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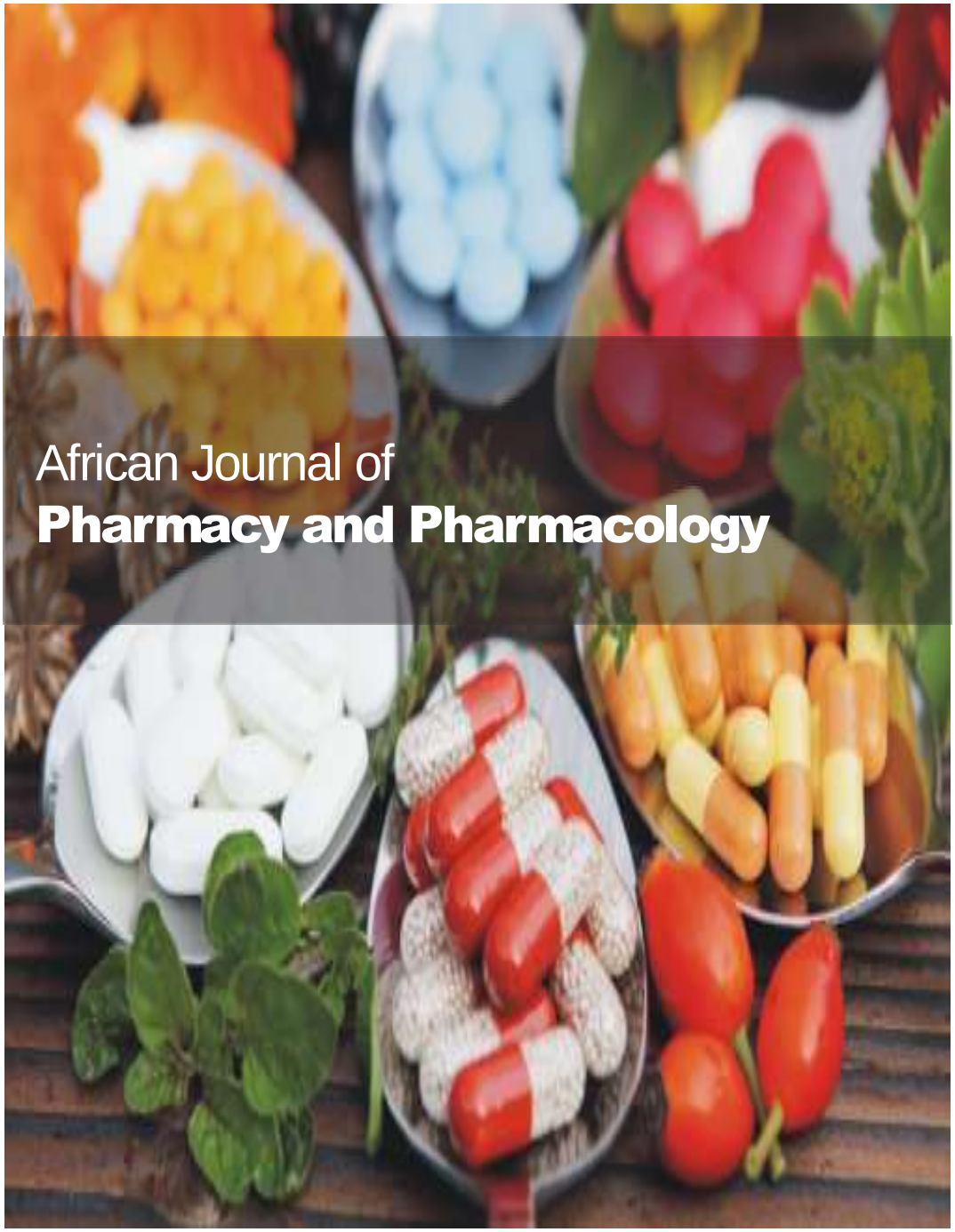

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Full Length Research Paper

Endothelium-independent vasorelaxation by dichloromethanolic fraction from *Anogeissus leiocarpa* (DC) Guill. Et Perr. (Combretaceae) bark of trunk on porcine coronary artery rings: Involvement of $[Ca^{2+}]_i$ decreased and phosphodiesterases inhibition

Belemnaba Lazare[1,2]*, Nitiéma Mathieu[2,3], Ouédraogo Sylvin[2], Auger Cyril[1], Schini-Kerth Valérie B.[1] and Bernard Bucher[1]

[1]UMR CNRS 7213, Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie Université de Strasbourg, Illkirch, France
[2]Institut de Recherche en Sciences de la Santé (IRSS/CNRST), Ouagadougou, Burkina Faso
[3]Université Ouaga I Pr Joseph KI-ZERBO, Ouagadougou, Burkina Faso

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***Anogeissus leiocarpa* (DC) Guill. et Perr. belongs to the Combretaceae family and was previously named *A. leiocarpa* (DC) Guill. Et Perr. It has been widely used in Burkina Faso by traditional medicine for the treatment of hypertension. Previous study showed that the dichloromethanolic fraction from the barks of trunk of *A. leiocarpa* (ALF) has induced an endothelium-independent and endothelium-dependent vasodilation effect and had the capacity to inhibit *in vitro*, purified cyclic nucleotide phosphodiesterases (PDEs) activity. The aims of this study were to better underline ALF-induced endothelium-independent vasorelaxation in an organ model. The results showed that ALF significantly reduce the contractile response to U46619 in porcine coronary artery rings without endothelium that were in concentration-dependent manner. In denuded rings, pretreatment by ALF (10, 30 and 100 µg/mL) did not affect relaxation to sodium nitroprusside (SNP), suggesting that relaxation to ALF was not due to its ability to be a nitric oxide donor. Moreover, SNP-induced relaxation had not been affected in the presence of Nimodipine (PDE1 inhibitor), EHNA (PDE2 inhibitor) or DMPPO (PDE5 inhibitor). In addition, the results showed a relaxation effect to isoproterenol in endothelium-denuded artery rings pretreated with ALF (3, 10, and 30 µg/mL) which were significantly affected suggesting a possible membrane hyperpolarization leading to the vasodilation. In the presence of the PDE3 specific inhibitor Cilostamide and ALF (30 and 100 µg/mL), the vasodilation effects of isoproterenol was enhanced and comparable. Moreover, various potassium channels were not involved in ALF-induced relaxation since tetraethylammonium chloride (non-selective K^+ channels inhibitor), iberiotoxin (voltage-sensitive potassium channels inhibitor) and Glibenclamide (K_{ATP} channels inhibitor) did not notably affect the relaxation effect to ALF in rings without endothelium. Taken together, ALF-induced endothelium-independent relaxation mainly involves a sustained decrease in $[Ca^{2+}]_i$ and may be due to PDE1, 3 and 5 inhibitions localized in the vascular smooth muscle cells. While, the involvement of the ions channels have not been clearly revealed in this experiment.**

Key words: Anogeissus leiocarpa, U46619, phosphodiesterases (PDEs), calcium, porcine coronary artery, sodium nitroprusside (SNP), isoproterenol.

INTRODUCTION

The traditional medicine has always been used in Burkina Faso for the care of the population and this for several generations. In cardiovascular diseases such as stroke, headache, and hypertension, vessels dilation is fundamental because it directly influences the arteries of the circulatory system. Accordingly, many researchers have investigated the vasorelaxant effects of various herbal medicines (Gan et al., 2016; Kassahun Gebremeskel et al., 2017; Cam et al., 2018; Khan et al., 2018; Vajic et al., 2018; Sanchez-Recillas et al., 2019). It was the case of a recent study which indicated the endothelium-dependent vasodilation effect of the aqueous extract of *Anogeissus leiocarpa* (Belemnaba et al., 2018).

Anogeissus leiocarpa is commonly named African birch. It has large ecological distribution ranging from the boarders of Sahara up to the out layer humid tropical forests. In West Africa, it expands from Senegal to Cameroon, with extension to Ethiopia and East Africa. It has been grown in dry and gallery forests. The tree was up to 30 m in height but typically, between 15 and 18 m with light green foliage. The base of the trunk is wider and occasionally striped. The colour of the bark is grey and becomes blackished depending on the age. It is fibrous with thin scales. It has a finely publescent stems and alternate to sub-opposite, elliptical to oval leaves which are 2 to 8 cm length and 1.5 to 3.5 cm wide (Arbonnier, 2009).

However, there have been fewer pharmacological studies and clinical data for *A. leiocarpa* in comparison to other medicinal plants used for their pharmacological effects through their molecules contain (*Notopterygii Rhizoma* and *Radix*; *Calotropis procera*, *Kaya senegalensis*, *Moringa oleifera*).

Therefore, more pharmacological and clinical studies are needed to support the continuous use of *A. leiocarpa* in traditional medicine for the treatment of hypertension.

Nevertheless, studies on *A. leiocarpa* extracts had already reported various pharmacological activities such as antioxidant, antimicrobial, anthelmintic activity and antihypertensive effect (Mann et al., 2008; Konaté et al., 2011; Soro et al., 2013; Ouedraogo and Kiendrebeogo, 2016). Many second messengers are involved in the regulation of the cardiovascular system with specifications depending on whether it is at the heart or vascular level. At the heart level, regulation involves several systems such as the orthosympathetic and parasympathetic systems in which intracellular Ca^{2+} as the main regulator of cardiac excitation-contraction coupling. An imbalance in the intracellular calcium concentration inevitably leads to heart dysfunction (Chung et al., 2016; Mora et al., 2017; Montalvo et al., 2018). At the vascular level, regulation in the presence of endothelium involves many second messengers such as nitric oxid, prostacyclin I_2, inhibition of phosphodiesterases (PDEs), endothelium-derived hyperpolarizing factors and many others messengers. In the absence of endothelium, this relaxation notably involves the potassium channels, calcium channels, inhibition of PDEs and other vasodilation factors (Alamgeer et al., 2018; Jia et al., 2018; Sanchez-Recillas et al., 2019). Specifically for *A. leiocarpa* fraction (ALF), previous studies have shown that this extract has induced an endothelium-dependent and endothelium-independent vasorelaxant effect on pig artery by involving mostly NO/sGC/cGMP and Na^+/k^+-ATPase pathways and in some extent a possible PDEs inhibition (Belemnaba et al., 2013). However, there was no published studies on this extract demonstrating that its vasorelaxation effect implicates the inhibition of PDEs in a vascular system. Indeed, the possible implication of the PDEs inhibition in the mechanism of vasodilation of ALF has been demonstrated but through PDEs purified according to a tube model (Belemnaba et al., 2013). It was therefore necessary to show this implication on a model using vessels which is close to reality. Moreover, it was well known that other ions channels were involved in vascular stone regulation especially in endothelium independent arteries. It was the case of large-conductance calcium-activated potassium channels (BK_{Ca}), K_{ATP} channels and K^+ channels (Gan et al., 2016; Greenberg et al., 2016; Li et al., 2018).

For this purpose, we have used pig isolated arteries rings to assess the implication of PDEs inhibitions in the ALF endothelium independent vasodilation effects by using various pharmacologicals agents [bradykinin, sodium nitroprusside (SNP, a NO donor), isoproterenol (Adenylate cylase activator)] and specifc inhibitors [Nimodipine (PDE1 inhibitor), EHNA (PDE2 inhibitor), Cilostamide (PDE3 inhibitor), DMPPO (PDE5 inhibitor), tetraethylammonium chloride (non-selective K^+ channels inhibitor), iberiotoxin (voltage-sensitive potassium channels inhibitor) and Glibenclamide (K_{ATP} channels inhibitor)].

MATERIALS AND METHODS

Plant and extraction

The plant materials consist of the barks of the trunk of *A. leiocarpa*. The collection method of *A. leiocarpa* has been previously described (Belemnaba et al., 2013).

*Corresponding author. E-mail: lbelemnaba2@gmail.com

Briefly, the barks of trunk of *A. leiocarpa* were collected in May 2006 in the East of Ouagadougou (zone of savana). A voucher specimen (Number 1544) was deposited at the Department of Forest Production of the National Centre for Scientific and Technological Research (CNRST) after identification by a botanist of this centre. Plant material freshly collected was dried in a greenhouse with air circulation. It was powdered in a pulverizer mil and a decoction was made (120 g in 2100 mL distilled water). After the decoction, ALF extract was obtained by an exhaustion into a methylene chloride solution, then concentrated into a rotavapor system and finally dried in an oven at 35°C.

Drugs and chemicals

Bradykinin, isoproterenol (Isop), indomethacin, sodium nitroprusside (SNP), glibenclamide (GLIB), iberiotoxin (IBTX), tetraetylammonium chloride (TEA), bradykinin and the cyclic nucleotides PDE inhibitors (EHNA, Cilostamide) were from Sigma-Aldrich (Saint Quentin Fallavier, France). Nimodipine and DMPPO were given by Bayer (Berlin). U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F2α) was from Cayman Chemical (Ann Arbor, MI, United States of America).

