental groups consisting of 6 rats per group: group one was given oral saline for 4 weeks and 2 weeks of DMSO subcutaneously twice a week (control group); group two was given 50 mg/kg aqueous rooibos extract (RE) orally for 4 weeks and DMSO subcutaneously twice a week in the final two weeks; group 3 was given oral dose of 50 mg/kg rooibos extract for 4 weeks and 0.7 mg/kg DEP extract subcutaneously for twice a week in the final two weeks; and Group 4 was given oral saline for 4 weeks and 0.7 mg/kg DEP extract subcutaneously for twice a week in the final two weeks. Twenty-four hours after the last treatment, the rats were sacrificed by cervical dislocation and liver biopsies were collected and stored at -80 °C for subsequent analysis.

RNA Extraction

Total RNA was isolated from liver tissues using TRI Reagent® (Zymo Research, USA) as

previously described (Ramalho et al., 2004). The isolated RNA was reconstituted in nuclease free water and quantified by measuring absorbance at 260 and 280 nm. The purity of the isolated RNA was calculated by the ratio of Absorbance at 260/ Absorbance at 280 nm (Table 1). The values between 1.8-2.1 were considered pure RNA samples (Wilfinger et al., 1997) and were used for downstream application.

Table 1: Quantity and Purity of Isolated RNA Samples

Treatmen t	RNA conc. (μg/ml)	RNA purity
Control	2200± 93.34	2.00± 0.03
RE (50 mg/kg)	6622± 93.34	2.05± 0.06
RE (50 mg/kg + DEP)	4543± 575.58	2.01± 0.01
DEP (0.7 mg/kg)	4818± 311.12	2.04± 0.07

Concentration of RNA sample= 44 μg/ml X A_{260} x dilution factor. RNA purity= A_{260}/A_{280}.

cDNA Synthesis

1 μg of the extracted RNA was used for the reverse transcription reaction to synthesise complementary DNA (cDNA) using ProtoScript II First Strand cDNA Synthesis Kit (BioLabs, New England) in a 3-step reaction condition: 65 °C for 5 min, 42 °C for 1 hr and 80 °C for 5 min.

Gene Expression Analysis by RT-qPCR

The Luna Universal qPCR Master Mix (BioLabs, New England) was used for the real time-quantitative PCR (qPCR) reaction and runs was performed on a StepOnePlus Applied Biosystem qPCR System according to the manufacturer's protocols. Primers to cDNA were purchased from Inqaba biotec (Hatfield, SA) and are listed in table 2. PCR conditions were as follows: 1 cycle at 95 °C for 3 min, 40 cycles at 95 °C for 15 sec, 40 cycles at 60 °C for 30 sec, and 40 cycles at 72 °C for 30 sec. The relative amount of cDNA was quantified by comparative cycle threshold (ΔΔCT) method and gene expression was normalised with β-actin gene as housekeeping gene.

Table 2: Primer Sequence for Real Time Quantitative PCR

Gene	Sequence
β-actin	Forward: CTCCCTGGAGAAGAGCTATGA Reverse: AGGAAGGAAGGCTGGAAGA
IL-8	Forward: GGGAGAAATCAGGGTGGATAAT Reverse: GGCAGCATCTGACAGAGTAAA
IL-10	Forward: TTGAACCACCCGGCATCTAC Reverse: CCAAGGAGTTGCTCCCGTTA
NF-κB	Forward: AGACATCCTTCCGCAAACTC Reverse: TAGGTCCATCCTGCCCATAA
I κB	Forward: CCACTCCATGTAGCTGTCATC Reverse: CACGTAGGCTCCGGTTTATT
I κKB	Forward: GAGAACAGGCCTTAGAGGATTT Reverse: CAATGATGTCACCTGAGCTTTC
TNF*α*	Forward: ACCTTATCTACTCCCAGGTTCT Reverse: GGCTGACTTTCTCCTGGTATG

IL, Interleukin; IκB, Inhibitory Kappa B; NF-κB, Nuclear Factor Kappa B; IκKB, Inhibitory Kappa B Kinase; TNFα, Tumor Necrosis Factor Alpha.

Agarose Gel Electrophoresis Analysis

The size and purity of the amplicon generated from the qPCR assay was further analysed using 0.8% agarose (prepared in 1x tris base, glacial acetic acid, EDTA, TAE, buffer). The samples and quick-load purple 100 bp DNA ladder (BioLabs, New England) were loaded on the ethidium bromide stained gel and run at 100 mA/gel. The gel was visualized under ultraviolet light and photographed.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Bonferroni post-hoc test for multiple comparisons between groups. GraphPad Prism 5 software was used for statistical analyses and data were expressed as mean±SEM. Differences were considered statistically significant at the p-value of <0.05.

