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Protective effect of neem leaf extract and dexamethasone in cigarette-smoke induced liver and kidney injury in Swiss mice: A comparative analysis

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Abstract

Cigarette smoke (CS) is one of the leading causes of lung injury where co-morbidity in different organs including liver and kidney can be observed. CS contributes as one of the causes of mortality across the globe. Dexamethasone, a corticosteroid is widely used as an anti-inflammatory medication despite being associated with several side effects with long-term usage. Therefore, the present study focused on the comparison between dexamethasone and *Azadirachta indica* leaf extract (AILE) for treating hepatic and renal injury caused by CS in mice. The phytochemical screening of AILE revealed the abundance of phytocompounds as sterols, proteins, alkaloids, flavonoids and diterpenes that might have antioxidant and anti-inflammatory activity. In this context, Swiss mice were treated with CS, dexamethasone and AILE. CS-induced mice expressed elevated inflammatory cells, Reactive Oxygen Species (ROS) with higher levels of both liver and kidney function markers: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and creatinine respectively which was reversed by AILE. No mortality was observed in CS or AILE-treated group while dexamethasone-treated mice exclusively resulted in 20% mortality. Moreover, CS-induced mice also exhibited declined SOD levels in the liver and kidney and higher Eosinophil peroxidase (EPO) which was modulated by AILE treatment. This is the first report demonstrating *in vivo* the effects of *A. indica* ethanolic leaf extract in treating CS-induced hepatic and renal chronic inflammation in an animal model which gave better results when compared to dexamethasone.

Keywords: *Azadirachta indica*; cigarette smoke; dexamethasone; kidney; liver; mice

Introduction

Inflammation is a primary defensive response expressed by living cells against noxious intracellular and extracellular agents. Every so often, these cellular responses continue to remain constant for a long, developing into chronic diseases which require potent pharmacological treatments (Demir, 2020). However, these

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treatments may have adverse effects and therefore alternative natural therapies are acknowledged to cure long-term illness. Various herbs and plants are persistently in use since the Vedic era for the treatment of severe diseases including blood and urine infections, myocardial infarction, kidney failure, lung diseases, and many more (Sen *et al*., 2017).

Although COPD is reported all these years to affect only lungs, the complexity of the disease is much higher and heterogeneous. It has intra- and extra-pulmonary components and shows variability among patients. So, it is likely that the systemic circulation of inflammatory reaction has numerous extra-pulmonary effects i.e., the so-called systemic effect of COPD.

Several reasons are beholding the cause of liver and kidney damage such as asthma, diabetes, alcohol consumption, and chronic obstructive diseases (Yong *et al*., 2020). Smoking causes architectural variations leading to the malfunctioning of the organ by increasing oxidative stress, and chronic inflammation in tissues and enhances the production of stress kinases, inflammatory cytokines, and lipid peroxidation which ultimately injures liver and also kidney tissues. Cigarette smoke (CS) alters the activity of NF-kB, a redox-sensitive transcription factor, which plays a significant role in oxidation regulation and inflammation. It modifies the NF-kB expression by increasing the expression of other regulatory genes in non-parenchymal hepatocytes and also renal cells. Moreover, cigarette smoking causes oxidative injury by reducing superoxide dismutase (SOD) activity in addition to elevation in eosinophil peroxidase and serum aminotransferases activity (Ponist *et al*., 2019). Under these circumstances, it becomes obligatory to diagnose liver and kidney damage which is facilitated by the examination of aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels (liver injury markers) and creatinine level (kidney injury marker) since RBCs are rich in AST that leaks into plasma before hemolysis (Raja *et al*., 2011). Dexamethasone is a commercial anti-inflammatory corticosteroid widely used for the treatment of chronic obstructive pulmonary disease (COPD), but despite that its long-term use or high doses has been reported to cause various harmful side effects including insulin resistance, hyperglycemia and weight change (Malkawi *et al*., 2018). As a consequence of this, there is a requirement of substitutes that could potentially be effective against aforesaid diseases with limited side- effects. Several plants have been exploited extensively in traditional culture (Ayurveda) which are of medicinal value and have been used to treat several diseases with the least side-effects (Balunas *et al*., 2005). *Azadirachta indica* (neem) is native to the Indian sub-continent and has been used widely for centuries as medicine to cure several diseases because of its antioxidant, antimicrobial, anti-inflammatory, anti-gastric ulcer, antipyretic, hypoglycemics and anti-tumour activities (Eid *et al*., 2017).

There are several bioactive compounds present in leaf extract of neem such as tannins, sterols, alkaloids, flavonoids, diterpenes, and proteins (Benisheikh *et al*., 2019). Research has shown that *A. indica* extract has safe nature that set the basis for its use over thousands of years. Flavonoids are reported to have antioxidant and free radical scavenging activity (Kanagasanthosh *et al*., 2015) and about this, it is assumed that the phytochemicals present in neem leaf extract probably are responsible for its potential anti-inflammatory activity. This study was designed to examine the effect of ethanolic leaf extract of neem in comparison to dexamethasone against renal and hepato-injury caused by cigarette smoke in mice.

Materials and Methods

Plant materials and animals

The 200 g fresh leaves of *A. indica* were collected from the BHU campus and then identified by Prof. NK Dubey (with a voucher number of Melia. 2019/1), Department of Botany, Institute of Science, Banaras Hindu University, Varanasi. During animal experiments animal ethical no. was issued by the institution i.e., "Dean/2015/CAEC/1410".