Vascular reactivity studies

The vascular reactivity of ALF was assessed as indicated previously (Belemnaba et al., 2018). Pig hearts were collected from the local slaughterhouse (Copvial, Holtzheim) and left circumflex coronary arteries were excised and then cleaned to remove all connectives tissues. Porcine coronary arteries were cut into rings of 3 to 4 mm and suspended in organ bath contained appropriated krebs bicarbonate solution (Composition in mM: NaCl 119, $CaCl_2$ 1.25, $NaHCO_3$ 25, $MgSO_4$ 1.18, KH_2PO_4 1.18, KCl 4.7, D-glucose 11, pH 7.4, 37°C) and oxygened with carbogen gas (mixture of oxygen 95% and carbon dioxide 5%). Rings were put into an initial isometric tension of 5 g for 1 h.

The presence of endothelium was confirmed pharmacologically by testing the response to bradykinin at 3×10^{-7} M under pre-contraction conditions with U46619. A relaxation about 90% of the contracted rings by bradykinin (3×10^{-7} M) was considered with endothelium. When required, the endothelium was removed by gently rubbing the intimal space with a stainless steel rod with a diameter equivalent to that of the arterial lumen. A relaxation about 10% of the maximal contracted rings by bradykinin (3×10^{-7} M) was considered without endothelium. For the assessment of ALF effect on rings, rings were contracted with U46619 to about 80% of the maximal contraction before the construction of the concentration-response curve to ALF. In some experiments, rings were pre-incubated with specific inhibitors or ALF (3, 10, 30 or 100 µg/mL) before contraction to U46619 and the subsequent construction of a concentration-response curve to an antagonist. Indeed, to determine the effect of the extract on U46619-induced vasoconstriction, rings were first incubated with ALF (3, 10, 30, 100 and 300 µg/mL) for 5 min before a cumulative of U46619. In other experiments, rings were pre-incubated with different concentrations of ALF (3, 10, 30, 100 and 300 µg/mL) for 5 min followed by U46619 to the maximum contraction and subsequent cumulative with SNP or isoproterenol. Other rings were first pre-incubated with PDEs inhibitors (Nimodipine, EHNA, Cilostamide, DMPPO) or with TEA, Glibenclamide or Iberiotoxin for 30 min followed by U46619 contraction and cumulative with ALF (3, 10, 30, 100 and 300 µg/mL).

Statistical analysis

All results were expressed as the mean ± standard error of mean (SEM) and *n* represents the number of rings from different pigs used in the experiments. The relaxation response was expressed as percentage of decreases in tension from the contracting level induced by U46619. The concentrations of substances induced 50% of maximal relaxation (EC_{50}) and their maximal relaxation (Emax) was determined with GraphPad Prism 5.00.288. Two way or one way ANOVAs (with post hoc Bonferroni's test) determined significant differences, if any, between concentration-relaxation curves and EC_{50}, respectively in different treatment groups. $P < 0.05$ was considered as the significant threshold.

RESULTS

Effect of ALF (3-100 µg/mL) on U46619-induced contraction in rings with endothelium

The thromoxane A2 mimetic U46619 (10^{-10} M to 3×10^{-7} M) has induced a concentration-dependent contraction of pig coronary rings (Figure 1). The magnitude of U46619-induced isometric tension development on rings without endothelium was in a concentration-dependent manner. The maximum contraction-response (Emax) to U46619 in rings pre-incubated with ALF 3, 10, 30 and 100 µg/mL was of 21.57±1.88, 20.73±1.81, 17.04±1.56 and 13.11±0.51 g, respectively (Figure 1B) while the control was 20.34±1.69 g. The pre-incubation by ALF 30 and 100 µg/mL causes a significant reduction in the contraction-induced by U46619 as compared to the control. Moreover, we noted a slight but not significant shift to the left and to the right contraction-curve when rings were preincubated with ALF 3 and 10 µg/mL, respectively compared to the control.

Effects of ALF (3-100 µg/mL) on SNP-induced vasodilation in porcine arteries rings

The results showed that the endothelium-independent vasodilator sodium nitroprusside (SNP, 10^{-11} to 10^{-5} M) was dose-dependently relaxed U46619-contracted pig coronary arteries rings without endothelium. In pre-incubated denuded artery rings with ALF (3, 10, 30 or 100 µg/mL), the vasodilator effect of SNP (a NO donnor) was significantly improved in a concentration-dependent manner compared to the control (Figure 2). The half-maximal effective concentration (EC_{50}) values were of 0.171±0.076 µM for the control, 0.054±0.019, 0.050±0.034, 0.043±0.017 and 0.005±0.002 µM in the presence of ALF 3, 10, 30 and 100 µg/mL, respectively. No significant difference was notified in the Emax values that were 99.51±1.72, 100.27±0.37, 101.62±1.78, 100.2±0.35, and 100±0, respectively for the control, ALF 3, 10, 30 and 100 µg/mL.

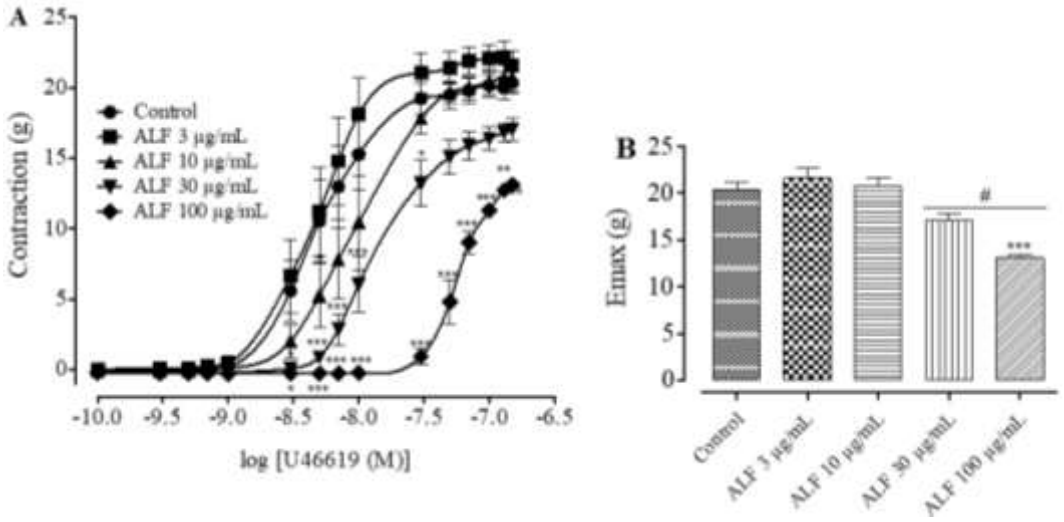

Figure 1. Effect of ALF on U46619-induced contraction : (A) Denuded porcine coronary artery rings were exposed to ALF (3, 10, 30, 10 µg/ml) 5 min before the addition of increasing concentration of U46619 ; (B) The respective maximal contraction obtained. Experiments were performed in the presence of indomethacin (n=3 to 5 different experiments; *P<0.05 vs. Control).

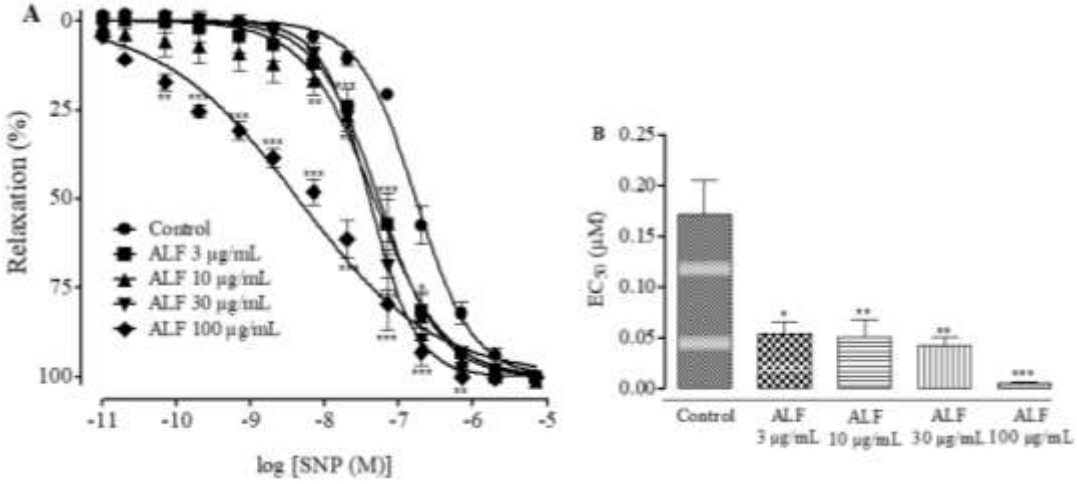

Figure 2. (A) Effect of ALF (3, 10, 30, and 100 µg/ml) on the relaxation-induced by sodium nitroprusside (SNP) in denuded porcine artery rings pre-contracted with U46619. (B) The histogram of the representative EC50 values.Experiments were performed in the presence of indomethacin (10 µM) to avoid endothelium derivative hyperpolarization factor (n=6 to 8 different experiments, *P<0.05 vs. Control).

Effects of Nimodipine (1 µM), EHNA (10 µM), and DMPPO (0.1 µM) on SNP-induced vasodilatation in porcine arteries rings

The concentration-relaxation curves to SNP in denuded rings contracted with U46619 were significantly shifted to the left when pre-incubated with Nimodipine (PDE1 specific inhibitor),EHNA (PDE2 specific inhibitor)or DMPPO(PDE5 specific inhibitor) compared to the control (Figure 3).

The EC_{50} values of SNP-induced endothelium-independent vasodilation on rings were of 0.063±0.040, 0.059±0.015 and 0.022±0.007 µM, respectively in the presence of Nimodipine (1 µM), EHNA (10 µM), and DMPPO (0.1 µM). When comparing the EC_{50} values, only the presence of DMPPO showed a significant difference compared to the control (EC_{50} = 0.171±0.076 µM) but their Emax was not affected.

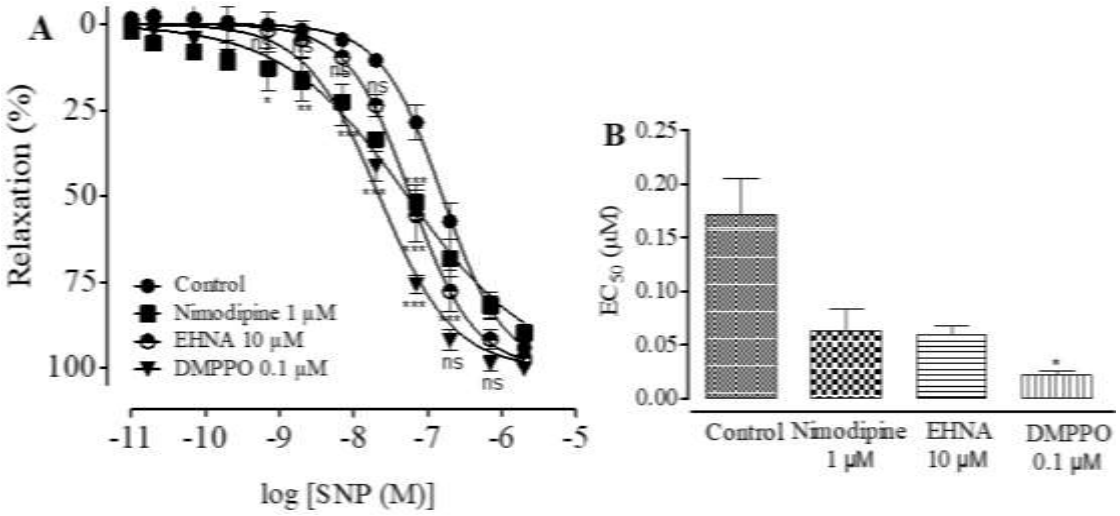

Figure 3. Characterization of SNP-induced relaxation in porcine coronary artery ring. (A) Rings without endothelium were exposed to EHNA (PDE2 specific inhibitor), Nimodipine (PDE1 specific inhibitor), and DMPPO (PD5 specific inhibitor) 30 min each, before a cumulative concentration of SNP (10^{-11} to 10^{-5} M). (B) The representative EC50 for the control and in the presence of Nimodipine (1 µM), EHNA (10 µM) and DMPPO (0.1 µM). Experiments were performed in the presence of indomethacin (10 µM) (n=4 to 5 different experiments, *P<0.05 vs. Control).