RESULTS

Rooibos Extract Exerts Anti-inflammatory Effects on Diesel Exhaust Particles-induced Inflammation

In order to assess the effects of rooibos extract on the gene expression of inflammatory genes in the presence of diesel exhaust particles, we determined the mRNA levels of IL-8 (Figure 1) and IL-10 (Figure 2) in the liver of rats exposed to 0.7 mg/kg DEP in the presence and absence of 50 mg/kg rooibos extract. The results showed that treatment with 50 mg/kg rooibos extract only, does not alter IL-8 gene expression compared to control (Figure 1A). Exposure to 0.7 mg/kg DEP caused an 8.93-fold significant ($p<0.001$) induction of IL-8 gene expression compared to control (Figure 1A). Also, pre-treatment with 50 mg/kg rooibos extract induced a 5.99-fold significant ($p<0.001$) increase in IL-8 mRNA levels compared to control (Figure 1A). However, the presence of 50 mg/kg rooibos extract attenuates diesel exhaust particles-induced IL-8 mRNA levels significantly ($p<0.001$) by 1.49-fold. The melting (Figure 1B) and the amplification (Figure 1C) curves and the agarose gel analysis (Figure 1D) of the IL-8 gene expression indicate that the gene of interest was amplified.

Figure 1

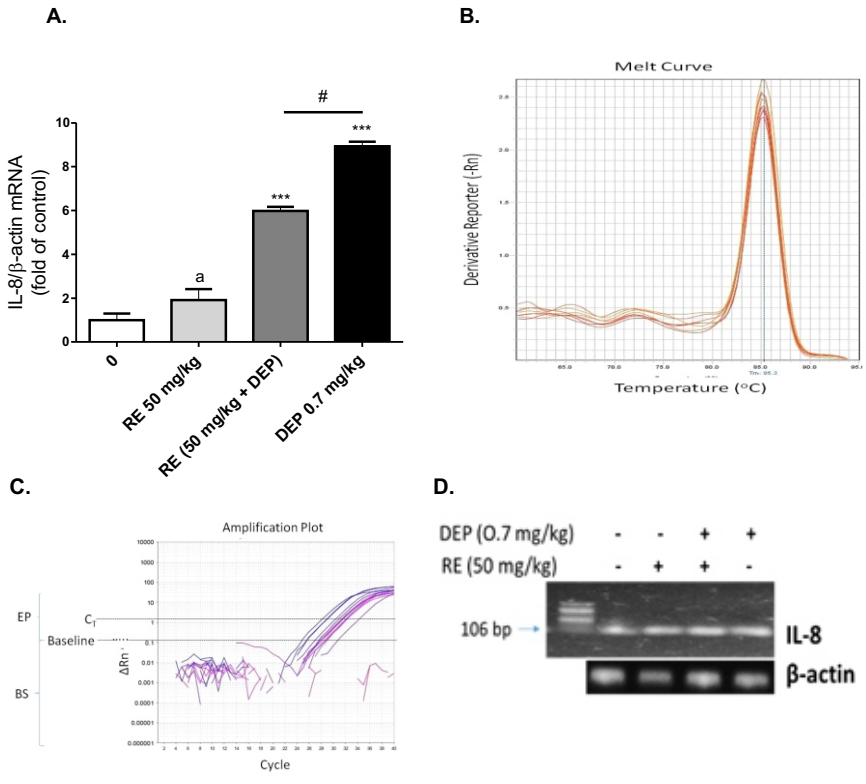

Figure 1. Effects of Rooibos Extract on Interleukin (IL)-8 Gene Expression in the Presence of DEP. (A) Relative quantification of IL-8 gene expression using $\Delta\Delta C_T$. Values are mean±SEM of three different experiments done in triplicate (n=3). *p<0.05, **p<0.01, ***p<0.001 as significant difference as compared to the controls. #p<0.001 as significant difference between rooibos extract pre-treatment vs DEP, ap<0.01 as significant difference between rooibos extract pre-treatment vs rooibos extract. (B) Instrument-derived melting curve. (C) Instrument derived amplification curve. (D) Agarose gel analysis of the qPCR products show single amplicons (106 bp) as a single band. BS, background signal; EP, exponential phase; C_T, cycle threshold; Rn, emission intensity of the reporter/emission intensity of passive reference (normalised reporter); ΔRn, the difference between the Rn at the end point and at the starting point and it is directly proportional to the DNA amount during the exponential phase.