Preparation of AILE

Plant leaves of 200 gm initially were grind using mortar-pestle and subjected to alcoholic extraction by Soxhlet apparatus. Five (5) gm of dried powdered leaves were taken in a cotton cloth and placed into a flat-bottom beaker of the Soxhlet containing 50 ml of 50% (v/v) ethanol and kept overnight. Ethanolic extract so prepared was then transferred into Petri plates followed by their incubation at 37 °C for 7-8 hours (Figure 1).

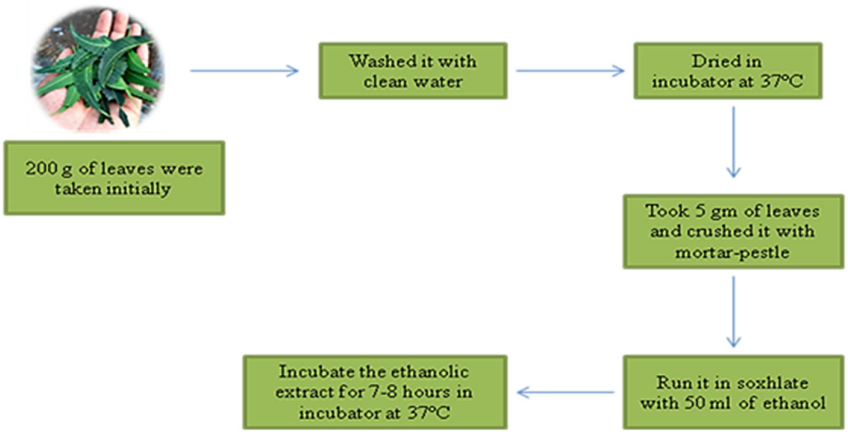

Figure 1. Flow-chart showing the complete procedure of Neem-leaf extract preparation

Phytochemical screenings

Test for tannins, saponins and sterols

Two (2) ml of AILE was taken to which 2 ml distilled water was added followed by the introduction of a few drops of Ferric chloride to confirm the presence of tannins. Green precipitates showed the presence of tannins (Sivakumar and Gajalakshmi, 2013). Distilled water and neem leaf extract were added into the test tube in a 1:1 ratio and shaken vigorously. The persistence of foam indicated the presence of saponins (Ijoma *et al*., 2017). One (1) mg of crude neem leaf extract was dissolved into 10 ml of chloroform followed by the addition of an equal volume of conc. sulfuric acid through the wall of the test tube. The upper layer turned red while the lower layer appeared yellowish with green fluorescence (Saklani *et al*., 2012).

Test for detection of proteins, phenols and alkaloids

Concentrated Nitric acid (HNO_3) was added to AILE. The appearance of yellow colour confirmed the presence of proteins (Sati and Kumar, 2015). A few drops of ferric chloride ($FeCl_3$) were added to the extract. The formation of bluish-black precipitates showed the presence of phenols (Ijoma *et al*., 2017). The extract was dissolved into diluted HCl. Subsequently, the mixture was filtered and later few drops of Wagner's reagent were added. Reddish-brown precipitates at the bottom of the test tube indicated the presence of alkaloids (De *et al.,* 2010).

Test for flavonoids and carbohydrates

A few drops of NaOH solution were added to a few ml of AILE followed by the addition of diluted acid. The intense yellow colour was observed with the addition of NaOH which turned colourless with the addition of diluted acid (Mohammed, 2019). A 5 ml of distilled water was added to AILE and the mixture was then subjected to filtration through Whatman filter paper No. 1. Benedict's solution was added to the filtrate so obtained. Orange-red precipitates gave positive results (Nadhiya *et al*., 2019).

Test for diterpenes and triterpenes

Neem leaf extract was added with water and a few drops of freshly prepared copper acetate solution. Emerald green precipitates at the bottom of the test tube confirmed the presence of diterpenes (Shahverdi *et al*., 2019). Chloroform was added to the AILE and then filtered. Concentrated sulphuric acid was added to the filtrate. Golden-yellow precipitates appeared in the test tube as per the method (Kumar *et al*., 2015).

Experimental animal

Swiss mice (6-8 weeks old, 18-28 gms) of either sex were acclimatized for a week in a clean animal house under standard conditions of temperature (24 ± 2 °C), light (photocycle of 12 h light and 12 h dark) and relative humidity (60 to 70%) in polypropylene cages, with rice autoclaved husk as the bedding material. Animals were maintained on pellet food containing crude protein, crude fibre, calcium, phosphorus, nitrogen-free extract enriched with stabilized vitamins A, C, D3, B12, thiamine, riboflavin, pantothenic acid, choline chloride, folic acid, minerals, and trace elements. Water was available ad libitium. Swiss mice were procured from the Zoology department and then identified with a reference number of Dean/2015/CAEC/1410 by Central Animal Ethical Committee, Banaras Hindu University, Varanasi, India.

Animal group

Swiss mice (18-28 g) of either sex were randomly divided into 5 groups with 8 mice per group (Table 1). The mice were housed in a plastic cage at room temperature (25 ± 2 °C) for the experiment. Mice were randomly divided into five groups: 1-Control, 2-CS induced, 3-Dexamethasone induced (standard drug), 4-AILE 100 and the last one was 5-AILE 400.

Administration of herbal drug and dexamethasone

The stock solution of dexamethasone was prepared by dissolving 10 mg in 1 ml of distilled water of which 250 ml was used as a working solution. The ethanolic neem leaf extract was used in two different concentrations which were 100 mg/kg body weight and 400 mg/kg BW in two mice groups respectively as shown in Table 1.

