THE PROTECTIVE EFFECTS ABELMOSCHUS ESCULENTUS PODS SKIN AND SEEDS AGAINST NICOTINE INDUCED LIVER, KIDNEY DAMAGE IN MICE’

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Keywords:
Okra, kidney disease, Cigarette smokers, mice, cholesterol, Urea, SGPT, seeds, pods, hyperlipidemia

ABSTRACT: Abelmoschus esculentus is one of the oldest urbane crops grown in many countries and widely distributed. Parts used of okra are fruit, leave seed, root wish showed to have numerous Medicinal significant uses. On the other hand, cigarette, smokers found to have a high a significant effect on liver kidney and other numerous organs in the body. Aim: The study aims to explore the possible protective effects of three parts of okra against nicotine-induced damage in many parts of the mice body. Methods: Fifty male Swiss albino mice were used throughout the study. After tow-weeks acclimatization period, the mice divided into 5 groups from the control group, nicotine-treated group, nicotine-okra pods extract -treated group, nicotine-okra skin extract treated group, and nicotine-okra seeds extract -treated group. Result: shown significant protective effects against nicotine damage effects as cholesterol level decrease in okra skin treatment with a significant difference in comparison with the positive group also okra protective effects on renal function have found that urea and creatinine levels decrease significantly in okra feeding mice. Liver function tests have also been evaluated, and SGPT levels of all okra parts extract have shown significant differences decrease compared to the positive control group. Conclusion: Abelmoschus esculentus possess effects like normalizing cholesterol levels, (antihyperlipidemic roles), suggesting that the consumption of okra may be of benefit in metabolic diseases.

INTRODUCTION: Okra (Abelmoschus esculentus) is a simply vegetable crop of contact in the Malvaceae family and is extremely popular in many countries in the world 1. It is one of the oldest urbane crops and currently grown in many countries and is widely distributed from Africa to Asia, southern Europe and America 2. Abelmoschus esculentus is a tropical to subtropical crop and is susceptible to weather changes and the development from different countries have sure modified distinctive characteristics specific to the country to which they related 3.

Okra usually consumed for its green tender fruits as a vegetable in a variety of ways. It's rich in vitamins, calcium, potassium and other mineral matters 4. The mature okra seed is a better source of oil and protein and has been known to have greater nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid, which is vital for human nutrition. Its mature fruit and stems contain crude fiber, that’s used in the paper industry 5. Parts used of okra are fruit, leave seed, root wish showed to have numerous medicinal uses as antispasmodic; demulcent; diaphoretic; a diuretic; emollient; stimulant; vulnerary 6. The roots are highly rich in mucilage, having a powerfully demulcent effect. This mucilage can be used as a plasma replacement. An infusion of the roots is useful in the management of syphilis. The juice of the roots is used topically in Nepal for the treatment of cuts, wounds and boils 7.
The leaves provide an emollient property, also it is used in the treatment of catarrhal infections, gonorrhea, and also dysuria. The seeds of okra found to have antispasmodic, cordial, and stimulant.

Okra has been found to have a Purgative possesses that is beneficial for bowel purification that’s related to okra fiber content, sufficient water levels in faces are ensured. This benefits the organism in general, as the toxins and bad cholesterol can induce various health conditions. Okra poses no risk to the life form, causes no addiction; it is completely safe and Reliable. Okra ensures healing from psychological and beavers conditions, like, depression and general weakness.

It’s also shown to be successful in the treatment of ulcers and joint fitness. It is used to neutralize the acids, due to its alkaline origin. It also protects the mucous membranes of the digestive system by layering them with additional covers. It is also good resource of iodine, which is helpful in the management of simple goiter; It is very useful genitourinary disorders, spermatorrhea and chronic dysentery. Some research found that an alcohol extract of okra leaves can eliminate oxygen free radicals, alleviate renal tubular interstitial diseases, reduce protein urea, and improve renal function. Okra fruit is mainly consumed fresh or cooked and is a major source of many vitamins and minerals also Iron and Iodine and viscous fiber.

Greenish-yellow okra oil is extracted from okra seeds; it has a nice taste and odor and is high in unsaturated fats such as oleic acid and linoleic acid. On the other day, Tobacco smoke contains many compounds, the important substances of medical significance being the carcinogens, irritant substances, nicotine, carbon monoxide, and other gases. Nicotine is considered the primary toxic chemical in tobacco that is responsible for continuous tobacco use and dependence. Studies had been relating lung carcinogenesis by nicotine to genetic variation in CYP2B6. Its continuous exposure with hyperoxia has been found to stimulate cancer in hamsters. Nicotine has been found to initiate lung tumorigenesis by inhibiting anti-apoptotic pathway.