The gene expression data for IL-10 showed that the presence of 50 mg/kg rooibos extract caused a significant ($p<0.001$) 11.12-fold induction in IL-10 mRNA levels compared to control (Figure 2A). Also, pre-treatment with 50 mg/kg rooibos extract induced IL-10 gene expression significantly ($p<0.05$) by 1.65-fold. However, exposure to 0.7 mg/kg DEP produced no significant change in IL-10 mRNA level compared to control (Figure 2A). Pre-treatment with 50 mg/kg rooibos extract prior to 0.7 mg/kg DEP exposures caused 1.65-fold significant ($p<0.05$) induction in IL-10 mRNA levels compared with animals exposed to 0.7 mg/kg DEP only (Figure 2A). We further analysed the efficiency of the RT-qPCR method used for the quantification of the IL-10 gene expression. The melting (Figure 2B) and amplification (Figure 2C) curves showed that IL-10 gene was the only amplicon amplified by the RT-qPCR method. Further analysis of the IL-10 gene expression on agarose gel electrophoresis confirmed the differential expression of IL-10 gene in agreement with the RT-qPCR analysis (Figure 2D).These set of data suggest that 50 mg/kg rooibos extract has the ability to protect against diesel exhaust particles-induced pro-inflammatory response by mitigating against the expression of pro-inflammatory gene and at the same time enhancing the expression of anti-inflammatory gene.

Figure 2

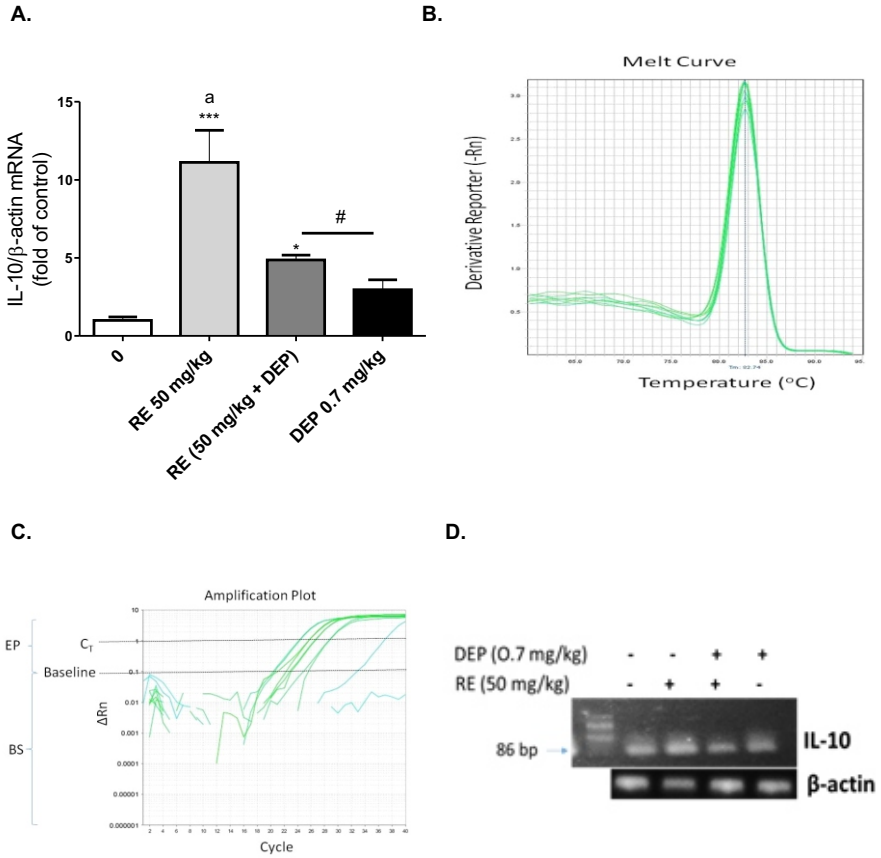

Figure 2. Effects of Rooibos Extract on Interleukin (IL) -10 Gene Expression in the Presence of DEP. (A) Relative quantification of IL-10 gene expression using $\Delta\Delta C_T$. Values are mean±SEM of three different experiments done in triplicate (n=3). *$p<0.05$, **$p<0.01$, ***$p<0.001$ as significant difference as compared to the controls. $^{\#}p<0.001$ as significant difference between rooibos extract pre-treatment vs DEP, $^{a}p<0.01$ as significant difference between rooibos extract pre-treatment vs rooibos extract. (B) Instrument-derived melt curve. (C) Instrument derived amplification curve. (D) Agarose gel analysis of the qPCR products show single amplicons (86 bp) as a single band. BS, background signal; EP, exponential phase; C_T, cycle threshold; Rn, emission intensity of the reporter/emission intensity of passive reference (normalised reporter); ΔRn, the difference between the Rn at the end point and at the starting point and it is directly proportional to the DNA amount during the exponential phase.