Table 1. Grouping of animal model

Group A	Control
Group B	Smoke-induced
Group C	Dexamethasone-induced smoker
Group D	Neem extract (100 mg/kg BW) induced smoker
Group E	Neem extract (400 mg/kg BW) induced smoker

Smoke exposure to develop COPD

Mice were placed in a smoke chamber (a rectangular box) with a partition wall dividing it into two halves, containing 16 holes in it. A cigarette puff plugged into a motor was adjusted on either side of the box for its ignition whose smoke was allowed to pass through these small holes towards the other side of the wall where mice were placed. CS exposure was continued for 5-7 minutes after the drug administration which was given 45 minutes before the CS exposure. This treatment was given thrice a week with an interval of two days and continued for 7 weeks.

Administration of drug into the experimental animal

Drugs from the working solution were administered to mice intraperitoneally 45 minutes before cigarette smoke exposure. This was repeated thrice a week for 7 weeks with an interval of two days.

Analysis of bodyweight and mortality rate of mice

The body weight of mice was noted every week to observe gain/reduction due to the administration of dexamethasone, neem extract, and cigarette smoke. It was observed from the first to the last day of the experiment. The differences in body weights of mice were calculated before sacrificing them. The mortality rate was observed during the whole experiment.

Sample collection

After the completion of experiments, mice were sacrificed by cervical dislocation and blood serum was collected from retro-orbital bleeding. Out of all the vitals, a lobe of the liver and kidney were taken for histopathology and another one is kept for homogenization and further process.

Estimation of eosinophil peroxidase (EPO) in liver and kidney

Mice tissue (100 mg) was homogenized (1:1) into 1.9 ml PBS followed by its centrifugation at 12000 × g for 10 minutes. The supernatant was taken for enzymatic assay and an equal amount of substrate (0.075 mmol/L Tris- HCl pH 8, 1.5 mmol/L O- phenylenediamine, and 6.6 mmol/L H_2O_2 in) was added. The further reaction was stopped with the addition of 50 µL of 1 mol/L H_2SO_4, and absorbance was recorded at 490 nm (Adamko *et al*., 2004).

Histopathology of liver and kidney

The histopathology of the liver and kidney was performed as per the previous protocol (Ragavan *et al*., 2006). Tissue from the liver and kidney was dissected and placed into 10% NBF (Neutral Buffered Formalin) followed by its dehydration using different grades of alcohol and further the blocks were prepared. Thin sections of both the liver and kidney were cut by microtome and further the slides were stained by H & E. The stained slides were focused and observed under a microscope for analyzing architectural changes.

Total protein content in liver and kidney tissue

Total protein concentrations were measured as mg/ml in serum by Folin's assay (Lowry's method) as per the previously defined procedure (Lowry *et al*., 1951). Reagents were added to samples and observed using UV-spectrophotometry at 650 nm.

Liver function test by quantifying AST and ALT in serum

The 5 ml serum was diluted up to 10 ml with distilled water. A reagent mixture was prepared from Autospan liquid gold standard kit (Arkray Healthcare Pvt. Ltd.) and ELISA was performed for the estimation of AST and ALT. 10 ml of serum as a sample was mixed in 100 ml reagent prepared according to assay kit (SGPT (DST) (IFCC Method) Rechon Diagnostics P. Ltd.) and ELISA was performed followed by its observation at 390 nm. The enzyme activity is expressed as International Units/Litre (IU/L).

Estimation of Reactive Oxygen Species (ROS) in the liver and kidney

Tissue homogenate (1:1) was prepared as per method (Shin *et al*., 2016) and was centrifuged followed by the addition of PBS and DCFDA to the pellet. Observations were taken at an absorbance of 485 nm and emission at 535 nm.

Estimation of superoxide dismutase (SOD) in liver and kidney

Phosphate Buffer was added for the homogenization of tissue and other reagents were mixed including L-methionine, hydroxylamine hydrochloride, hydrogen peroxide, and EDTA followed by incubation for 10 mins under the white fluorescent light inside an aluminium foil-wrapped box. Freshly prepared Griess reagent was added and absorbance was measured at 543 nm (Das *et al*., 2006).

Statistical analysis

Experimental data were expressed as mean ± SEM (n=8/group). The result obtained from each experimental group was analyzed by applying a student t-test and one-way ANOVA test with CRD design. Statistical analysis was calculated by SPSS Software and P-value <0.05 was considered as significant.

Results

Phytochemical analysis of an ethanolic extract of A. indica

Phytochemical investigation of ethanolic AILE revealed the presence of tannins, sterols, proteins, alkaloids, flavonoids and diterpenes while saponins, phenols, triterpenes and carbohydrates were found to be absent (Table 2). The presence of these biologically active compounds in AILE probably holds the accountability for its anti-inflammatory property.

Table 2. Phytochemical analysis of ethanolic extract from the leaves of neem (*A. indica*)

Bioactive compounds	Present (+)/ Absent (-)
Tannins	+
Saponins	-
Sterols	+
Proteins	+
Phenols	-
Alkaloids	+
Flavonoids	+
Diterpenes	+
Triterpenes	-
Carbohydrates	-

Body-weight analysis

Bodyweight of mice was analyzed for 7 weeks after their treatment with dexamethasone and two different doses of *A. indica*, *viz* 100 mg and 400 mg (Figure 2). During the first week, the weight of mice remained constant in the control, smoke-induced and dexamethasone-administered groups as well. However, a subtle weight decline was observed during the fourth week in the CS-induced mice group while the dexamethasone-treated mice group resulted in a gradual weight reduction during the second and fourth week. Additionally, the body weight of mice decreased during the first week in both ethanolic neem extract-induced groups, while further, it remained constant throughout the experiment.