Risk of chronic kidney disease in smokers is significant. Cigarette smokers found to have a high albumin excretion in urine, decrease glomerular filtration rate, causes an increased incidence of renal artery stenosis which is associated with high mortality in patients suffering from end-stage renal disease.

At the side of the toxic effects mentioned above; smoking causes a variety of dangerous effects on organs that have no direct contact with the smoke itself, such as the liver. The liver is an essential organ that controls many metabolic processes. It is also responsible for metabolizing drugs, alcohol, and other toxins to eliminate them from the body. Heavy smoking found to expose highly to toxins that found to induce necroinflammation and raise the severity of hepatic lesions (fibrosis) when occupied with hepatitis C virus (HCV) or hepatitis B virus (HBV) infection. Cigarette smoking associated with a high risk of developing chronic liver disease (CLD) patients independently of liver status. Association of smoking with hepatocellular carcinoma (HCC) irrespective of HBV status has been reported. The study aimed to explore the possible protective effects of many parts of okra against nicotine-induced damage in many parts of the mice body.

MATERIALS AND METHODS: The fresh okra were purchased from a market. Two portions of 2.5 kg fresh okra pods were arranged. One portion lyophilized directly to get a dried okra pod (251.3 g) another portion was separated into okra seeds and okra skins, lyophilized to have, correspondingly, dried okra seeds (50.1 g) and dried okra skins (200.5 g).

The okra seeds, pods, and the skins were grounded and extracted separately with 1500 mL boiling water each for 1 h (3 times). Each filtered liquid was combined and concentrated under vacuum, to give up residues of okra pods (OPD 107.2 g), okra seeds (OSD, 20.7 g) and okra skins (OSK, 83.4 g), respectively. Therefore, the ration of an extract of OPD: OSK: OSD is about 5:4:1. All samples were stored at −20 °C.

Experimental Animals: Fifty male Swiss albino mice aged nine to 12 weeks and weighing 25 to 30 g were used throughout the study. Animals were
fed by the same diet ingredient and had free access to tap water. All mice were kept under the same experimental condition, fed standard diet, and water was available ad libitum.

After the two-week acclimatization period, the selected animals of nearly a similar weight were divided into five experimental groups to keep more or less the same mean body weight within the individual groups. The selected animal groups (ten animals per each group) were treated as follows:

Control group (saline-treated group), nicotine-treated group (NI) (positive control), nicotine-okra pods extract-treated group, nicotine-okra skin extract-treated group, nicotine-okra seeds extract -treated group.

Chemicals: The treated chemicals in the experiment were nicotine ((S)-3-(1- methyl-2-pyrroli- diny) pyridine). Nicotine was supplied as a colorless liquid, from Baghdad College of pharmacy. The mean LD₅₀ for intraperitoneal nicotine to 8- week-old (29.6 g) mice were reported as 12.5 mg/kg or/and a dose of nicotine equals to 1/5 of LD₅₀.

Blood samples of the experimental from each group were taken from the heart, and then it sacrificed by cervical dislocation at the end of two weeks of the experimental period, liver and kidney were sampled and kept in aqueous bouin for histological, histochemical and morphometrical examinations The whole study was approved by the animal ethical committee of our institute.

RESULTS: In related to cholesterol results There is a significant difference between the positive and negative control group, cholesterol level shown a decrease in okra skin with a significant difference in compare with the positive group on the other hand triglyceride level has been increasing with a positive control group to 111.33 ± 1.15. All part of okra (skin, seeds, pods) shown significant differences in comparison to the positive control group, HDL level shown nonsignificant differences in comparison to the positive group with no significant difference between the positive and negative group. LDL, VLDL positive control level has been increased significantly in the comparison between the positive and negative group. All other extracts have been failed to decrease the level to near the normal level.

Urea levels has been increased very significantly from 23.1 ± 1 to 53.66 ± 1.5 in the positive control group, and all okra extract shows a highly significant differences in comparison to positive control also, on the other hand, creatinine level show a highly significant differences in comparison between positive and negative group and all okra extract show significant differences.

SGPT levels increased from 56.6 ± 4.9 to 77.6 ± 3.2 with very high significant differences and all okra parts extract has shown a significant differences decrease in compared to positive control group, on the other hand SGOT levels shown an increase in the concentration in compare between positive and negative control group and only okra seeds and pods has shown a significant differences decrease compared to positive control group also ALP levels increased from 158.3 ± 8 to 170.3 ± 5.7 and all okra parts extract has shown a significant differences decrease in compared to positive control group.