Rooibos Extract Represses Diesel Exhaust Particles-induced NF-κB Pathway Activation

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) mediates pro-inflammatory signalling pathway and controls the expression of other pro-inflammatory genes (Gosh et al., 1998; Lawrence, 2009). In this study, we examined the role NF-κB pathway played in the anti-inflammatory effects of rooibos extract on diesel exhaust particles-induced inflammation in the liver (Figure 3-5). Data showed that 50 mg/kg RE caused a 2.13-fold significant ($p<0.01$) decrease in NF-κB mRNA level compared to control (Figure 3A). Exposure to 0.7 mg/kg DEP induced a 1.36-fold significant ($p<0.05$) increase in NF-κB gene expression compared to control (Figure 3A). Though pre-treatment with 50 mg/kg rooibos extract did not produce any significant change in NF-κB gene expression, however, it significantly ($p<0.05$) attenuates (by 1.27-fold) the increased NF-κB mRNA level caused by 0.7 mg/kg DEP exposures (Figure 3A). The melting (Figure 3B) and the amplification curves (Figure 3C) and the agarose gel analysis (Figure 3D) showed that the qPCR system amplified a single product (amplicon).

Figure 3

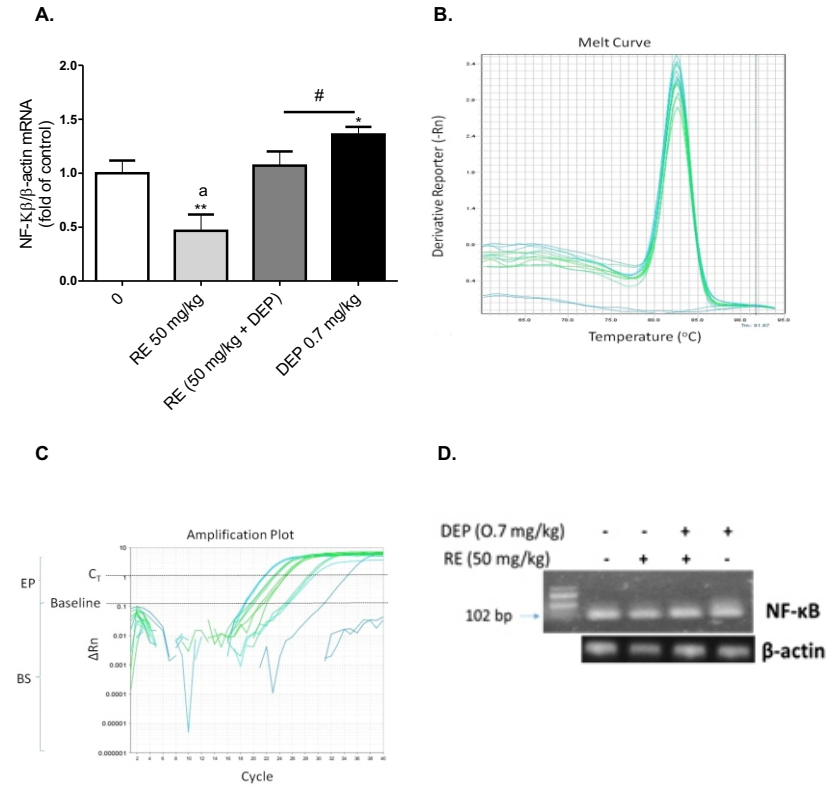

Figure 3. Effects of Rooibos Extract on DEP-induced Nuclear Factor Kappa B (NF- κ B) Gene Expression. (A) Relative quantification of NF- κ B gene expression using $\Delta\Delta C_T$. Values are mean±SEM of three different experiments done in triplicate (n=3). *$p<0.05$, **$p<0.01$, ***$p<0.001$ as significant difference as compared to the controls. $^{\#}p<0.001$ as significant difference between rooibos extract pre-treatrmentvs DEP, $^{a}p<0.01$ as significant difference between rooibos extract pre-treatment vs rooibos extract. (B) Instrument-derived melt curve. (C) Instrument derived amplification curve. (D) Agarose gel analysis of the qPCR products show single amplicons (102 bp) as a single band. BS, background signal; EP, exponential phase; C_T, cycle threshold; Rn, emission intensity of the reporter/emission intensity of passive reference (normalised reporter); ΔRn, the difference between the Rn at the end point and at the starting point and it is directly proportional to the DNA amount during the exponential phase.