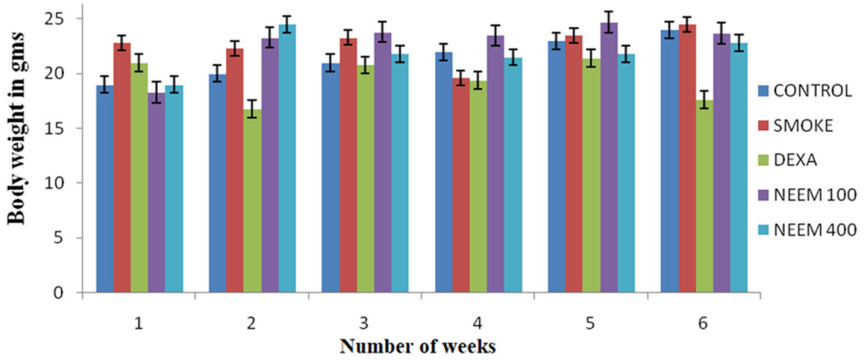

Figure 2. Graphical representation of the effect of the synthetic drug and herbal extract on the bodyweight of the model organism. Bar represents the ± SE.

Mortality rate analysis

The mortality rate was observed weekly among all the mice groups during the experiment (Figure 3). Dexamethasone treated group exhibited a 20% mortality rate in the fifth week of the experiment while no mortality was observed in the rest of the mice groups as illustrated (Figure 3).

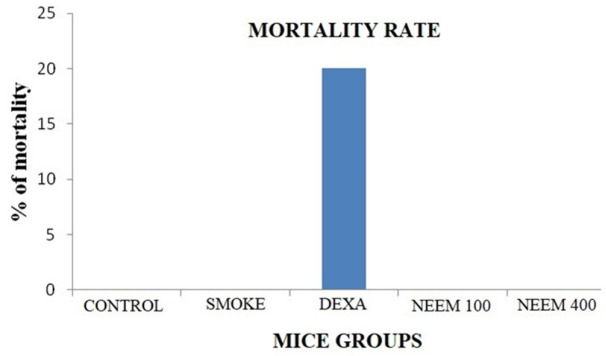

Figure 3. Effect of dexamethasone and neem leaf extracts on mortality rate of mice.

Eosinophil peroxidase activity in the liver and kidney (EPO)

EPO activity was analyzed to examine the recruitment and activation of eosinophils. The activity and level of eosinophil peroxidase elevated in the liver and kidney in the smoke-induced group when compared to its control (Figure 4). In parallel, the dexamethasone-administered group also showed a rise in EPO activity which was comparable to the smoke-induced group while AILE treated group exhibited declined EPO activity in both liver and kidney tissue.

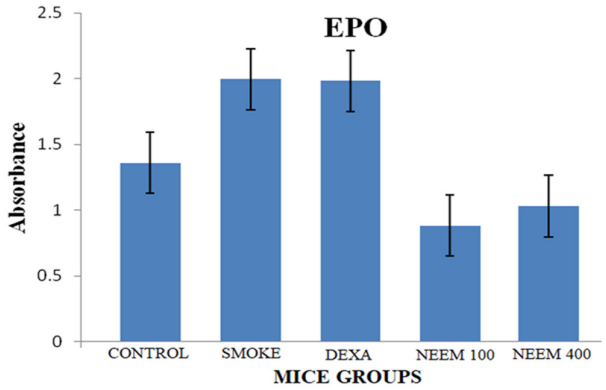

Figure 4. Effect of dexamethasone and neem leaf extracts on eosinophil peroxidase activity in smoke-induced liver injury in mice. Bar represents the ± SE.

Histopathology of liver and kidney

The liver and kidney tissues of mice from all the groups were sectioned appropriately and stained with Hematoxylin-Eosin staining. The histological images presented pathological variations in CS-induced and dexamethasone-treated mice tissues (Figure 5B and 5C respectively) when compared to normal tissue from the control mice group (Figure 5A). Cellular inflammation and disrupted architecture were seen in altered liver and kidney tissues of mice. On the contrary, liver and kidney tissues from the mice group treated with 100 mg dose of ethanolic neem leaf extract showed reduced inflammatory cells (Figure 5D) and furthermore, tissues from the mice group treated with 400 mg herbal dose resulted in the least cellular inflammation and disruption, thereby tissue recovery (Figure 5E).

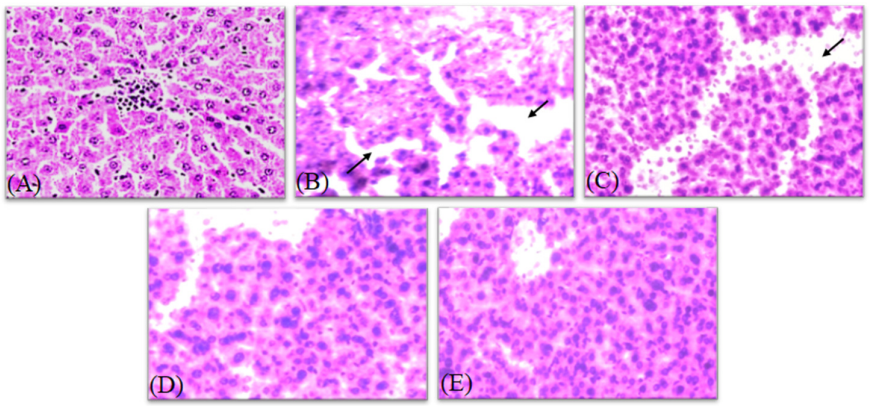

Figure 5. Morphology of liver tissue by Haematoxylin & Eosin stainin
A. Normal control mice liver; B. Highly disruption observed in smoke-induced mice liver; C. Cells disruption and the presence of inflammatory cells were found in Dexa-induced mice liver; D. Reduction in inflammatory cells was found in low dose neem treated mice liver; E. Damage recovery in high dose neem leaf extract induced liver