Effects of ALF (3-100 µg/mL) on isoproterenol-induced vasorelaxation in porcine arteries rings

Isoproterenol caused concentration-dependent relaxation of U46619 pre-contracted in pig coronary artery without endothelium (EC_{50} = 0.148±0.027 µM). Pre-incubation with ALF (3, 10, 30 and 100 µg/mL) has been potentiated significantly in the relaxation effect of isoproterenol in a concetration-dependent manner compared to the control. The EC_{50} was of 0.08±0.03, 0.04±0.009, 0.02±0.01 and 0.01±0.002 µM in the presence of ALF 3, 10, 30 and 100 µg/mL, respectively. However, the Emax values were not significantly modified compared to the control (Figure 4).

Effects of ALF (3 µg/mL), nimodipine (10 µM) and DMPPO (0.1 µM) on isoproterenol-induced vasodilatation in porcine arteries rings

The results shown in Figure 5 indicated that the vasodilation effect to isoproterenol was not notably improved when rings were preincubated either with nimodipine (PDE1 specific inhibitor) or with DMPPO (PDE5 specific inhibitor). The shape of their relaxation curves was similar to that following the pre-incubation with ALF 3 µg/mL. The EC_{50} values of isoproterenol were of 0.148±0.027 µM for the control and of 0.076±0.030, 0.123 ±0.091 and 0.112±0.076 µM in the presence of ALF 3 µg/mL, nimodipine and DMPPO, respectively but without significant difference to the control.

Effects of ALF (30 and 100 µg/mL) and cilostamide (10 µM) on isoproterenol-induced vasodilation in porcine arteries rings

The results showed that the vasodilation effect to isoproterenol was higly and significantly improved when rings were pre-incubated with cilostamide (a PDE3 specific inhibitor, 10 µM; Figure 6). This effect was relatively similar to those obtained in the presence of ALF30 and 100 µg/mL. The EC_{50} of isoproterenol was of 0.030±0.007, 0.023±0.007 and 0.014±0.002 µM in the presence of cilostamide, ALF 30 and 100 µg/mL, respectively. Thus, the rank orders for relaxant effect of isoproterenol when pre-incubated with ALF and cilostamide were ALF 100 µg/mL > ALF 30 µg/mL > Cilostamide.

Effects of TEA (1 µM), IBTX (10 µM) and GLIB (0.44 µM) on ALF-induced vasodilation in porcine arteries rings

In endothelium denuded artery rings, results showed that the non-selective blockade of potassium channels with TEA was not affected. ALF (0.1 to 30 µg/mL)-induced vasodilation effect that was superposable to those of the control (Figure 7). When pre-incubated with IBTX or Glibenclamide, the relaxation of ALF in rings were slighly shifted but not significantly different to the control. The Emax values were 97.41±6.84% for the control and 100%

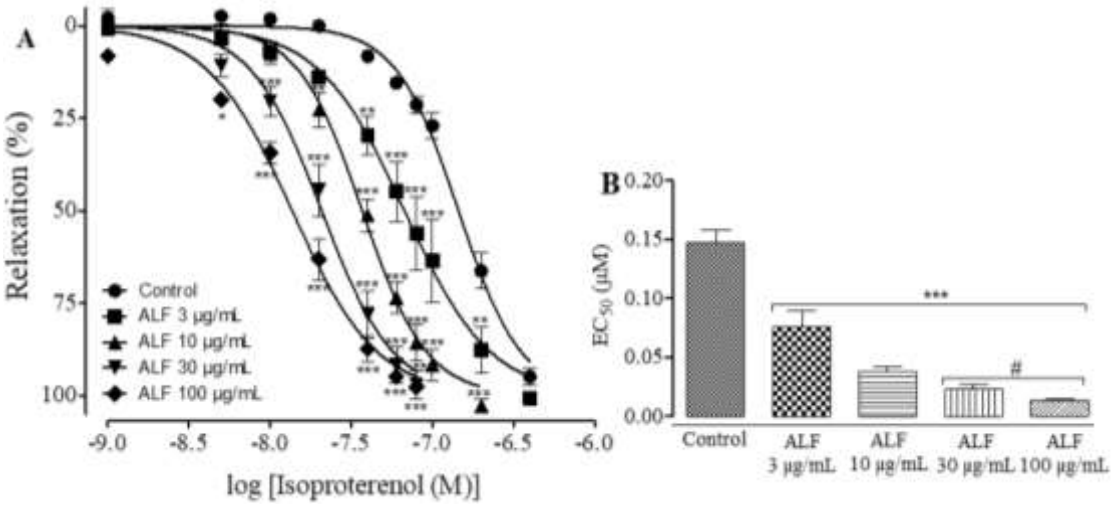

Figure 4. (A) Characterization of isoproterenol-induced relaxation in porcine coronary artery ring: endothelium-denuded rings were exposed to ALF (3, 10, and 30 µg/ml) 5 min before the addition of increasing concentration of isoproterenol (10^{-9} to 4 $\times 10^{-7}$ µM). (B) Histogram representing the relative EC50. Experiments were performed in the presence of indomethacin (n=3 to 8 different experiments, *P<0.05 vs. Control; *P<0.05 vs. ALF 3 µg/ml).

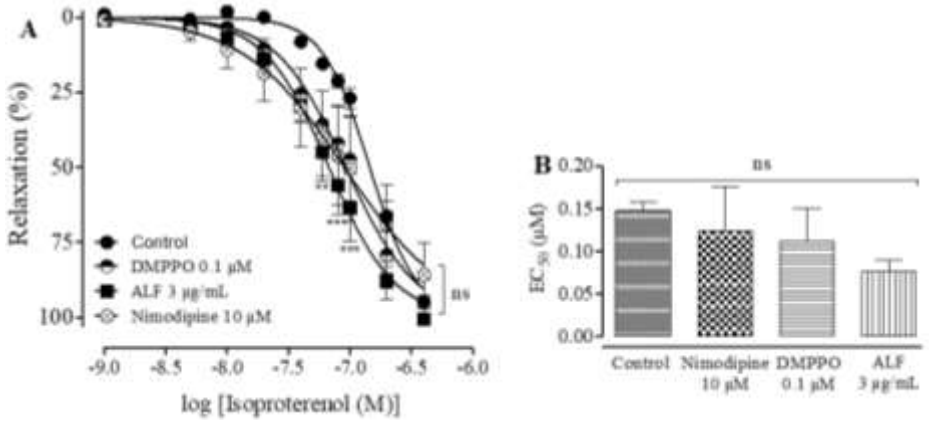

Figure 5. Effect of Nimodipine (PDE1 inhibitor, 30 min) and DMPPO (PDE5 specific inhibitor) on isoproterenol-induced relaxation in coronary artery rings without endothelium. For comparison, the effect of ALF (3 µg/ml) is induced in the graphs. (B) Histogram represent the relative EC50 for the control and in the presence of Nimodipine, DMPPO and ALF. All experiments were performed in the presence of indomethacin (10 µM) (n=4 to 8 different experiments, *P<0.05 vs. Control).

in the presence of the tested inhibitors.

DISCUSSION

In previous study, it was demonstrated that ALF has been induced as an endothelium-dependent and endothelium-independent vasodilation effect in porcine coronary arteries rings that was mediated via NO/sGC/cGMP and cAMP pathways, potassium (K^+) and partly Na^+/K^+-ATPase channels opening (Belemnaba et al., 2013). Moreover, these findings indicated that ALF was a powerful inhibitor of PDE1, 2, 4 and PDE5 with a less activity on PDE 3. While these tests were conducted on tubes assay and on purified PDEs.

In the present study, experiments were focus on the endothelium-independent vasodilaion effect of ALF in order to better characterized its underline mechanism of

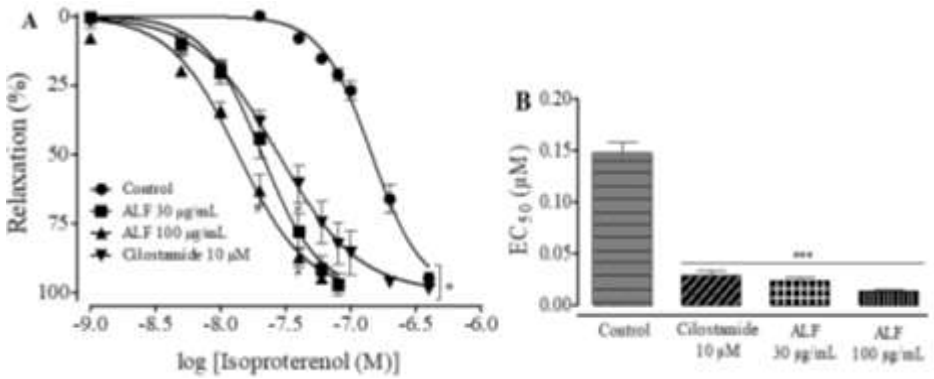

Figure 6. (A) Effect of Cilostamide (PDE3 inhibitor, for 30 min) on isoproternol-induced relaxation in denuded coronary artery rings. For comparison, the effect of ALF (3 and 100 µg/ml) were included in the graph. (B) Histogram represent the relative EC50. All experiments were performed in the presence of indomethacin (10 µM); n=4 to 8 different experiments (*P<0.05 vs. Control).

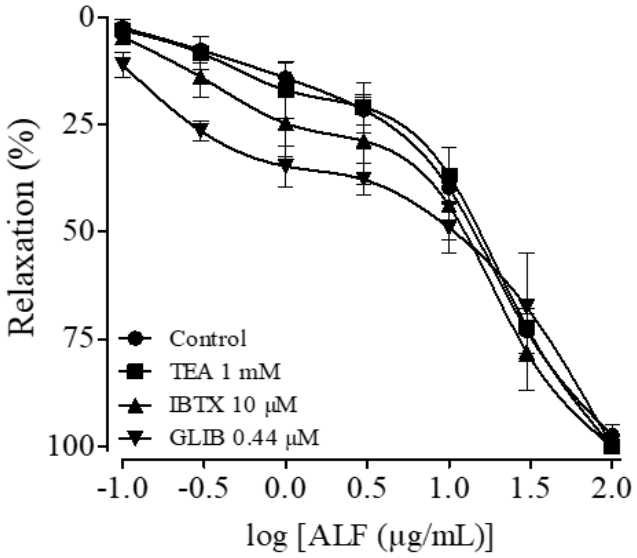

Figure 7. Cumulative-response of ALF in porcine coronary artery rings without endothelium. Rings were pre-incubated with TEA (non-selective inhibitor of potassium channels, 1 µM), IBTX (Large conductance Ca^{2+} activated K^+ channel blocker or voltage-sensitive potassium channels, 10 µM) and Glib (K_{ATP} channels inhibitor, 0.44 µM) 30 min before the addition of ALF. Experiments were performed in the presence of indomethacin (n=4 to 6 different experiments; *P<0.05 vs. Control).

vasodilation and also to show the implication of PDEs inhibiton activity by this fraction of *A. leiocarpa* in an organ model.

Thus, in endothelium-denuded coronary artery ring, vasoconstriction was induced by the thromboxane A2 analogue U46619. The thromboxane A2 (TXA2) was a pharmacological agonist known to be a potent constrictor of artery smooth muscle as well as an inducer of platelet aggregation but also a major cyclooxygenase-dependent endothelium-derived contracting factor (Fu et al., 2017; Minuz et al., 2018; Xu et al., 2018). Because thromboxane A2 was an unstable component, the stable U46619 was

used to contract the coronary arteries rings in the presence or absence of active substances. Indeed, the U46619 was a full TXA2 receptor agonist that induced a susbtained contraction in porcine coronary arteries rings by an influx of Ca^{2+} transmembrane leading to the increase of intracellular Ca^{2+} (Hanasaki et al., 1988; Han et al., 1995; Cogolludo et al., 2003; Hahnenkamp et al., 2004; Sinharoy et al., 2017). Other studies have been also indicated that the important mechanism by which TXA2 elicits its role in pulmonary vasoconstriction was through the involvement of an increase in $[Ca^{2+}]_i$ in the smooth muscle cells (Chakraborti et al., 2009).

The present findings showed that ALF had significantly reduced the U46619-induced vasoconstriction in porcine coronary artery rings without endothelium and interestingly, this effect was in a concentration-dependent manner (Figure 1). For that, ALF effect could be explained by a possible decrease in $[Ca^{2+}]_i$ release in the vascular smooth muscles cells (VSMC) since U46619 was known to induce vasoconstriction in vessels by activation of PLC via Gaq, followed by an increase in intracellular free calcium concentration through an inositol-3-phosphate stimulation sensitive receptors (Hirata et al., 1991; Somlyo and Somlyo, 2000; Bhattacharya et al., 2005).

In order to determine the possible implication of Ca^{2+} decrease in ALF-induced vasodilation effect in vascular system, the effect of SNP was investigated in denuded artery rings pre-incubated with different concentrations of ALF and contracted with U46619 (Figure 2). In this study, results showed that in the functional vascular study using wire myograph and SNP which acts directly on VSMC, that ALF acutely displays an increase in endothelium-independent relaxation in a concentration-dependent manner compared to the control.