In contrast to NF-κB gene expression, 50 mg/kg rooibos extract did not alter the gene expression of inhibitory κ B (IκB) compared to control (Figure 4A). Rooibos extract pre-treatment induced a 1.74-fold significant ($p<0.01$) increase and diesel exhaust particles caused a 2.78-fold significant ($p<0.05$) decrease in IκB mRNA levels compared to control (Figure 4A). The presence of 50 mg/kg rooibos extract significantly ($p<0.001$) mitigates against (by 4.77-fold) diesel exhaust particles suppression of IκB gene expression. The efficiency of the qPCR for the amplification of the IκB gene was seen in the single melting point (Figure 4B), the amplification curve (Figure 4C) and the agarose gel analysis (Figure 4D). These figures showed that there was doubling of the product (amplicon) at the exponential phase.

Figure 4

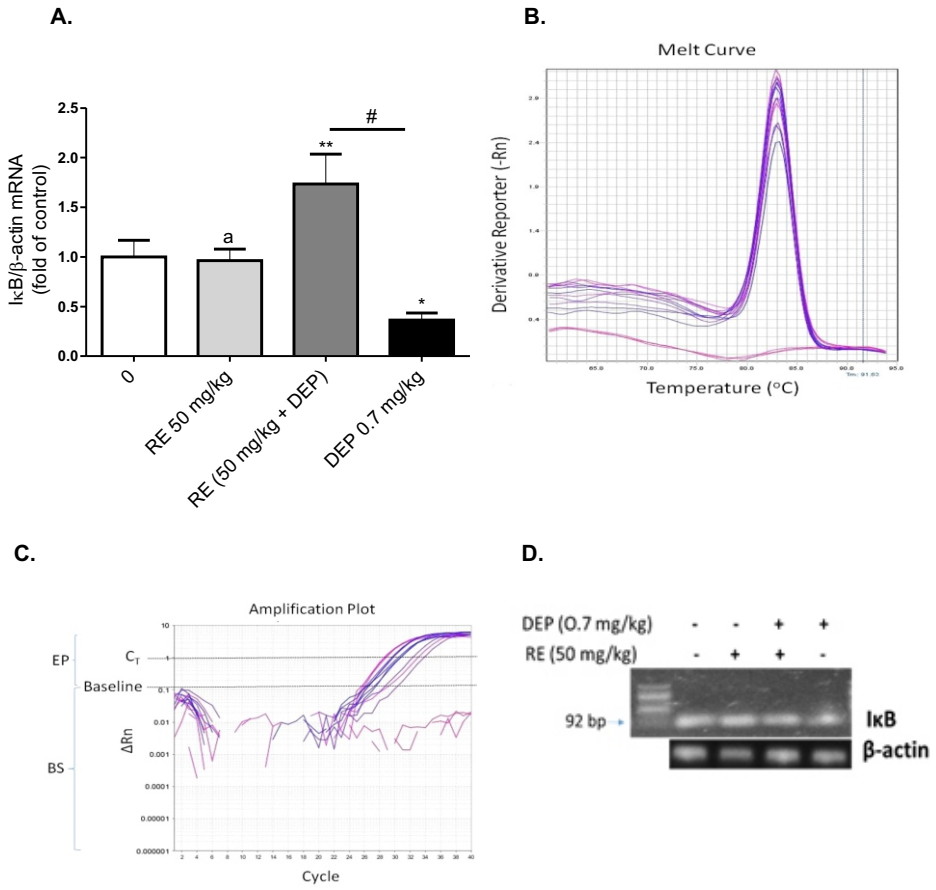

Figure 4. Effects of Rooibos Extract on Inhibitory Kappa B (I κ B) Gene Expression in the Presence of DEP. (A) Relative quantification of I κ B gene expression using $\Delta\Delta C_T$. Values are mean±SEM of three different experiments done in triplicate (n=3). *$p<0.05$, **$p<0.01$, ***$p<0.001$ as significant difference as compared to the controls. $^{\#}p<0.001$ as significant difference between rooibos extract pre-treatment vs DEP, $^{a}p<0.01$ as significant difference between rooibos extract pre-treatment vs rooibos extract. (B) Instrument-derived melt curve. (C) Instrument derived amplification curve. (D) Agarose gel analysis of the qPCR products show single amplicons (92 bp) as a single band. BS, background signal; EP, exponential phase; Rn, emission intensity of the reporter/emission intensity of passive reference (normalised reporter); C_T, cycle threshold; ΔRn, the difference between the Rn at the end point and at the starting point and it is directly proportional to the DNA amount during the exponential phase.