Protein content in serum

The protein content in the smoke-induced group of mice is higher compared to the normal group, while it is lower in the group administered with dexamethasone. Whereas, there may be the formation of protective protein in the mice administered with a high and low dose of neem extract as illustrated (Figure 6).

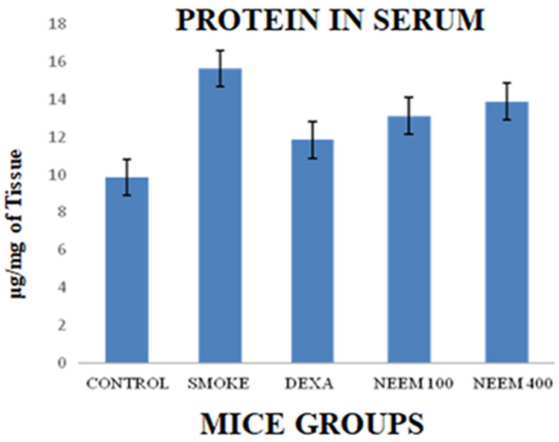

Figure 6. Effect of dexamethasone and neem on the protein content in the serum of a smoke-induced liver injury in mice

Aspartate aminotransferase (AST) activity

Effects of tobacco smoke, dexamethasone and leaf extract of *A. Indica* on the activity of aspartate aminotransferase (AST) was analyzed. The examination revealed a higher serum AST activity in the smoke-induced group at 68.00 IU/L in comparison to the control group of mice. AST level in the dexamethasone-treated mice group, 56.00 IU/L, was comparable to the mice group treated with CS while the activity of AST was minimum in the 400 mg herbal extract-treated group at 10.20 IU/L (Figure 7).

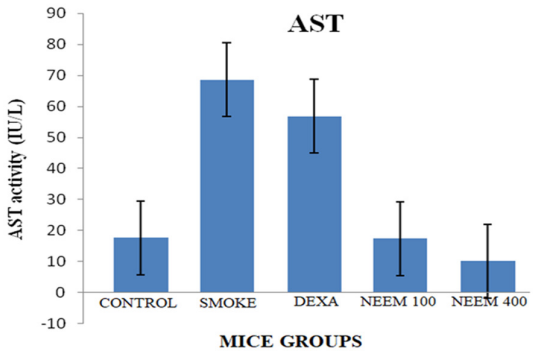

Figure 7. Effect of dexamethasone and neem on aspartate aminotransferase (AST) activity in serum of smoke-induced liver injury in mice. Bar represents the ± SE.

Alanine aminotransferase (ALT) activity

Alanine aminotransferase (ALT) activity was investigated to check the impacts of neem leaf extract. The results demonstrated higher activity of ALT in the smoke-induced and dexamethasone-treated group at 72.50 IU/L and 64.90 IU/L respectively as compared to the normal group, while serum ALT was highly reduced in 400 mg dose of neem leaf extract with 15.90 IU/L level, as shown in Figure 8.

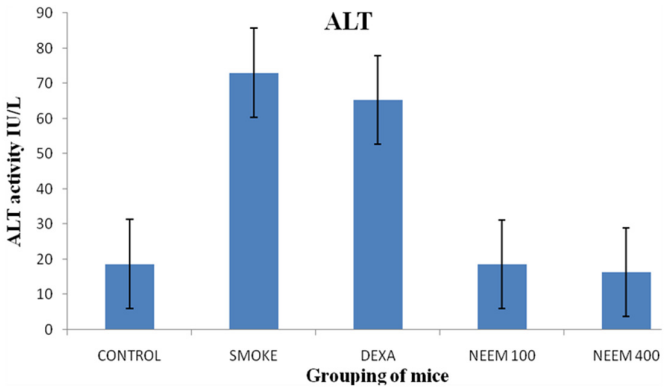

Figure 8. Effect of dexamethasone and neem on alanine aminotranferase (ALT) activity in serum of smoke-induced liver injury in mice. Bar represents the ± SE.

Estimation of Reactive Oxygen Species (ROS) in mice liver and kidney

Reactive Oxygen Species (ROS) were analyzed to examine the free radical generation during smoke-induced inflammation and the effect of neem extract on it. ROS level were found to be enhanced in the liver and kidney of CS-induced group when compared with its control group. Dexamethasone-induced group showed high ROS content while AILE treatment declined the ROS level in both the liver and kidney (Figure 9).