In the literature, the SNP (a NO donor) was a well-known arterial and venous vasodilator used in clinical practice to lower blood pressure and usually used to increase the endothelium-independent vasodilatation effect by a cGMP independent mechanism (Otsuka et al., 1988; Hottinger et al., 2014; Basrali et al., 2015). Indeed, several studies demontrated that in vascular smooth muscle, the NO has activated the soluble guanylate cyclase (sGC) that stimulate the production of cyclic GMP, which induced a reduction in the concentration of cytosolic Ca^{2+} with consequent vascular relaxation (Rapoport and Murad, 1983; Hottinger et al., 2014; Zhao et al., 2015; Montfort et al., 2017). Because ALF has enhanced SNP effect suggested that ALF and SNP may have possible synergetic actions to promote cGMP accumulation and Ca^{2+} decreased in VSMC.

Moreover, the endothelium-independent relaxation to isoproterenol (a β-adrenoceptor agonist) was enhanced by the presence of ALF which was in a concentration-dependent manner (Figure 4). Because isoproterenol was known to reduce VSMC vasoconstriction state by stimulating intracellular cAMP level leading to vasodilation suggested a possible synergetic action between ALF and isoproterenol vasorelaxant pathway (Mokkapatti et al., 1998; Xu et al., 2006; Townsend et al., 2012; Ruiz-Medina et al., 2018). The present findings are in accordance with those of other authors which have shown that extract of *Thymus linearis* Benht significantly reduced the contraction effect of U46619 in endothelium-denuded porcine coronary rings in concentration dependent (Alamgeer et al., 2018). In addition, the extract of this plant has significantly improved the effect of isoproterenol, which indicated a certain implication of the cAMP relaxation pathway that was in line of those of ALF. Likewise, the ALF effect might also be due to other mechanisms of actions such as the PDEs inhibition pathway and confirm our previous study (Belemnaba et al., 2013). In fact, the previous study indicated that ALF endothelium-independent effect might be explained by a stimulation of the K^+, Na^+/K^+-ATPase channels but also via inhibition of PDEs activity.

The inhibition of PDEs fosters the accumulation of second messengers according to their family. Under normal physiological conditions, PDE1 was activated by the Ca^{2+}-calmodulin complex. On the one hand, PDE2 and PDE1 have used cAMP and cGMP as substrates for their activity following a decrease in the two second messengers normally responsible for the vasodilatation of VSM (Lugnier, 2006, 2011; Keravis and Lugnier, 2012; Bobin et al., 2016). On the other hand, the PDE5 have used cGMP as substrate and their inhibition leads specifically to the accumulation of cGMP at the smooth muscle level (Lugnier, 2006). In the present findings, the results have shown that the effects of SNP in the presence of nimodipine (PDE1 specific inhibitor) and EHNA (PDE2 specific inhibitor) were slightly shifted to the left but not significantly different as compared to the control.

These results suggest that, in the presence of EHNA, cGMP accumulated by the action of SNP on sGC reactivates PDE2 present in VSM which degrades this substrate thus reducing the effect of sGC activator. However, nimodipine did not significantly improve the effect of SNP compared to the control and its effect in the presence of EHNA. These results indicate a possible involvement of PDE5 which degrade the cGMP produced by the SNP in the case of PDE2 inhibition by EHNA; insofar as in the presence of DMPPO, this effect was significantly different from the control (Figure 3). Thereby, these results corroborate those of others studies in which, in rat lungs precontracted with U46619, the DMPPO amplifies the vasodilator effects of sodium nitroprusside (Eddahibi et al., 1998).

Furthermore, in the present study, the results showed that the effect of isoproterenol on rings pre-incubated with ALF 3 µg/mL is comparable to those obtained in the presence of nimodipine and DMPPO (Figure 5). On the

other hand, this effect in the presence of ALF (10, 30 or 100 µg/mL) is improved and comparable to that obtained with rings pre-incubated with cilostamide (PDE3 inhibitor, Figure 6). Thus, at high concentrations, ALF would also inhibit PDE3 to ensure its vasodilator effect.

Because PDE3 was well known to have a high affinity for cAMP and hydrolyzes cAMP with a rate 10-fold greater than for cGMP hydrolysis despite its capacity to hydrolyze both cAMP and cGMP, this study suggests a possible increase of cAMP and cGMP in VSM after ALF treatment (Beavo, 1995). These results are in line with other studies performed in denuded rat aorta which led to vasodilation and this effect was enhanced by the cilostamide (PDE3 inhibitor) and by the combinaison of SNP plus DMPPO (Delpy et al., 1996; Cui and Green, 2003).

Moreover, the large-conductance calcium-activated potassium channels (BK_{Ca}) were an important potassium ion channels in the VSMC membrane. A depolarization of VSMC membrane potential was followed by an increase of BK_{Ca}, leading to the increase of the intracellular potassium efflux responsible of membrane hyperpolarization. Since then, L-type-calcium channel opening was reduced and was followed by the intracellular calcium concentration which decreased, responsible to vasodilation (Qian et al., 2017). In this study, results showed that ALF has no significant effect on denuded rings pre-incubated with IBTX indicating that BK_{Ca} has not been involved in ALF endothelium independent vasodilation effect.

Likewise, investigation of ALF effect with the K_{ATP} channels inhibitor (Glibenclamide), showed that this effect was slightly but not significantly enhanced compared to the control. That result suggests that K_{ATP} pathway appears not to be involved in the ALF vasodilation process (Figure 7). The same observation was obtained with the non-selective K^+ channel blocker (TEA) claiming that ALF would induce its effect by preferentially inhibited by the PDE1, 3, and 5 activity than K^+, K_{ATP} and BK_{Ca} channels involved.

Conclusion

The present findings demonstrated that ALF obtained from barks of trunk of *A. leiocarpa* was able to induce endothelium-independent vasodilation effect in denuded porcine coronary arteries rings pre-contracted with U46619. This effect might be due to a possible decrease in $[Ca^{2+}]_i$ and the reduction of the membrane hyperpolarization. In addition, ALF endothelium-independent effect was more due to its capacity to inhibit PDE1, 3 and PDE5 in the VSMC but not the inhibition of the PDE2. Moreover, it also appears that the implication of openned channels (BK_{Ca}, K^+ and K_{ATP}) was too lessier than those of the inhibition of PDEs and the Ca^{2+}-decrease in vessels. Then, the efficacy for acute and chronic usage of ALF needs to be investigated in *in vivo* model.

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CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Rats cholestasis instigated by α-Naphthylisothiocyanate and the impacts of green tea (*Camellia sinensis*) antioxidant

Wael M. Al-Amoudi

Department of Biology, Faculty of Applied Sciences, Umm Al-Qura University, Makkah 21955, Saudi Arabia.

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α-Naphthylisothiocyanate (ANIT) is known to provoke liver damage with intrahepatic cholestasis. This ANIT is accepted to be important for assessing the effect of medicine instigated cholestasis. This assessment investigated the effect of green tea (*Camellia sinensis*) in contrast to that of Vitamin C (VC) against the hepatotoxicity of ANIT. Rats were orally treated with green tea portion (GT50, 75, 100 mg/kg) and CV (250 mg/kg) following 12 h of ANIT mixture (75 mg/kg). Rats were killed 24 h after treatment. Rats treated with ANIT demonstrated hepatocyte damage and cholestasis appeared as changes in serum biomarker levels, among others; increase in entire cholesterol, triglycerides, phospholipids, and lipid peroxide, and furthermore, an extension in hepatic lipid peroxide, a decline in glutathione and myeloperoxidase activity and a decrease in hepatic superoxide dismutase. The utilization of GT (75 mg/kg) to rats treated with ANIT blocked hepatic cell mischief and cholestasis and alleviated these serum and hepatic biochemical changes, while the use of GT (50 or 100 mg/kg) was less impressive. Both VC and ANIT-treated rats kept up a vital separation from liver cell hurt, yet not cholestasis, and reduced serum lipid peroxide, hepatic lipid peroxide, and myeloperoxidase action. These results demonstrate that the GT guarantees ANIT-affected liver harm (Cholestasis in the rat) more satisfactorily than VC.

Key words: Antioxidant, α-Naphthylisothiocyanate, cholestasis, green tea, Vitamin C.

INTRODUCTION

Green tea is the most expended drink and can deter the impact of cancer *in vivo* (Yang et al., 2009; Fujiki et al., 2018) Green tea (*Camellia sinensis*) has cell fortifying and weakening properties. The protective impact was credited to the cell reinforcement limit of the concentrate (Erba et al., 1999; Somia et al., 2017) and likely because of the gift of hydrogen by green tea polyphenols (Anderson et al., 2001). Correspondingly, green tea extricate anticipated H_2O_2-actuated cell demise just like manner bladder malignancy and urothelial cells (Coyle et al., 2008). Polyphenols in green tea are believed to be in charge of the protection impacts of disease. Every day

E-mail: wmamoudi@uqu.edu.sa.

utilization of polyphenols from green tea is high in a few nations (Lorenzo et al., 2016; Fujiki et al., 2018). The phenolic mixes of green tea with the most astounding focus are gallic acid, gallocatechin, catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, p-coumaroylquinic corrosive and gallocatechin-3-gallate (Shishikura and Khokhar, 2005; Kwon et al., 2015; Riley et al., 2018). Green tea likewise contains dense and hydrolyzable tannins (Okuda and Ito, 2011; McAlpine et al., 2016) and has the most astounding centralization of polyphenols contrasted with different teas, including epigallocatechin gallate (EGCG), which might be the reason green tea can prompt apoptotic cell passing in malignant growth than different teas (Lin et al., 2003; Riley et al., 2018; Miyata et al., 2018). Green tea polyphenols have been exhibited against malignant growth action in various investigations that could be intervened by the antioxidant or pro-oxidant system in various rats models of human disease (Davalli et al., 2012; Naponelli et al., 2017; Fujiki et al., 2018). These polyphenols, for example, EGCG hinders cell feasibility and actuate apoptosis in various malignant growth cell lines, for example, osteogenic sarcoma (Ji et al., 2006), lymphoblastoid cells (Noda et al., 2007), leukemia cells (Nakazato et al., 2005), melanoma cells (Nihal et al., 2005), immune system microorganisms (Li et al., 2000), antibladder cancer (Miyata et al., 2018) and laryngeal carcinoma (Lee et al., 2010). Green tea polyphenols have appeared to lessen the separation of malignant growth cells into slower multiplying cells (Zhou et al., 2004). Be that as it may, the ramifications of oxidative or cell antioxidant instruments is vague; it is as yet indistinct whether green tea has a defensive impact against intense liver damage with cholestasis *in vivo* in rats.

α-Naphthylisothiocyanate (ANIT) is a substance usually used to start cholestasis by harming epithelial cells of the bile conduits and causing serious cholangitis and intrahepatic cholestasis (Yan et al., 2017;Wu et al., 2017; Han et al 2018). A one of a kind treatment of rats explored different avenues regarding α-naphthylisothiocyanate (ANIT) which can harm the liver with intrahepatic cholestasis (Plaa and Clerical, 1976; Kossor et al., 1993; Golbar et al., 2017). It is imagined that this hepatic injury with ANIT-initiated cholestasis is valuable for examining the procedures associated with medication instigated cholestasis, since hepatic sores and cholestasis exist on account of the application of certain explicit medications (e.g. Erythromycin estolate, Chlorpromazine, and so forth) to rats and people by emulating the application of ANIT to rats (Kossor et al., 1993; Yan et al., 2017;Han et al.,2018). ANIT instruments with cholestasis have been proposed; however, it has not yet been completely explained. It has been prescribed that lessened hepatic glutathione (GSH) be added to the movement of hepatic inclusion related with ANIT-prompted cholestasis because of its capacity to deliver a reversible ANIT-S conjugate which is basic for the vehicle of ANIT into the bile where it is discharged at high fixations and most likely lethal (Roth and Dahm, 1997; Yan et al., 2017; Wu et al., 2017). Likewise, it has been proposed that neutrophil-intervened aggravation might be added to the enhancement of ANIT-initiated cholestatic liver damage in rats (Roth and Dahm, 1997). Besides, in rats treated once with ANIT, it was affirmed that lipid peroxidation instigated by responsive oxygen species (ROS) made by methods for attacking neutrophils in the liver tissues is engaged with the movement of liver sores with cholestasis (Kongo et al., 1999; Cullen et al., 2016). Moreover, it has appeared in ANIT-treated rats that the intrusion of the hepatic disease hindering action framework adds to enhanced cholestasis-related liver harm (Ohta et al., 2001; Nakamura et al., 2013; Wu et al., 2017).