**TABLE 1: REPRESENT THE EFFECTS OF OKRA SKIN, SEEDS, AND PODS ON LIPID PROFILE AT THE END OF THE EXPERIMENT**

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol level mg/dL</th>
<th>Triglyceride level mg/dL</th>
<th>HDL level mg/dL</th>
<th>LDL level mg/dL</th>
<th>VLDL level mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>110.6 ± 10.06*</td>
<td>111.33 ± 1.15xx</td>
<td>38. ± 2.64</td>
<td>44.6 ± 1.15*</td>
<td>20.8 ± 2.0*</td>
</tr>
<tr>
<td>Negative control</td>
<td>74.33 ± 11.15</td>
<td>101.1 ± 1.7</td>
<td>34.6 ± 5.5</td>
<td>25.6 ± 2.6</td>
<td>18.3 ± 2.8</td>
</tr>
<tr>
<td>Okra skin</td>
<td>94 ± 5.657*</td>
<td>85 ± 10*</td>
<td>27. ± 5.2</td>
<td>44 ± 5.25**</td>
<td>22.25 ± 0.9*</td>
</tr>
<tr>
<td>Okra seeds</td>
<td>116.33 ± 5.5*</td>
<td>90.33 ± 10.6*</td>
<td>42.33 ± 4.16</td>
<td>40.6 ± 4*</td>
<td>20.33 ± 1</td>
</tr>
<tr>
<td>Okra pods</td>
<td>117.4 ± 3.5**</td>
<td>91 ± 5.2*</td>
<td>28.2 ± 3*</td>
<td>42.6 ± 15.7*</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

* represent significant differences in compare to positive control group ** represent highly significant differences in comparison to positive control group, * represent significant differences in comparison between positive and negative control group xx represent highly significant differences in comparison between positive and negative control group. All values are mean ± SD data were analyzed by using spss 22 statistical test.
TABLE 2: REPRESENT THE EFFECTS OF OKRA SKIN, SEEDS, AND PODS ON RENAL FUNCTION TEST AT THE END OF THE EXPERIMENT

<table>
<thead>
<tr>
<th></th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>53.66 ± 1.5**</td>
<td>0.89 ± 0.08***</td>
</tr>
<tr>
<td>Negative control</td>
<td>23.1 ± 1</td>
<td>0.42 ± .002</td>
</tr>
<tr>
<td>Okra skins</td>
<td>36.0 ± 2.64***</td>
<td>0.68 ± 0.028***</td>
</tr>
<tr>
<td>Okra seeds</td>
<td>44.0 ± 1**</td>
<td>0.56 ± 0.15*</td>
</tr>
<tr>
<td>Okra pods</td>
<td>33.3 ± 5**</td>
<td>0.55 ± 0.05**</td>
</tr>
</tbody>
</table>

* represent significant differences in compare to positive control group **represent highly significant differences in comparison to positive control group *** represent highly Significant differences in comparison between positive and negative control group • represent significant differences in comparison to negative control group •• represent highly significant differences in comparison to negative control group. All values are mean ± SD data were analyzed by using spss 22 statistical test.
TABLE 3: REPRESENT THE EFFECTS OF OKRA SKIN, SEEDS, AND PODS ON LIVER FUNCTION AT THE END OF THE EXPERIMENT

<table>
<thead>
<tr>
<th></th>
<th>SGPT</th>
<th>SGOT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>77.6 ± 3.4**</td>
<td>60.6 ± 5.1*</td>
<td>170.3 ± 5.7*</td>
</tr>
<tr>
<td>Negative control</td>
<td>56.6 ± 4.9</td>
<td>58.3 ± 14.1</td>
<td>158.3 ± 8</td>
</tr>
<tr>
<td>Okra skin</td>
<td>75.6 ± 3 *</td>
<td>55. ± 19.1</td>
<td>140.33 ± 10.1***</td>
</tr>
<tr>
<td>Okra seeds</td>
<td>54.6 ± 0.8*</td>
<td>48. ± 12.7*</td>
<td>149.6 ± 0.5*</td>
</tr>
<tr>
<td>Okra pods</td>
<td>63.6 ± 2.5**</td>
<td>53 ± 12.8**</td>
<td>149 ± 10.4*</td>
</tr>
</tbody>
</table>

* represent significant differences in compare to positive control group ** represent highly significant differences in comparison to positive control group, ‘represent significant differences in comparison between positive and negative control group ▪ represent significant differences in comparison to negative control group ▪▪ represent highly significant differences in comparison to negative control group. All values are mean ± SD data were analyzed by using spss 22 statistical test.