Inhibitory kappa B kinase (IκKB) catalyses the phosphorylation of IκB to activate NF-κB migration into the nucleus, thus enhancing pro-inflammatory genes transcription. Our results showed that while treatment and pre-treatment with 50 mg/kg rooibos extract did not produce any significant change in IκKB gene expression compared to control, exposure to 0.7 mg/kg significantly ($p<0.001$) induced IκKB mRNA level by 7.79-fold compared to control (Figure 5A). Pre-treatment with 50 mg/kg rooibos extract, however, caused a 3.54-fold reduction in IκKB gene expression induced by 0.7 mg/kg diesel exhaust particles (Figure 5A). The melting and amplification curves showed that a single amplicon product was amplified during the qPCR runs (Figure 5B and C). The agarose gel analysis confirmed a single product having an amplicon size of 95 bp (Figure 5D). These data suggest that the anti-inflammatory effects of rooibos extract on diesel exhaust particles-induced inflammation may be exerted via the suppression of NF-κB pathway.

Figure 5

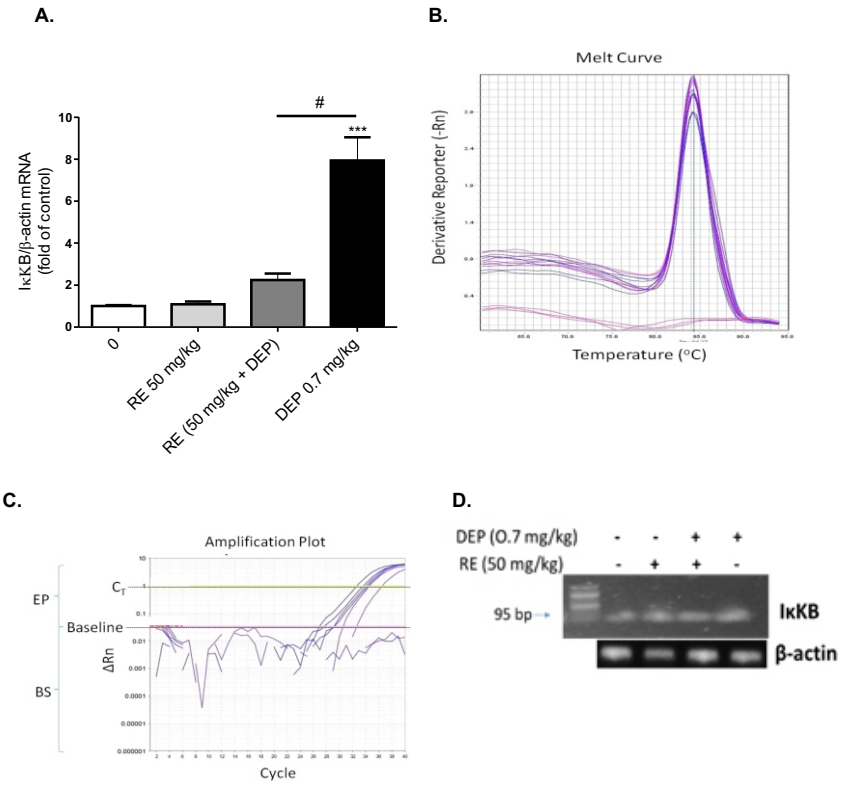

Figure 5. Effects of Rooibos Extract on Inhibitory Kappa B Kinase (I κ KB) Gene Expression in the Presence of DEP. (A) Relative quantification of I κ KB gene expression using $\Delta\Delta C_T$. Values are mean±SEM of three different experiments done in triplicate (n=3). *$p<0.05$, **$p<0.01$, ***$p<0.001$ as significant difference as compared to the controls. $^{\#}p<0.001$ as significant difference between rooibos extract pre-treatrmentvs DEP, $^{a}p<0.01$ as significant difference between rooibos extract pre-treatment vs rooibos extract. (B) Instrument-derived melt curve. (C) Instrument derived amplification curve. (D) Agarose gel analysis of the qPCR products show single amplicons (95 bp) as a single band. BS, background signal; EP, exponential phase; C_T, cycle threshold; Rn, emission intensity of the reporter/emission intensity of passive reference (normalised reporter); ΔRn, the difference between the Rn at the end point and at the starting point and it is directly proportional to the DNA amount during the exponential phase.