The destructive ascorbic acid, also called vitamin C (VC), is prominent for applying antioxidant and reducing exercises (Sorice et al., 2014). This investigation demonstrates that green tea extricates control weight actuated liver harm, in any event to a lesser degree, through its cell reinforcement and calming exercises identified with VC (Reddy et al., 2017). Furthermore, VC has been proven to hinder the harm to liver cells (Yang et al., 2018), yet not cholestasis, in rats treated with a solitary ANIT treatment, at any rate to some degree, through its cancer prevention agent and calming exercises (Ohta et al., 2006; Han et al., 2018). In this vein, this investigation inspected the guarded impact of green tea extricate on ANIT-actuated liver damage and cholestasis in rats contrasted with that of VC.

MATERIALS AND METHODS

Chemical

α-Naphthylisothiocyanate (ANIT), 3,3', 5,5'- tetramethylbenzidine (TMB), cow-like serum egg whites, p-coumaric corrosive, RRR-α-tocopherol (α-Toc), superoxide dismutase (Grass) decontaminated from ox-like erythrocytes, yeast glutathione reductase, NADPH, chlorogenic corrosive, epigallocatechin gallate (EGCG), L-ascorbic corrosive, cinnamic corrosive, chrysin, N, N-dimethylformamide (DMF), α'- dipyridyl, 5.5 Dithiobis (2-nitrobenzoic corrosive) (DTNB), ethylenediaminetetraacetic corrosive (EDTA), Folin-Ciocalteu reagent (FCR), gallic corrosive, kaempferol, quercetin, glutathione decrease (GSH), 2-thiobarburic acid, trichloroacetic acid (TCA), Tween 80 and different synthetic concoctions were bought from Sigma (St. Louis, Missouri, USA); α-Toc and delta-tocopherol standard utilized for the assurance of the CV.

Preparation of GT extract and determination its composition

A green tea extricate (GT) was set up by drenching about 30 g of dried green tea leaves homogenized in 100 ml of 95% ethanol at 90°C for 60 min. The cooled blend was sifted through a 0.45 µm millipore nylon channel before the examination. The sifted test was dried at 45°C.

The polyphenol substance of the GT was controlled by the Folin-Ciocalteau colorimetric system referenced by Ahn et al. (2004). The essential constituents of GT were decayed by HPLC as shown by the method portrayed by Friedman et al. (2006). HPLC was performed utilizing a Hitachi display 665-II fluid chromatograph with an autosampler (Demonstrate 655A-40, Hitachi Ltd., Tokyo, Japan). The section was (250 mm x 4.0 mm i.d.). The section temperature of 30°C was kept up utilizing a Shimadzu CTO-10vp segment heater (Shimadzu, Kyoto, Japan). The slope of the versatile stage was a blend of 100% acetonitrile and 20 mM KH_2PO_4. The stream rate was 1 mL/min. The photodiode cluster UV-VIS locator (SPD-10Avp model, Kyoto, Japan) was set somewhere in the range of 200 and 600 nm. The tea extricates (10 µL) was infused into the section. Recognition of every catechins, theaflavins, alkaloid, and artepillin C in the GT extricate were performed at 280 nm and the constituent of every substance was assessed. The constituent of strong parts was assessed at 14.3%. The catechins and the segments of the other two fundamental green tea, caffeine and theanine, are confirmed as just the extricated concentrates of 58, 25 and 12 mg/g of catechins, caffeine, and theanine, referenced by Chang et al. (2000).

Experimental animals and protocol

Six-week-old male rats were acquired from King Fahad Exploration Center, King Abdul Aziz Higher Institution, Jeddah, Saudi Arabia. The rats were housed in temperature-controlled pens (25 ± 2°C) and relative stickiness (52 ± 5%) with 12 h of light. The rats were kept with free access to rats sustenance and faucet water for seven days. All rats got kind consideration as per Umm Al-Qura Higher Institution rules.

The rats were isolated into 6 factions (n = 5 each) as shown below:

i) Control faction: The rats did not get ANIT, GT or VC.
ii) ANIT faction: The rats were dealt with just with ANIT.
iii) ANIT + GT faction (50): The rats were treated with ANIT and managed after GT application at a quantity of 50 mg/kg.
iv) ANIT + GT faction (75): The rats were treated with ANIT and BPEE at a quantity of 75 mg/kg was applied hereafter.
v) ANIT + GT faction (100): The rats were treated with ANIT and along these lines managed by GT at a quantity of 100 mg/kg.
vi) ANIT + VC faction: The rats were treated with ANIT and post-managed with VC. ANIT has been broken down in olive oil. All rats were fasted for 15 h before treatment and got water voluntarily amid the tests. Seven-week-old rats from all factions got an intraperitoneal (I.P.) infusion of ANIT at a portion of 75 mg for each kg of body weight, as portrayed by Kongo et al. (1999), Ohta et al. (2001, 2006). The control faction and alternate factions were treated with a similar volume of olive oil. Following 12 h of starting an infusion of ANIT or olive oil, the rats treated with ANIT were partitioned into 4 factions; Three factions of them were orally attended to, with GT at various quantities (50, 75 and 100 mg/kg) of green tea extricate with ethanol. The last faction of ANIT was treated with VC at a quantity of 250 mg/kg body weight. The quantity of VC utilized in this examination was resolved dependent on information introduced by Kaida et al. (2010). Following 24 h, the treated rats were yielded for histological examination and biochemical investigation.

Sample preparation

24 h after the main infusion of ANIT or vehicle, each rat was gauged and relinquished under ether anesthesia, and the blood was expelled from the mediocre vena cava. The serum was disconnected from the blood gathered by centrifugation. Not long after the killing, every liver was washed with super cold 0.9% NaCl to empty the blood into the tissues, at that point weighed subsequent to being wiped on a channel paper. The liver and serum tests were kept at - 80°C until its utilization.

Serum biomarkers investigation

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were directed utilizing a transaminase II test pack. γ-Glutamyl transpeptidase (γ-GTP) was settled in the serum utilizing the γ-GTP C-Test pack. The bilirubin and bile acid totals were inspected utilizing the Bilirubin BII-Test and Aggregate bili basic analysis units, individually. Serum adds up to cholesterol (T-Chol), while triglycerides and phospholipids were directed by the cholesterol test E test, the triglyceride test G packs, and the phospholipid C test, individually. The packs were obtained from Wako, Richmond, VA, USA. Lipid peroxide (LPO) was assessed using serum fluorometry by the methodology of thiobarbituric acid, as depicted by Yagi (1979). The excitation and transmission wave lengths of this gauge were 515 and 553 nm. The estimation of LPO in serum is communicated as malondialdehyde proportionate (MDA).

Determination of hepatic biomarkers

The liver was gathered and isolated from every trial faction and weighed independently. The heaviness of the liver was assessed by their relative weight (g/100 g B.W). Disconnected hepatic tissue was homogenized in 9 volumes of super cold 50 mM Tris-HCl support (pH 7.4), containing 1 mM EDTA, to set up a 10% homogenate utilizing a Fisherbrand™ Q700 sonicator (Fisher, Hampton, New Hampshire, USA). In the wake of keeping the remaining on the ice for one moment, the homogenate was centrifuged at 4°C (10,000xg) for 20 min. A microdialysis device was utilized to dialyze the supernatant against 100 volumes of a comparative transporter at 4°C for 1 h (Fisher, Hampton, New Hampshire, USA). For estimation of GSH, α-Toc, and LPO, the subsequent liver homogenate was utilized. The DTNB procedure of Sedlak and Lindsay (1968) was utilized to gauge the dimension of GSH in liver homogenate. HPLC was utilized to assess α-Toc in the liver homogenate utilized as an interior standard, as demonstrated by Kamiya (2005). The spectrophotometer was utilized to evaluate LPO in a homogenate utilizing the thiobarbituric acid strategy depicted by Ohkawa et al. (1979). The estimation of liver LPO accounted for is like that of MDA reciprocals. Hepatic SOD movement was settled utilizing a business SOD test unit (Sigma, St. Louis, Missouri, USA). The techniques utilized by Cohen (1970) and Kamata et al. (1994) were utilized to decide catalase (Feline) and Se-glutathione peroxidase (Se-GSH-px), individually. Myeloperoxidase (MPO) was assessed as depicted by Suzuki et al. (1983), where MPO was utilized as a record of neutrophil invasion in tissues. The action of the MPO in the liver tissue supernatant was controlled by brooding of the supernatant at 60°C for 2 h to construct recuperation of MPO in liver tissues as revealed by Schierwagen et al. (1990). MPO movement in the warmed liver tissue test was evaluated by estimating the oxidation of the hydrogen peroxide-subordinate TMB at 37°C. The TMB was decayed to N, N-dimethylformamide. One unit (U) of this compound is described as estimating the catalyst, causing a change of the absorbance of 1.0 every moment at 655 nm. The protein in the supernatant was evaluated utilizing the Protein Test Fast unit (Wako, Richmond, VA, USA). The protein level in the liver tissue

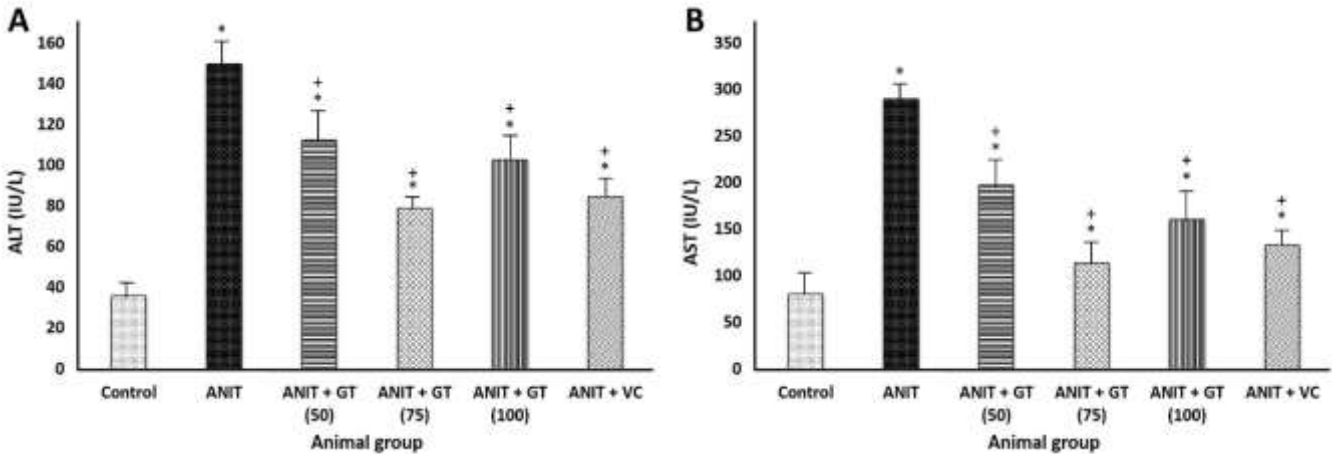

Figure 1. Impacts of GTAE and VCs applied on serum ALT (A) and AST (B) exercises in ANIT-treated rodents. Fasted rodents in the ANIT, ANIT + GTAE (50), ANIT + GTAE (75), ANIT + GTAE (100) and ANIT + VC factions were applied orally with the vehicle (Tween 80-EtOH), 50 mg/kg of GTAE, 75 mg/kg of GTAE, 100 mg/kg of GTAE and 250 mg/kg of VC, individually, 12 h after treatment with ANIT disintegrated in olive oil (75 mg/kg, IP). The fasted rodents in the control faction got olive oil and Tween 80-EtOH as a vehicle just previously and 12 hours after ANIT treatment, individually. ALT and AST in serum were resolved 24 h after ANIT treatment. Each amount is an average Â± SD (n = 5 for the control faction, n = 5 for each faction for every one of the factions treated with ANIT). $*P$<0.05 (contrasted with the control faction); +P <0.05 (versus ANIT faction).

supernatant was resolved and the cow-like serum egg whites were utilized as a benchmark.

Histological study

Rat liver specimens treated with ANIT and GT or VC and untreated control rats were gathered and settled in phosphate cradled saline for 10 h with 10% formalin. The specimen was washed with faucet water, and afterward got dried out in liquor. The tissue was inserted in paraffin and cut into 5-7 µm thick areas. They were mounted on glass slides and recolored with hematoxylin and eosin (H&E). The histological perceptions were inspected under an optical magnifying lens.

Statistical analysis

The outcomes acquired are communicated as mean ± standard deviation (S.D.). The measurable investigation was resolved to utilize StatView statistical programming. Each mean was thought about by one-way analysis of variance (ANOVA). Numerous correlations between factions were then performed utilizing the Tukey-Kramer test, and the distinction of P < 0.05 was viewed as measurably huge.