FIG. 7: REPRESENT THE MEAN DIFFERENCE OF SGPT LEVELS BETWEEN POSITIVE (NICOTINE TREATED GROUP, NEGATIVE (NORMAL SALINE TREATED GROUP), OKRA SKIN EXTRACT PLUS NICOTINE, SEEDS EXTRACT PLUS NICOTINE AND PODS EXTRACT PLUS NICOTINE ON THE END OF THE EXPERIMENT

FIG. 8: REPRESENT THE MEAN DIFFERENCE OF SGOT LEVELS BETWEEN POSITIVE (NICOTINE TREATED GROUP, NEGATIVE (NORMAL SALINE TREATED GROUP), OKRA SKIN EXTRACT PLUS NICOTINE, SEEDS EXTRACT PLUS NICOTINE AND PODS EXTRACT PLUS NICOTINE ON THE END OF THE EXPERIMENT

FIG. 9: REPRESENT THE MEAN DIFFERENCE OF ALKALINE PHOSPHATASE (ALP) LEVELS BETWEEN POSITIVE (NICOTINE TREATED GROUP, NEGATIVE (NORMAL SALINE TREATED GROUP), OKRA SKIN EXTRACT PLUS NICOTINE, SEEDS EXTRACT PLUS NICOTINE AND PODS EXTRACT PLUS NICOTINE ON THE END OF THE EXPERIMENT

DISCUSSION: The goal of this project was to clarify the antioxidant effect of *Abelmoschus esculentus* on liver, kidney & lipid profile, since folklore medicine claims *Abelmoschus esculentus* can help in hyperlipidemia and has hepatoprotective and nephroprotective effect.

Presently, these conditions are treated or controlled using pharmacologic agents and nonpharmacologic methods, such as diet and exercise. But, all the pharmacologic agents are not cleared from adverse effects, and this enhances researchers to explore a new drug from all possible sources, including traditional medicines, which might be less toxic when compared to the available drug therapy.

Fangbo Xia et al., in their research "Antioxidant and anti-fatigue constituents of okra" found that the antioxidant part of okra could be related to okra seed (OSD), and the active constituents of okra seed (OSD) were found to be polyphenols and flavonoids due to their antioxidant activity.

With the Larger Consumer Demand for Functional: Food, much more attention is the highlight to *Abelmoschus esculentus*, for its special functional and nutrition value. Therefore, it is meaningful to research the chemical compositions of *Abelmoschus esculentus* and to develop its health function.
A research "Analysis and comparison of the active components and antioxidant activities of extracts from *Abelmoschus esculentus* L." done by Haibing Liao *et al.*, has recognized that there are total phenols (TP) and total flavonoids (TF) contents in all the extracts of the plant organ, and the content amount may be varies. The results also demonstrate that there is more total phenols (TP) and total flavonoids (TF) content in the extract of the *Abelmoschus esculentus* L. flower than in the other parts. 25. Meanwhile, the contents of total phenols (TP) and total flavonoid activities of neutrophils and myeloperoxidase, provide new insights into the adsorbable organic halide (AOX), anti-radical, anti-inflammatory, and modulating properties of *Abelmoschus esculentus* “inflammation like” conditions 26.

Adelakun *et al.*, in their research "Influence of pre-treatment on yield chumulation and antioxidant properties of a Nigerian okra seed (*Abelmoschus esculentus* Moench) flour," establish that okra seed is a hopeful source of a lot of antioxidant and the Pre-treatment using soaking and blanching lead to enhancement of the results. Consumption of okra seed could be helpful in the prevention of chronic diseases 27.

Hyperlipidemia is one of the major risk factors that could lead to dangerous disorders like cardiovascular disease and metabolic disorders; it is usually characterized by an increase in total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C). Many studies and research have recommended that the consumption of foods high in flavonoid compounds could decrease the risk of obesity and hyperlipidemia, highlight that the consumption of okra may be of benefit in metabolic diseases 28. Hong Wang *et al.*, found in their research which continues for eight weeks "Hypolipidemic activity of okra is mediated through inhibition of Lipogenesis and Ureupregation of Cholesterol Degradation," that *Abelmoschus esculentus* is beneficial in managing hyperlipidemia and its associated metabolic disorders 29.