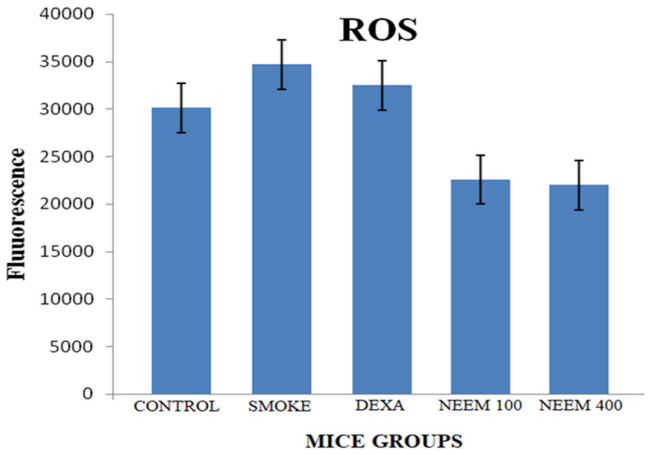

Figure 9. Effect of dexamethasone and neem on ROS production in serum of smoke-induced liver injury in mice. Bar represents the ± SE.

Estimation of Superoxide Dismutase (SOD)

Superoxides are produced as a by-product of oxygen metabolism and, if not regulated, can cause cell damage. SOD constitute a vital antioxidant defence against oxidative stress in the body. As illustrated in Fig. 9, the SOD activity in the liver and kidneys was higher in control mice. The SOD activity declined in CS-induced mice which were recovered on treatment with 400 mg dose of neem leaf extract in both liver and kidney tissue. Dexamethasone treated group also resulted in elevation in SOD activity yet it was not comparable to herbal extract (Figure 10).

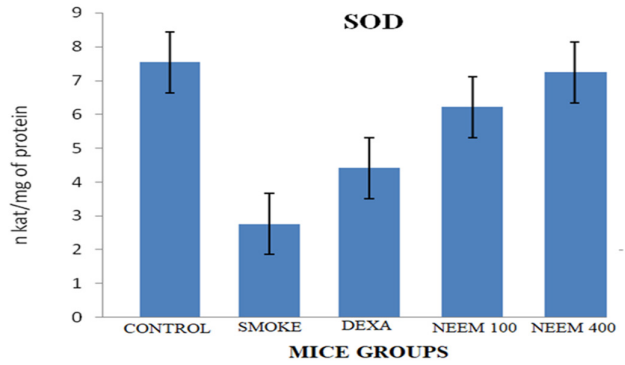

Figure 10. Effect of dexamethasone and neem on SOD activity in smoke-induced liver injury in mice. Bar represents the ± SE.

Discussion

Smokers are at considerable risk of cardiovascular diseases (ischaemic heart disease, hypertension), respiratory disorders (bronchitis, emphysema, chronic obstructive lung disease, asthma), cancer (lung, pancreatic, breast, liver, bladder, oral, larynx, oesophagus, stomach, and kidney), peptic ulcers and gastroesophageal reflux disease (GERD), male impotence and infertility, blindness, hearing loss, bone matrix loss, and hepatotoxicity. CS is responsible for a variety of adverse and hazardous effects on organs that do not have direct exposure to it such as the liver and kidney, and that's why it is prone to toxic immunological and

oncogenic effects (El-Zayadi, 2006). It yields numerous chemicals that are structurally distinct involving free radicals having cytotoxic potential and is responsible for the increase in necro-inflammation and fibrosis. Moreover, tobacco smoke contributes to the development of secondary polycythemia which might be a contributing factor to secondary iron overload disease-promoting oxidative stress of hepatocytes (Gutteridge *et al*., 1989).

In the present study, the effects of neem extract and dexamethasone on Cigarette Smoke induced hepato-injury were analyzed. Leaf extract of *A. indica* (100 mg/kg and 400 mg/kg) was administered to mice which had anti-inflammatory and antioxidative roles in treating kidney and liver injury (Biswas *et al*., 2002). The phytochemical screening of the leaf extract of *A. indica* revealed the presence of several bioactive compounds involving tannin, saponin, sterols, protein, phenols, alkaloids, flavonoids, diterpenes, triterpenes, and carbohydrates (Vinoth *et al*., 2012). Although, a flavonoid is a large group of naturally occurring compounds that exhibit anti-inflammatory and antioxidant activity, so far terpenes and saponins also get hold of some anti-inflammatory activity (Sultana *et al*., 2017). Within the realm of possibility, the phytochemicals present in AILE were accountable for manifesting anti-inflammatory as well as antioxidant properties in Cigarette Smoke-induced liver and kidney injury as demonstrated in the animal model. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine marker were found to be commonly used biomarkers for liver and kidney damage respectively (Alsalhen *et al*., 2014). However, the presence of AST also in cardiac muscle, skeletal muscle and erythrocytes obliged ALT to be the most specific marker for liver damage (Goorden *et al*., 2013). In this study, the levels of AST and ALT were examined for the functioning of the liver where CS caused the liver injury was indicated by the elevated levels of AST and ALT while the rising level of creatinine showed kidney injury. The mice group treated with dexamethasone resulted in lower levels of AST and ALT yet it was not comparable to the *A. Indica* extract treatment showed highly diminished levels of AST and ALT suggesting that the liver damage was controlled in more proportion to administration of *A. indica*. The inflammation was confirmed by observing the enhanced activity of EPO, a marker of eosinophilic inflammation (Saidani *et al*., 2019). In this study, the activity of EPO enhanced in smoke-induced mice was suppressed on administration of *A. indica* extract at both doses and perhaps the bioactive compounds of *A. indica* were responsible for the anti-inflammatory effect. Besides, another inflammatory marker was protein concentration which shared similar results as that of EPO. An advanced antioxidant system has been developed to relieve oxygen stress, however, an excessive amount of reactive oxygen species (ROS) disrupts homeostasis which develops oxidative stress that ultimately leads to hepatic disorders by bringing irremediable changes in lipids, proteins and DNA content (Li *et al*., 2015). Redox state constitutes an essential background for numerous liver and kidney disorders because it is associated with the course of inflammatory, metabolic, and proliferative liver and kidney diseases (Cichoz *et al*., 2014). In our investigation, the generation of ROS was found to be elevated in the smoke-induced group providing the basis for inflammation in liver and kidney tissues. Furthermore, ROS production in this study was enhanced by the suppressed activity of superoxide dismutase (SOD) as observed in the liver and kidney tissues of *the Labeorohita* during the toxicity test (Bojan *et al*., 2017). The enzyme served as an anti-inflammatory agent and also prevented precancerous cell changes (Karimi *et al*., 2017). The analysis of SOD activity resulted in the inhibition of ROS generation on treatment with *A. indica* extract demonstrating the anti-inflammatory and anti-oxidative function of herbal dose.