RESULTS

Effects of GT and VC on liver cell damage and cholestasis

Serum ALT and AST demonstrate hepatocyte harm. In the ANIT faction, γ-GTP activity, a collection of bilirubin and bile acid obsession, and cholestasis were considerably higher than in the control faction (Figures 1 and 2). In the ANIT + GT (50), ANIT + GT (75) and ANIT + GT (100) factions, ANIT-driven serum ALT, AST, and γ-GTP action augmentations were recognized, while ANIT instigated serum delta ALT and AST movement was considerably weakened in the ANIT + VC faction (Figures 1A and 2B). The impact of ANIT + GT (50) on the expansion in ANIT-instigated serum ALT was in a general sense higher than in the ANIT + GT (75) and ANIT + GT (100) factions. The impact of ANIT-incited increments in AST and γ-GTP movement in the ANIT + GT (50) gathered was more articulated than in the ANIT + GT (75) and ANIT + GT (100) factions (Figures 1B and 2A). The impact of ANIT + GT (75) on the compression of expanded ANIT-prompted serum ALT and AST movement was tantamount to that of the ANIT + VC faction (Figure 1). In the ANIT + GT (75) faction, ANIT-prompted increments in serum add up to bilirubin and aggregate bile acid focuses were essentially weakened (Figures 2B and 2C). In the ANIT + GT (75) faction, ANIT-instigated increments in serum total bilirubin and bile acid obsession were basically restricted (Figures 2B and 2C). Be that as it may, in the ANIT + GT (50) faction, the ANIT-prompted increment in serum total bilirubin and aggregate bile acid obsession was not constricted (Figures 2B and 2C). In spite of the fact that the expansion in serum adds up to bilirubin fixation actuated by ANIT which was totally constricted in the ANIT + GT (100) faction, the weakening impact of ANIT + GT (100) totals was basically as low as that of ANIT + GT (75) (P < 0.05) (Figures 2B and 2C). In the ANIT + VC faction, the

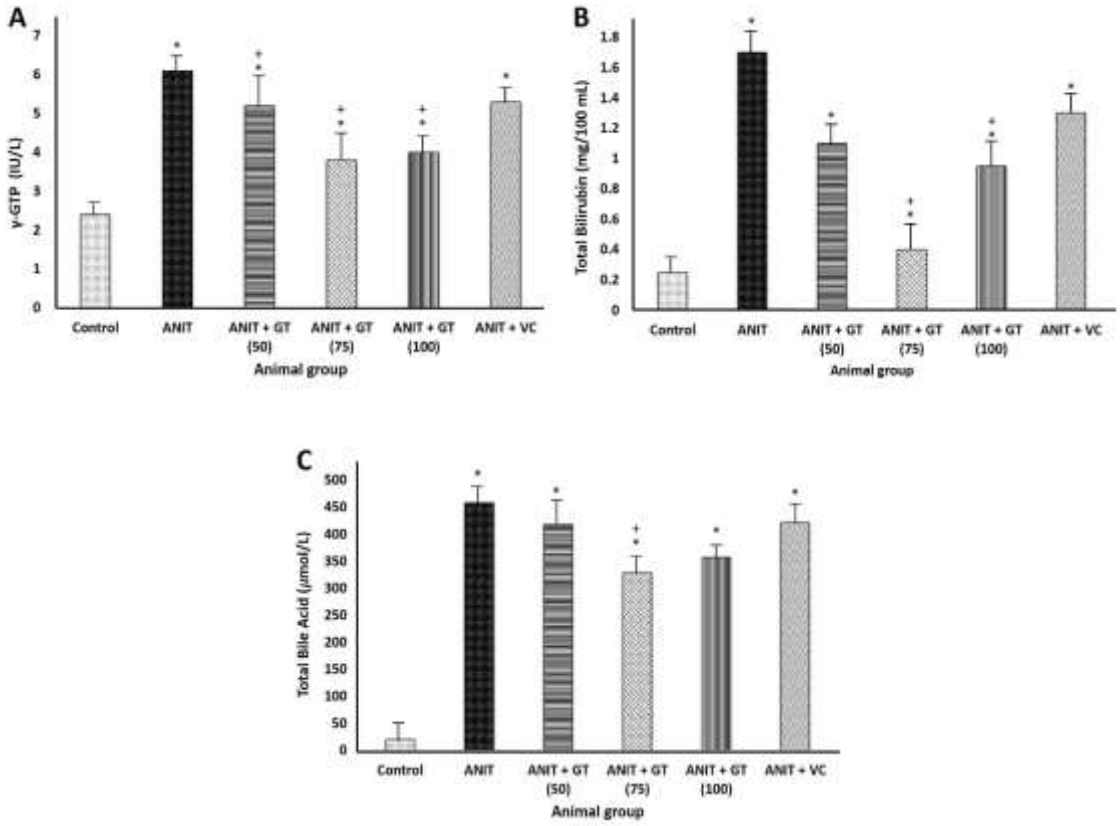

Figure 2. Impacts of applied GTAE and VC on serum Se-GSH-px (A) action and aggregate bilirubin (B) and aggregate bile acid (C) fixations in ANIT-treated rodents. Se-GSH-px, add up to bilirubin, and aggregate bile acid in serum was resolved at 24 h after ANIT treatment. Each amount is a mean ± S.D. (n = 5 for Control faction; n = 5 for every each faction for all factions with ANIT treatment). $*P$ < 0.05 (versus control faction); $+P$ < 0.05 (versus ANIT faction).

ANIT-initiated increment in serum add up to bilirubin and accumulated bile acid obsession was not constrained at all (Figures 2B and 2C).

Effects of GT and VC on live histological changes

In the control faction, the ANIT faction, the ANIT + GT (75) faction, and the ANIT + VC faction, the hematoxylin and eosin recolored liver regions were exposed to histopathological changes, necrosis, and aggravation tests. The control aggregate demonstrated the ordinary histological structure and no progressions were watched. Be that as it may, the faction treated with ANIT indicated huge necrotic and degenerative changes with outrageous provocative cell penetration. The ANIT + GT (75) and ANIT + VC bunches demonstrated a critical decrease in necrotic and degenerative changes with less fiery cell invasion (Figure 3). In addition, the histological changes in the ANIT + GT (75) aggregate was to some degree like the ANIT + VC faction (Figures 3C and 3D).

Effects of GT and VC on serum lipid profile concentrations

Serum T-Chol, triglyceride and phospholipid fixations were fundamentally lifted in the ANIT faction contrasted with the control faction (Figure 4). In the faction treated with ANIT + GT (75), an expansion in ANIT enlistment was seen in serum T-Chol, and triglyceride and phospholipid levels were altogether diminished (Figure 4). Nonetheless, in the ANIT + GT (50) faction, just ANIT enlistment with expanding serum triglyceride focus was essentially weakened. No noteworthy impact on ANIT-prompted serum T-Chol, triglyceride and phospholipid fixations were seen in the ANIT + GT (100) and ANIT + VC factions (Figure 4).

Effects of GT and VC on relative liver weight

The relative liver load of the ANIT faction was essentially higher than that of the control faction (Figure 5). In the

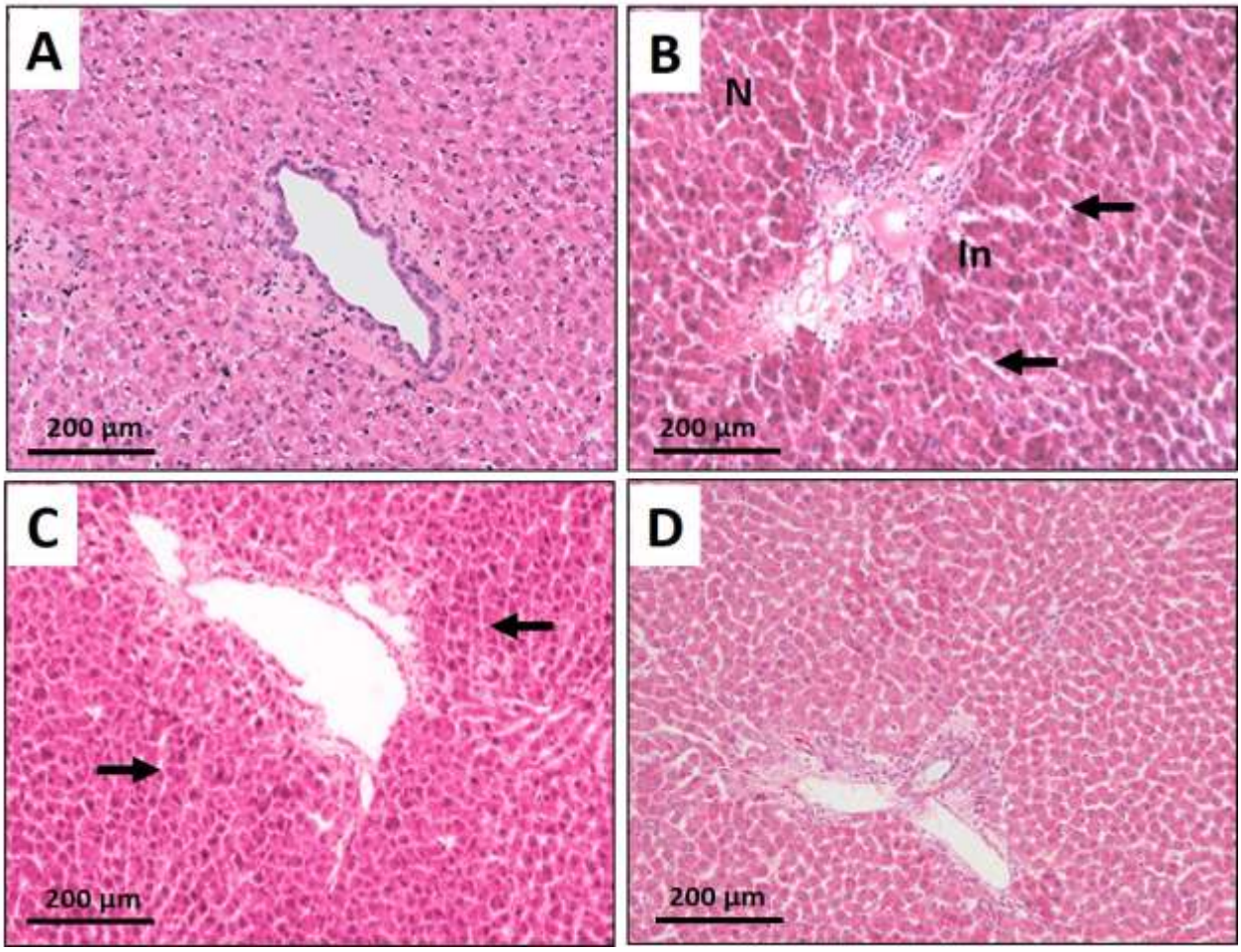

Figure 3. Histological figures of hepatic tissue of untreated control rodents and ANIT-treated rodents with and without GTAE or VC. (A) Control faction: no histological changes were detected. (B) ANIT faction: necrotic (N) and degenerative changes with serious incendiary cell invasion (In) were detected (arrow). (C) ANIT + GTAE faction (75): There was an emotional decline in necrotic and degenerative changes and incendiary cell penetration (arrow). (D) The ANIT + VC faction demonstrates a major decline in necrotic and degenerative changes and incendiary cell invasion (H and E, starting amplification Ã— 100).

ANIT GT (75) faction, ANIT initiated a critical decline in relative liver weight, while in ANIT GT (50), ANIT GT (100) no huge impact was seen on the relative liver weight gain and the ANIT VC faction (Figure 5).

Hepatic LPO concentrations and serum affected by GT and VC

Serum and liver LPO focuses were altogether higher in the ANIT faction than in the control rats (Figure 6). In the ANIT GT (75), ANIT GT (100) and ANIT VC factions, serum ANIT-prompted increments, and liver LPO focuses were essentially weakened, yet no critical impacts were found in the ANIT GT (50) faction (Figure 6). The decrease in serum and liver LPO focuses in the ANIT GT (75) faction was like that in the ANIT VC faction, however, essentially higher than in the ANIT GT (100) faction ($P < 0.05$) (Figure 6).