Rooibos Extract Mitigates Against Diesel Exhaust Particles-induced Tumor Necrosis Factor α (TNFα) Gene Expression

Pro-inflammatory cytokines, such as TNFα and IL-1, triggered the activation of NF-κB in a mechanism that involves activation of IκKB, with consequent phosphorylation of IκB (Lawrence, 2009). To establish the involvement of TNFα in the anti-inflammatory effects of rooibos extract on diesel exhaust particles-induced inflammation, we determined the expression of TNF*α* gene in liver of animals exposed to 0.7 mg/kg diesel exhaust particles in the presence and absence of 50 mg/kg rooibos extract (Figure 6). The results showed that 0.7 mg/kg diesel exhaust particles caused 1.54-fold significant ($p<0.001$) induction in TNF*α* mRNA level compared to control (Figure 6A). Pre-treatment with 50 mg/kg rooibos extract before diesel exhaust particles exposure significantly ($p<0.001$) mitigates against diesel exhaust particles -induced TNF*α* gene expression by 2.85-fold. Figure 6B and C showed the melting and amplification curves for the qPCR amplicon product. Agarose gel analysis of the qPCR product indicates a single amplicon product (Figure 6D). The data suggest that the anti-inflammatory effects of rooibos extract on the pro-inflammatory effects of diesel exhaust particles may involve the repression of TNFα gene expression, thereby blocking IκKB activation and IκB phosphorylation.

Figure 6

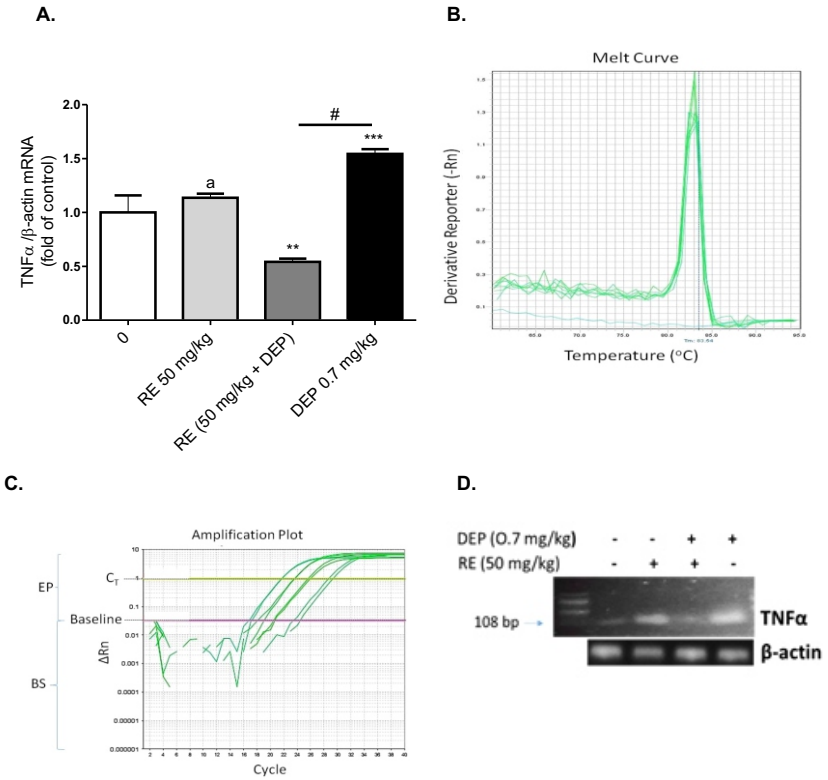

Figure 6. Effects of Rooibos Extract on Tumor Necrosis Factor **α** (TNF***α***) Gene Expression in the Presence of DEP. (A) Relative quantification of TNF*α* gene expression using $\Delta\Delta C_T$ Values are mean±SEM of three different experiments done in triplicate (n=3). *$p<0.05$, **$p<0.01$, ***$p<0.001$ as significant difference as compared to the controls. $^{\#}p<0.001$ as significant difference between rooibos extract pre-treatrmentvs DEP, $^{a}p<0.01$ as significant difference between rooibos extract pre-treatment vs rooibos extract. (B) Instrument-derived melt curve. (C) Instrument derived amplification curve. (D) Agarose gel analysis of the qPCR products show single amplicons (108bp) as a single band. BS, background signal; EP, exponential phase; C_T, cycle threshold; Rn, emission intensity of the reporter/emission intensity of passive reference (normalised reporter); ΔRn, the difference between the Rn at the end point and at the starting point and it is directly proportional to the DNA amount during the exponential phase.