Conclusions

Corticosteroids are anti-inflammatory drugs used to treat numerous conditions. Dexamethasone is one of them that have side effects on body organs. The herbal approach could be its best alternative being a source of medicines from ancient times. The current investigation demonstrates that the doses of ethanolic leaf extract

of *A. indica* are relatively more protective than dexamethasone to cure both renal and hepato-injury caused by cigarette smoke (CS) on account of no mortality and no severe toxic effects appeared in model organisms on its administration based on the biochemical parameters and histopathology. Elicited from this research it can be concluded that *A. indica* has a high margin of safety than dexamethasone in treating cigarette smoke-induced liver and kidney damage yet further studies with different doses of extract are needed.

Authors' Contributions

Formal analysis: S and AV; Investigation: S, AV and VS; Methodology: AV and S; Resources: S; Supervision: S and VS; Validation: S and VS; Writing-original draft: AS, VS and AS; Writing-review and editing: AS and VS. All the authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

All the procedures performed in studies involving animals were in accordance with the ethical standards of central animal ethical committee and the code were issued i.e., "Dean/2015/CAEC/1410"

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Adamko DJ, Wu Y, Gleich GJ, Lacy P, Moqbel R (2004). The induction of eosinophil peroxidase release: improved methods of measurement and stimulation. Journal of Immunological methods 291(1-2):101-108. *https://doi.org/10.1016/j.jim.2004.05.003*

Alsalhen KS, Abdalsalam AR (2014). Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida City, Libya. International Journal of Current Pharmaceutical Research 3(7):291-295. *https://doi.org/10.3329/ICPJ.V3I7.19077*

Balunas MJ, Kinghorn AD (2005). Drug discovery from medicinal plants. Life Sciences 78:431-441. *https://doi.org/10.1016/j.lfs.2005.09.012*

Benisheikh AAG, Muhammad FM, Kelluri H, Aliyu ZM, Mallam UB, Jibrin MW (2019). Phytochemical extraction and antimicrobial studies on crude leaf extract of *Azadirachta indica* (Neem) in semi-arid region of Borno state, Nigeria. International Journal of Current Research and Review 6(12):516-522. *https://doi.org/10.15446/rcciquifa.v50n1.95447*

Biswas K, Chattopadhyay I, Banerjee R, Bandhopadhyay U (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). Current Sciences 82(11):1336-1345.

Bojan N, Hemalatha D, Rangasamy B, Maharajan K, Ramesh M (2017). Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeorohita* exposed to organophosphorus pesticide profenofos. Biocatalysis and Agricultural Biotechnology 12:185-190. *https://doi.org/10.1016/j.bcab.2017.09.006*

Cichoż-Lach H, Michalak A (2014). Oxidative stress as a crucial factor in liver diseases. World Journal of Gastroenterology 20(25):8082-8091. *https://doi.org/10.3748/wjg.v20.i25.8082*

Das K, Samanta, Chainy GBN (2000). A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Indian Journal of Biochemistry and Biophysics 37:201-204. *https://doi.org/10.4018/978-1-7998-3594-3.ch001*

De S, Dey YN, Ghosh AK (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). International Journal of Biological & Pharmaceutical Research 1(5):150-157.

Demir S (2020). The process of acute and chronic inflammation: biomarkers and their relationship with diseases. Role of nutrition in providing pro-/anti-inflammatory balance: emerging research and opportunities. IGI Global, pp 1-23.

Eid A, Jaradat N, Elmarzugi N (2017). A review of chemical constituents and traditional usage of Neem plant (*Azadirachta indica*). Palestinian Medical & Pharmaceutical Journal 2:75-81.

El-Zayadi AR (2006). Heavy smoking and liver. World Journal of Gastroenterology 12(38):6098-6101. *https://doi.org/10.3748/wjg.v12.i38.6098*

Goorden SM, Buffart TE, Bakker A, Buijs MM (2013). Liver disorders in adults: ALT and AST. Nederlandse tijdschrift voor geneeskunde 157(43):A6443.

Gutteridge JMC, Halliwell B (1989). Iron toxicity and oxygen radicals. Bailliere's Clinical Haematology 2(2):195-256. *https://doi.org/10.1016/s0950-3536(89)80017-4*

Ijoma KI, Ajiwe VIE (2017). Phytochemical screening of Dialium indum leaf extract (*Velvet tarmarind*). International Journal of Phytopharmacy 7(1):06-13.

Kanagasanthosh K, Shanmugapriyan S, Kaviranjan V (2015). Evaluation of acute toxicity, anti-inflammatory activity and phytochemical screening of ethanolic extract of *Azadirachta indica* leaves. International Journal of Research and Development in Pharmacy & Life Sciences 4(5):1737-1742.