Hepatic antioxidant enzyme systems affected by GT and VC

In spite of the fact that there was no noteworthy contrast in liver CAT and Se-GSH-px movement between the two factions, the liver SOD action of the ANIT faction was considerably lower than that of the control faction (Figure 7). In the ANIT GT (50), ANIT GT (75), ANIT GT (100) and ANIT VC factions, an ANIT-instigated decrease in

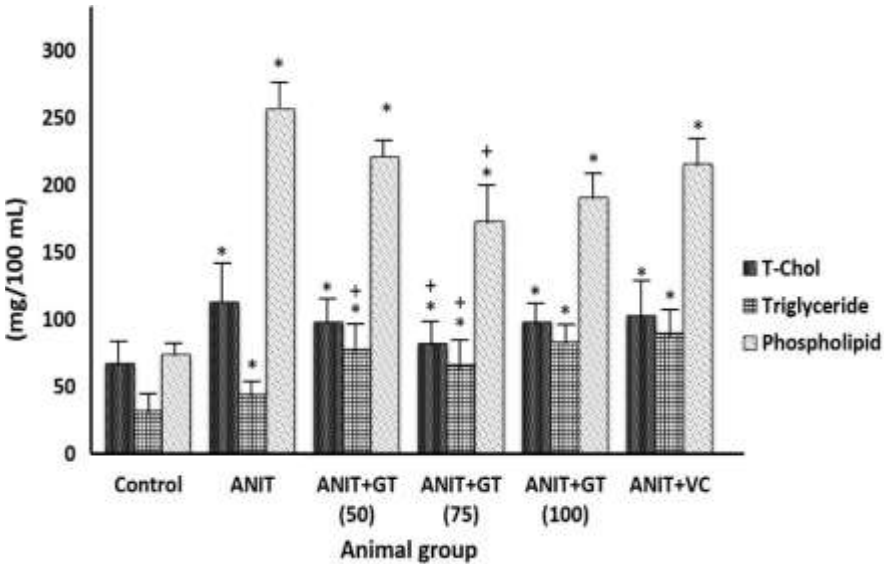

Figure 4. Impacts of the BPWs and VCs applied on serum dimensions of T-Chol, triglycerides, and phospholipids in ANIT-treated rodents. T-Chol, triglycerides, and phospholipids in the serum were measured 24 hours after ANIT treatment. Each amount is an average Â± SD (n = 5 for the control faction, n = 5 for each faction for every one of the factions treated with ANIT). $*P$ <0.05 (contrasted with the control faction); +P <0.05 (versus ANIT faction).

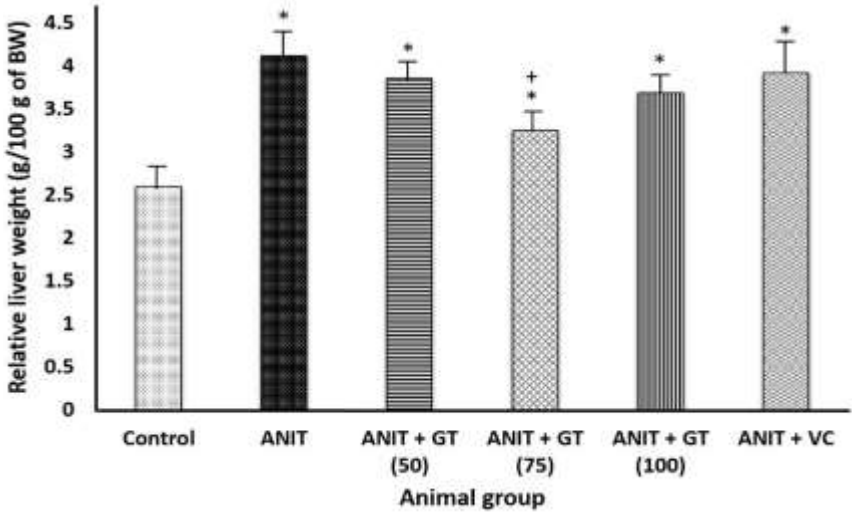

Figure 5. Impacts of controlled WGE and VC on liver load in ANIT-treated rodents. The liver load of each rodent was assessed utilizing its relative weight (g/100 g body weight) 24 h after ANIT treatment. Each amount is an average Â± SD (n = 5 for the control faction, n = 5 for each faction for every one of the factions treated with ANIT). $*P$<0.05 (contrasted with the control faction); +P<0.05 (versus ANIT faction).

liver SOD action was fundamentally lessened, despite the fact that the ANIT GT (75) aggregate exhibited this. The best constriction was found in the three ANIT GTAW factions (Figure 7A). Besides, the liver SOD movement of the ANIT GT (75) or ANIT GT (100) faction did not vary from the liver SOD action of the control faction (Figure

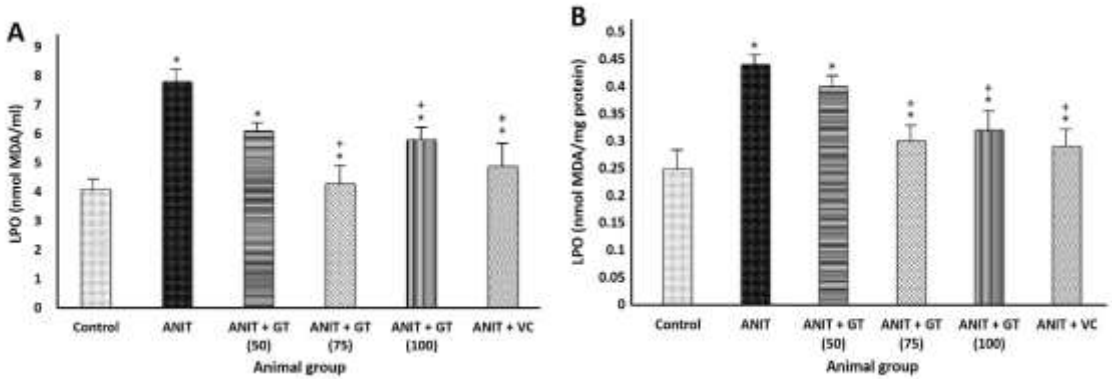

Figure 6. Impacts of the BPWs and VCs regulated on the LPO fixations in the serum (A) and liver (B) of rodents treated with ANIT. LPO in serum and liver tissues was tried 24 hours after ANIT treatment. Each amount is an average Â± SD (n = 5 for the control faction, n = 5 for each faction treated with ANIT). $*P$ <0.05 (contrasted with the control faction); $+P$ <0.05 (versus ANIT faction).

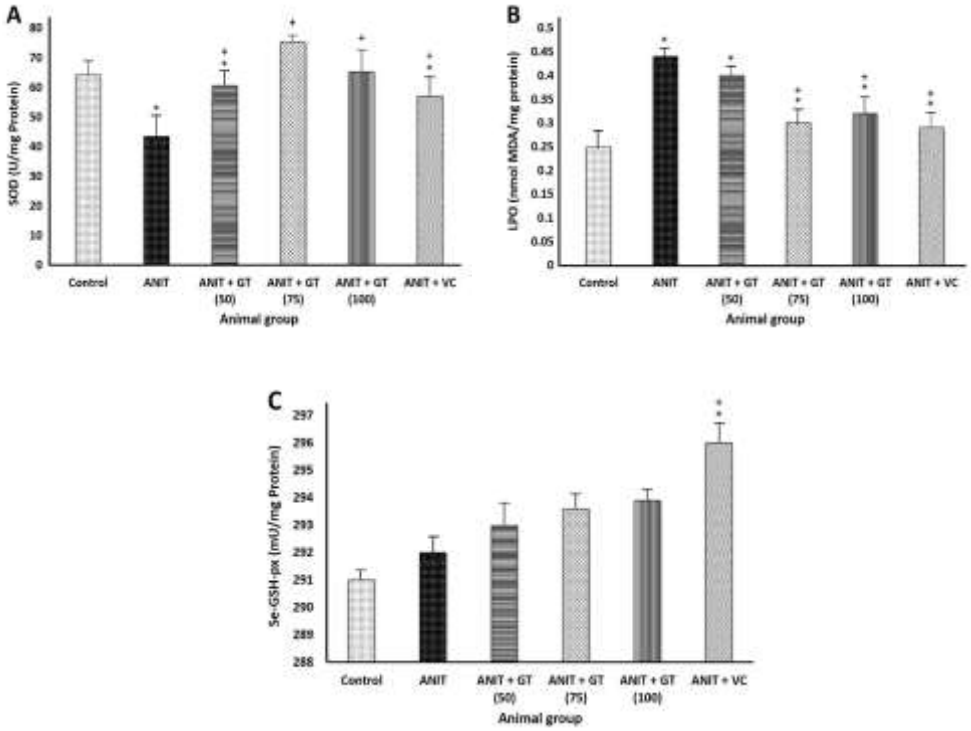

Figure 7. Impacts of the BPWs and VCs applied on liver exercises SOD (A), catalase (B) and Se-GSH-px (C) in ANIT-treated rodents. SOD, catalase, and Se-GSH-px in liver tissues were evaluated 24 hours after ANIT treatment. Each amount is an average Â± SD (n = 5 for the control faction, n = 5 for each faction for every one of the factions treated with ANIT). $*P$ <0.05 (contrasted with the control faction); $+P$< 0.05 (versus ANIT faction).

7A). The ANIT GT (50), ANIT GT (75) and ANIT VC factions had no noteworthy impact on liver catalase action; however, the compound action of the ANIT GT (100) faction was essentially decreased (Figure 7B). The ANIT GT (50), ANIT GT (75) or ANIT GT (100) factions had no huge impact on liver Se-GSH-px movement, yet the ANIT VC amass had a noteworthy increment in this action (Figure 7C).

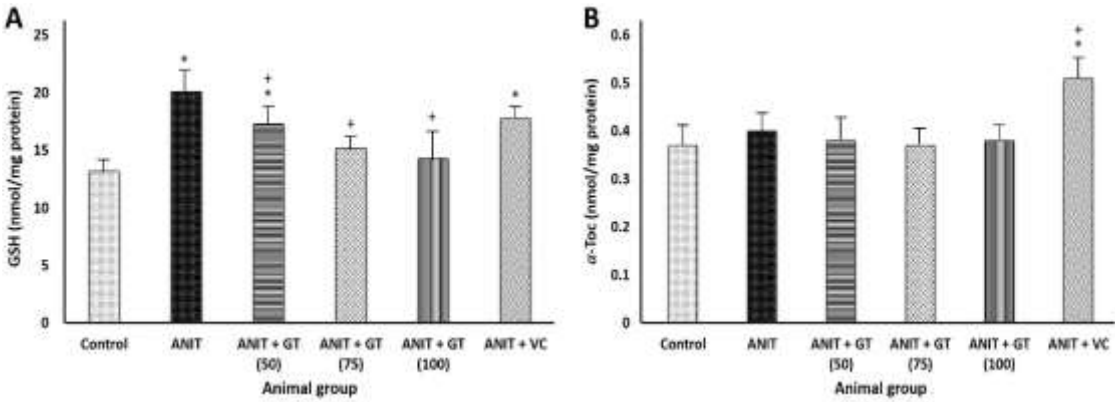

Figure 8. Impacts of applied GTAE and VC on hepatic GSH (A) and α -Toc (B) fixations in ANIT-treated rodents. GSH and α -Toc in liver tissues were evaluated at 24 h after ANIT treatment. Each amount is a mean ± S.D. (n = 5 for Control faction; n = 5 for each faction for all gatherings with ANIT treatment). * P < 0.05 (versus control faction); + P < 0.05 (versus ANIT faction).

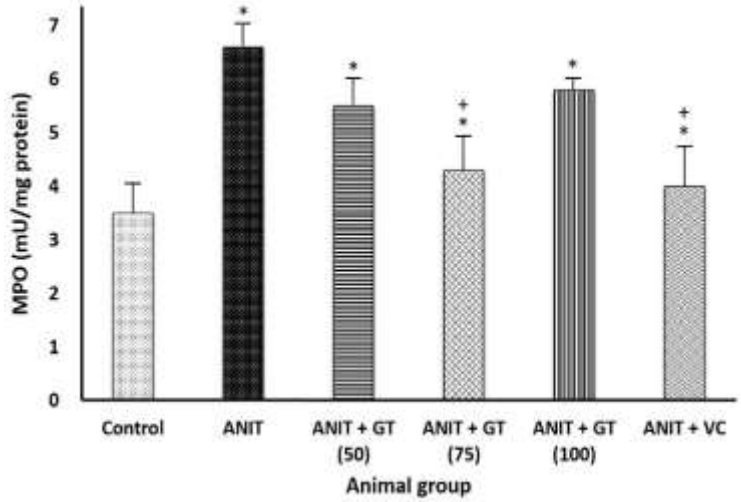

Figure 9. Impacts of applied GTAE and VC on hepatic MPO action in ANIT-treated rodents. MPO in liver tissues was resolved at 24 h after ANIT treatment. Each amount is a mean ± S.D. (n = 5 for the control faction; n = 5 for each faction with ANIT treatment). * P < 0.05(versus Control faction); + P < 0.05 (versus ANIT faction).