DISCUSSION

Our data indicate that while diesel exhaust particles induced significant pro-inflammatory effect in the hepatic tissue via the NF-κB pathway, the presence of rooibos extract attenuates this effect by a mechanism, which involved the de-activation of the 'canonical' pathway of NF-κB activation.

Several studies have reported on the inflammatory effect of diesel exhaust particles in hepatic tissues (Yin et al., 2013; Ito et al., 2016; Lawal, 2018). A study has shown that the hepatotoxic effects of diesel exhaust particles may involve the induction of inflammation in the hepatic cell (Kim et al., 2014) and increased pro-inflammatory cytokines, such as IL-6, IL-1β, as seen in rats' livers exposed to diesel exhaust particles (Kim et al., 2014; Ito et al., 2016). The results from our present study are in agreement with these studies. Our study shows that diesel exhaust particles induced the mRNA level of pro-inflammatory cytokine, IL-8, while decreased the mRNA level of anti-inflammatory cytokine, IL-10. The effects of diesel exhaust particles on NF-κB activation were highlighted in several studies (Lawal et al., 2015, Lawal, 2017). For instance, Ma et al (2004) and Ito et al (2016) reported an activation of NF-κB in the hepatic tissue of mice exposed to diesel exhaust particles. In agreement with these findings, our study shows a significant increase in NF-κB gene expression in the liver of the exposed Wistar rats, which may be responsible for the inflammatory cytokines production observed in our model.

We further investigated the role of TNFα/IκKB/IκB/NF-κB signalling pathway in diesel exhaust particles-induced inflammation in hepatic tissue. NF-κB mediated inflammatory response is enhanced by increased in TNF*α* and IκKB genes expressions, and decreased in IκB gene and protein expressions (Lawrence, 2009). Chaisson et al (2002) showed that TNFα caused nuclear translocation of NF-κB in hepatocyte but IκB hinder this translocation. Consistent with these findings, our data showed that while diesel exhaust particles increased TNF*α* and NF-κB gene expressions, it caused concomitant decrease in IκB mRNA. The increased TNFα may be responsible for the increased level of pro-inflammatory cytokine-IL-8 production due to the increased nuclear migration of NF-κB. In addition, the induction in IκKB mRNA level could be as a result of the increased TNFα leading to the consequent increased in IκB phosphorylation and enhanced NF-κB nuclear migration.

Some studies have shown the use of pharmaceutical and phytochemical agents to reverse the injurious inflammatory effects of diesel exhaust particles (Lawal et al., 2015; Tseng et al., 2015). However, this is the first study that will report on the anti-inflammatory effects of aqueous rooibos extract on diesel exhaust particles-induced inflammation. The anti-inflammatory properties of flavonoids from plants, including rooibos, have been reported to be exerted through the blockage in the TNFα-induced NF-κB gene expression, by inhibiting IκKB activity, at the IκB/NF-κB phosphorylation and degradation level (Ruiz and Haller, 2006). The present study confirmed this earlier report. Our data showed that rooibos tea extract exerts its anti-inflammatory effects on diesel exhaust particles-induced hepatic inflammation using the TNFα/IκKB/IκB/NF-κB signalling pathway. In this study, we found that rooibos extract mitigates against diesel exhaust particles-induced hepatic TNF*α*, IκKB and NF-κB gene expressions, while causing elevation in IκB mRNA level resulting in the inhibition of NF-κB nuclear translocation.

Conclusion

Our current study provides insight into the possible involvement of NF-κB signalling pathway in the anti-inflammatory effect of aqueous extract from commercially available rooibos tea on diesel exhaust particles-induced hepatic inflammation. Thus, inhibition of the NF-κB activation, by rooibos tea, may serve as an effective therapeutic target for ameliorating the adverse health effects caused by the pro-inflammatory effect of air particulate matter.

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