Karimi-Khouzani O, Heidarian E, Amini SA (2017). Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. Pharmacological Reports 69(4):830-835. *https://doi.org/10.1016/j.pharep.2017.03.011*

Kumar RK (2015). Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn. Journal of Pharmacognosy and Phytochemistry 4(1):7-9.

Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y (2015). The role of oxidative stress and antioxidants in liver diseases. International Journal of Molecular Sciences 16(11):26087-26124. *https://doi.org/10.3390/ijms161125942*

Malkawi AK, Alzoubi KH, Jacob M, Matic G, Ali A, Al Faraj A, Abdel Rahman A M (2018). Metabolomics based profiling of dexamethasone side effects in rats. Frontiers in Pharmacology 9:46. *https://doi.org/10.3389/fphar.2018.00046*

Mohammed IMI (2019). Isolation, characterization of flavonoids from *Terminalia brownie*, *Ziziphus abyssinica*, *Cassia sieberiana* roots and evaluation of antimicrobial activity. Doctoral Dissertation, Sudan University of Science and Technology.

Nadhiya R, Sengottuvel T, Gopalasatheeskumar K, Ariharasivakumar G (2019). Phytochemical analysis and antioxidant activity of hydroalcoholic fruit extract of *Cucumis dipsaceus*. European Journal of Biomedical Research 6(12):281-286.

Ponist S, Zloh M, Bauerova K (2019). Impact of oxidative stress on inflammation in rheumatoid and adjuvant arthritis: damage to lipids, proteins, and enzymatic antioxidant defense in plasma and different tissues. Animal Models in Medicine and Biology, IntechOpen. *https://doi.org/10.5772/intechopen.89480*

Rady MY, Johnson DJ, Patel B, Larson J, Helmers R (2006) Corticosteroids influence the mortality and morbidity of acute critical illness. Critical Care 10(4):1-9. *https://doi.org/10.1186/cc4971*

Ragavan B, Krishnakumari S (2006). Effect of *T. arjuna* stem bark extract on histopathology of liver, kidney, and pancreas of alloxan-induced diabetic rats. African Journal of Biomedical Research 9:189-197. *https://doi.org/10.4314/ajbr.v9i3.48904*

Raja MMM, Raja A, Imran MM, Santha AMI, Devasena K (2011). Enzymes application in diagnostic prospects. Biotechnology 10(1):51-59. *https://doi.org/10.3923/biotech.2011.51.59*

Saidani C, Béchohra L, Laraba-Djebari F, Hammoudi-Triki D (2019). Kidney inflammation and tissue injury induced by scorpion venom: comparison with a nephrotoxic model. Toxin Reviews 38(3):240-247. *https://doi.org/10.1080/15569543.2018.1446028*

Saklani S, Mishra AP, Sati B, Sati H (2012). Pharmacognostic, phytochemical and antimicrobial screening of *Aphanamixis polystachya*, an endangered medicinal tree. International Journal of Pharmacy and Pharmaceutical Sciences 4(3):235-240.

Sati SC, Kumar P (2015). Assessment of Himalayan juniper, *Juniperus squamata* buch–hamex d. don for phytochemical screening and antimicrobial potential against some infection causing pathogens. World Journal of Pharmaceutical Research 4:998-1011.

Sen S, Chakraborty R (2017). Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. Journal of Traditional and Complementary Medicine 7(2):234-244. *https://doi.org/10.1016/j.jtcme.2016.05.006*

Shahverdi MA, Omidi H, Tabatabaei SJ (2019). Stevia (*Stevia rebaudiana* Bertoni) responses to NaCl stress: Growth, photosynthetic pigments, diterpene glycosides and ion content in root and shoot. Journal of the Saudi Society of Agricultural Sciences 18(4):355-360. *https://doi.org/10.1016/j.jssas.2017.12.001*

Shin IS, Ahn KS, Shin NR, Lee HJ, Ryu HW, Kim JW, Oh SR (2016). Protective effect of EC-18, a synthetic monoacetyldiglyceride on lung inflammation in a murine model induced by cigarette smoke and lipopolysaccharide. International Immunopharmacology 30:62-68. *https://doi.org/10.1016/j.intimp.2015.11.025*

Sivakumar T, Gajalakshmi D (2013). *Invitro* antioxidant and chemical constituents from the leaves of *Ormocarpum cochinchinensee lumbotti*. American Journal of Plant Physiology 8(3):114-122. *https://doi.org/10.3923/ajpp.2013.114.122*

Sultana B, Anwar F, Przybylski R (2007). Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. Trees. Food Chemistry 104(3):1106-1114. *https://doi.org/10.1016/j.foodchem.2007.01.019*

Vinoth B, Manivasagaperumal R, Rajaravindran M (2012). Phytochemical analysis and antibacterial activity of *Azadirachta indica* A Juss. International Research Journal of Plant Science 2(3):50-55.

Waterborg JH, Matthews HR (1994). The Lowry method for protein quantitation. Methods Molecular Biology 32:1-4. *https://doi.org/10.1385/0-89603-268-X:1*

Yong SH, Leem AY, Kim YS, Park MS, Chang J, Kim SU, Jung JY (2020). Hepatic fibrosis assessed using fibrosis-4 index is predictive of all-cause mortality in patients with chronic obstructive pulmonary disease. International Journal of Chronic Obstructive Pulmonary Disease 15:831-839. *https://doi.org/10.2147/COPD.S242863*

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