Hepatic antioxidant concentrations affected by GT and VC

The glutathione fixation in the ANIT faction was considerably higher than that in the control rats, in any case, there was no huge contrast in liver α-Toc focus (Figure 8). In the ANIT GT (50), ANIT GT (75) or ANIT GT (100) factions, ANIT-prompted increments in liver GSH fixations were generously diminished, while the ANIT VC mass had no critical impact on liver GSH focuses (Figure 8A). The ANIT GT (50), ANIT GT (75) and ANIT GT (100) factions had no noteworthy impact on liver α-Toc fixations; however, the liver α-Toc focuses were essentially and altogether expanded in the ANIT VC faction (Figure 8B).

Effects of GT and VC on neutrophil infiltration

Liver MPO action was essentially higher in the ANIT faction than in the control faction (Figure 9). In the ANIT GT (75) and ANIT VC factions, ANIT-actuated increments

in the liver MPO movement were fundamentally weakened, albeit neither the ANIT GT (50) faction nor the ANIT GT (100) aggregate had any critical impact on liver MPO action (Figure 9). The decrease in the ANIT-initiated increment in hepatic MPO action was essentially less in the ANIT GT (75) faction than in the ANIT VC faction ($P < 0.05$) (Figure 9). Liver MPO impacts are a lot higher in ANIT totals than in the control faction (Figure 9). In the ANIT GT (75) and ANIT VC faction, the expansion in ANIT preparation in liver MPO practice was totally constricted, in spite of the fact that the ANIT GT (50) total and the ANIT GT (100) mass did not have any perceptible impact on the extension of liver MPO. (Figure 9). In the ANIT GT (75) polymerization, the impact of ANIT-instigated increment in liver MPO action was commonly not as much as that of ANIT VC total ($P < 0.05$) (Figure 9).

DISCUSSION

In the GT, epigallocatechin gallate (EGCG), quercetin (Qu), caffeine (Cf), theanine, theobromine, and catechin (C) are available in the investigation. The water extraction planning of green tea (*C. sinensis*) was affirmed by HPLC examination, and the substance of (EGCG) and catechin (C) were the most noteworthy among the substance of the six parts examined. This outcome is predictable with past reports (Kravchenko et al., 2011; Lan-Sook et al., 2014; Lorenzo and Munekata, 2016; Fujiki et al., 2018).).

It has been reported that rats treated with ANIT (75 mg/kg, IP) have liver damage and cholestasis, as estimated by serum ALT and AST levels, hepatocyte damage file, γ-GTP, add up to bilirubin and aggregate bile acid, files of biliary cell harm and cholestasis at 24 h, yet not at 12 h of treatment (Ohta et al., 2006; Nakamura et al., 2013). In the present investigation, ANIT-treated rats were given a solitary oral portion of GT or VC 12 h after ANIT treatment, as this deferred application of GT or VC was viewed as valuable for giving the impact of the concentrate or close clinical conditions. On account of vitamins, there is cholestasis of ANIT-actuated liver harm. Changes in hepatocyte harm and serum ALT and AST action were diminished after the application of GT at a portion of 50, 75 or 100 mg/kg 12 h after ANIT treatment. Subsequently, GT can be utilized as a cell reinforcement against ANIT-initiated hepatocyte harm in rats. Be that as it may, the defensive impact of GT was higher at its portion of 75 mg/kg than at the portion of 50 or 100 mg/kg, demonstrating that the defensive impact of GT on ANIT-incited hepatocyte damage was lessened at 100 mg/kg. Rats regulated with ANIT at a rate of 12 h after treatment (250 mg/kg) diminished the expansion in serum ALT and AST action, and these impacts were like GT (75 mg/kg). This outcome is like the outcomes announced by Nakamura et al. (2013) who detailed an expansion in ALT and AST levels in VC after treatment with ANIT. The consequences of the present investigation demonstrate that ANIT causes an expansion in serum T-Chol, triglyceride and phospholipid focus 24 h after treatment, and a huge increment in serum phospholipid fixation in most treated rats, an outcome like that obtained by Yoshizumi et al. 2005**;** Wu et al., 2018).

Liver LPO has appeared to add to the advancement of liver harm caused by cholestasis in rats treated once with ANIT. Also, it was reported that rat treated with ANIT (75 mg/kg, IP) demonstrated a huge increment in liver and serum LPO fixations following 24 h of treatment, despite the fact that there was a huge increment in LPO focus in the liver, yet no critical increment in the liver. Serum of ANIT-treated rats was found to have occurred 12 h after treatment (Zimmerman et al., 1997; Guangjun et al., 2002; Silvia et al., 2004; Ohta et al., 2006; Rahal et al., 2014). In the present examination, a solitary oral portion of GT (75 or 100 mg/kg) regulated to ANIT following 12 h of treatment brought about a noteworthy decline in liver and serum LPO fixations, notwithstanding that the GT portion which was 75 to 100 mg/kg was increasingly viable. As detailed by Kaida et al. (2010), controlled VC (250 mg/kg) caused a huge decline in liver and serum LPO focuses 24 h after ANIT treatment.

It has been shown in rats treated with ANIT (75 mg/kg) that liver SOD action diminished following 24 h, and otherwise without following 12 h of treatment, while liver CAT and Se-GSH-px movement expanded at 12 h in spite of an expansion. CAT and Se-GSH-px movement came back to the dimension of untreated control rats following 24 h (Ohta et al., 1999, 2001;Wu et al., 2018). In the present examination, a solitary oral application of GT (50, 75 or 100 mg/kg) for ANIT treatment 12 h after ANIT treatment brought about a critical decline in liver SOD movement found following 24 h, however with GT (75 mg/kg). The most astounding impact was accomplished and the decrease in hepatic SOD action was completely reestablished to the dimension of untreated control rats. The regulated GT (50 or 75 mg/kg) had no impact on liver CAT movement discovered 24 h after ANIT treatment, whereas the application of GT (100 mg/kg) caused a huge decline in liver CAT action. All dosages of GT had no impact on liver Se-GSH-px movement discovered 24 h after ANIT treatment. Interestingly, the application of VC (250 mg/kg) to ANIT-treated rats did not influence liver CAT movement, but rather caused a huge increment in liver Se-GSH-px action, despite the fact that application of VC somewhat lessened ANIT-incited liver decrease of SOD exercises. In this manner, the oral application of GT was found to enhance the SOD related disturbed hepatic enzymatic cancer prevention agent barrier framework in ANIT-treated rats.

Rats treated once with ANIT (75 mg/kg, IP) had an

expanded liver GSH focus 24 h after treatment, and liver α-Toc fixations did not change at 12 and 24 h (Ohta et al., 1999, 2001, 2006). Moreover, the liver GSH grouping of the ANIT-treated rats given GT (75 or 100 mg/kg) did not contrast from the untreated control rats. It has been recommended that liver GSH applies a causal or lenient impact in ANIT-incited rat cholestasis liver damage by shaping a reversible GSH conjugate of ANIT in hepatocytes and transporting the GSH conjugate of ANIT into bile. Separation of free ANIT and GSH was done according to Roth and Dahm (1997). As per Jean et al. (1995), an expansion in bile GSH and ANIT focuses occurred before an increment in liver GSH fixation in rats orally treated with ANIT (100 mg/kg). In this way, GT directed to ANIT-treated rats can possibly improve the discharge of GSH from liver tissue into the circulatory system, bringing about a decline in the expansion in GSH fixation in liver tissue (Basu et al., 2013).

Green tea is a drink that is famous everywhere throughout the world and has numerous pharmacological impacts, for example, anti-proliferative, anti-cancer impacts, anti-mutagenic, and is increasingly imperative for this investigation. It is known to apply a mitigating activity by hindering neutrophil penetration and the creation of ROS in actuated neutrophils (Kwon et al., 2015; Sharifzadeha et al., 2017; Torello et al., 2018; Miyata et al., 2018). It has been reported that the polyphenol segment in green tea extricates, along these lines, has a few times higher cancer prevention agent movement than vitamin C and E (Rice-evans et al., 1995; Sharifzadeha et al., 2017). Vitamin C has anticancer action in different tumor cell lines. Be that as it may, its particular system of activity stays obscure. Nonetheless, VC is known to go about as a mitigating specialist by restraining the generation of ROS in actuated neutrophils and neutrophil penetration (Alpekin et al., 1998**;** Bozonet et al., 2015). Penetrating neutrophils in rat liver tissue treated with ANIT have been shown to assume a key job in the improvement of ANIT-instigated cholestatic liver damage (Roth and Dahm, 1997; Kongo et al., 1999). Kongo et al. (1999) and Ohta et al. (2006) have demonstrated that neutrophil penetration in rat liver tissue treated with ANIT (75 mg/kg) is expanded 12 h after treatment, such as before the rise.

Cholestatic causes liver harm and increments further following 24 h. In the present examination, a solitary oral application of ANIT-treated rat GT (75 mg/kg) at 12 h after treatment brought about a critical decline in liver MPO movement, which is a pointer of tissue neutrophil penetration (Krawisz et al., 1984). In spite of the fact that the equivalent regulated GT (50 or 100 mg/kg) had no huge impact on the expansion in liver MPO movement, it was found at 24 h (Mullane et al., 1985). As recently detailed by Kaida et al. (2010), regulated VC (250 mg/kg) likewise created a huge lessening of the expansion in liver MPO movement, in spite of the fact that the controlled VC was more powerful than the GT applied at a quantity of 75 mg/kg. These outcomes are in great concurrence with the histological perceptions of the above hepatocytes. In this manner, the oral application of GT (75 mg/kg) was found to repress neutrophil invasion into the liver tissue of ANIT-treated rats, as on account of oral VC. Initiated neutrophils have been shown to intervene in lipid peroxidation by the generation of ROS by NADPH oxidase in cells (Zimmerman et al., 1997). It has additionally been demonstrated that MPO intercedes on lipid peroxidation within the sight of hydrogen peroxide and halide particles (Stelmaszynska et al., 1992; Han et al., 2018). Subsequently, these discoveries enable us to bring up that osmotic neutrophils in rat liver tissue treated with ANIT by the oral application of GT may work against oxidative harm related to intemperate ROS creation, potentially through mitigating within the sight of the oral VC impact. Orally controlled GT may add to its defensive impact against oxidative harm caused by neutrophils invaded in ANIT-treated rat liver by repressing the movement of MPO in penetrating neutrophils. During this investigation, GT (75 mg/kg) directed to ANIT-treated rats were shown to restrain neutrophil invasion, enabling it to deliver H_2O_2 into liver tissue by means of enacted NADPH oxidase, steady with different examinations (Ohta et al., 2001; Okado and Fridovich, 2001; Gottfredsen et al., 2013; Forester and Lambert, 2011).

Epigallocatechin gallate (EGCG) is available as a noteworthy segment of GT utilized in this examination. EGCG has been responsible for application of cancer prevention agent impacts by searching ROS and hindering LPO (Senthil et al., 2008; Tachibana, 2009; Lambert and Elias, 2010; Fujiki et al., 2018). Along these lines, it was recommended that the lessening impact of GT application in the expansion of LPO fixation and SOD movement in ANIT-treated rat liver might be essential because of the oxidative activity of EGCG present in the concentrate.

Conclusion

The results shown in this study indicated that a single oral administration of GT to rats treated once with ANIT before the onset of apparent liver damage with cholestasis protects against liver damage with cholestasis, although this protective effect of GT diminishes at its high dose. The present results also suggested that the protective effect of GT against ANIT-induced liver damage with cholestasis could be due to the antioxidant, anti-inflammatory, anti-hyperlipemic, and anti-hypertrophic actions of the extract. The orally administered GT (75 mg/kg) was found to be more effective in protecting against ANIT-induced liver damage with cholestasis than the similarly administered VC (250 mg/kg). However, further investigation is needed to clarify the exact mechanism underlining the protective effect of

GT against liver damage with cholestasis in rats treated with ANIT.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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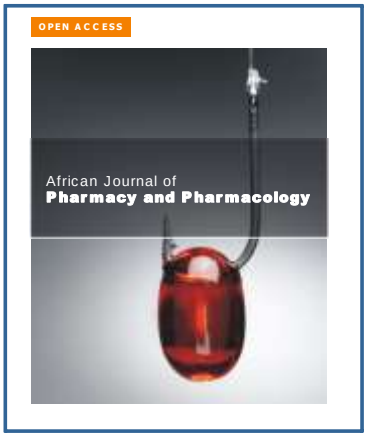

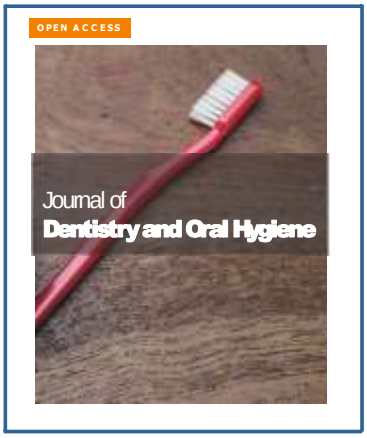

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