Shengjie Fan *et al.*, established in the research "Extract of okra lowers blood glucose and serum lipids in high-fat-diet-induced obese C57BL/6 mice", the extract of okra enhanced metabolic disorders in high fat (HF) diet-induced obese mice and additionally, the antioxidant activity of okra may relate to the inhibition of peroxisome proliferator-activated receptor (PPARγ) Messenger Ribonucleic acid (mRNA), and its target gene expression may account for its essential mechanism. The data propose that okra may be functional as a potential dietary therapy for metabolic disorders such as hyperglycemia and hypertriglyceridemia 30. V. Sabitha *et al.*, in their research "Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench in streptozotocin-induced diabetic rats", that the study, for the first time, confirms that *Abelmoschus esculentus* (AE) peel and seed possess blood glucose lipid profiles lowering action in diabetic condition 31. In this research, it was found that all extract of okra (skin, seeds, and pods) triglyceride level has been found to decrease significantly from the positive control group.

In our study it was found cholesterol level results has shown no significant decrease in compare to positive control level only okra skin decrease cholesterol significantly, triglyceride has been increased very significantly in compare between positive and negative control group and this increment decrease significantly by administered all types of okra skin, pods and seeds (significant differences in compare to positive control), HDL, LDL, VLDL level show un-significant deference in compare between okra extract and positive control group these differences may be due to the duration of the experiment (our experiment last for 2 weeks while other experiment last for 8 weeks).

The treatments of liver disease could be associated with serious adverse effects, especially when given for a prolonged period on the other hand herbs, and other medicinal plant products with improved its effectiveness are needed as an alternative for chemical drugs. It has also been recognized that high consumption of some vegetables and fruits are beneficial to health and in combating the onset of liver diseases 32.

S. I. Alqasoumi found in his research" ‘Okra’ *Hibiscus esculentus* L. A study of its hepatoprotective activity", that the protective effects of ethanolic extract of okra (EEO) against
liver injury were evaluated in rodents using carbon tetrachloride-induced hepatotoxicity model ethicalanolic extract of okra (EEO), exerted significant dose-dependent hepatoprotection and this effects are found to be compatible with standard silymarin. The ability of okra extract to protect chemically induced liver damage may be attributed to its potent antioxidant property. Subramanian Saravanan et al., also found in "Hepatoprotective role of Abelmoschus esculentus on carbon tetrachloride-induced liver injury," that the incubation of human liver cancer cell line with CCl₄ decreased the cell viability and increased the leakage of transaminases and Pre-treatment with the extract significantly restored the cell death and reduced the levels of transaminases.

These results are compatible with our results which found that a significant decrease in all hepatic enzymes has been achieved in all extract of okra part which reflects a powerful protective effect of okra against nicotine-induced liver damage. The mechanisms of nicotine-induced renal damage are not widely understood but are likely due to both vascular and tubular effects. Oxidative stress which induced by smoking could lead to endothelial and vascular injury. Studies have found that it also increased renal vascular resistance (and decreased glomerular filtration rate, and biochemical evidence of smoking or chronic nicotine-induced renal toxicity.

Increased oxidative stress and morphological abnormalities have also been recognising in the proximal tubular epithelium after exposure to chronic cigarette smoke or nicotine, and low-grade injure of proximal tubules has also been experimental among chronic smokers, these alterations may accelerate the kidney to acute ischemic progression.

While the dangerous effects of smoking may be due to many different components of tobacco smoke, one of the more likely cause is the alkaloid nicotine, it is excreted by glomerular filtration and tubular secretion and it detected in high concentration in the serum and kidneys of smokers. Chronic exposure to nicotine could lead to increases oxidative stress in the kidney and this connecting smoking and nicotine to renal injury as a result; smoking exposure might exacerbate acute renal injury through increasing oxidative stress.

In our study it was found that urea levels have been increased very significantly from 23.1 ± 1 to 53.66 ± 1.5 in the positive control group and all okra extract show a highly significant differences in comparison to positive control also on the other hand creatinine level show a highly significant differences in comparison between positive and negative group and all okra extract show a significant differences this powerful protective effects of okra could be related to its antioxidant effects against free radicals initiated by nicotine administration.

CONCLUSION: Abelmoschus esculentus possess effects like normalizing cholesterol levels, anti-hyperlipidemic roles, flavonoid compounds that could reduce the risk of obesity, hyperlipidemia, suggesting that the consumption of okra may be of benefit in metabolic diseases, it also owns an antioxidant property that is active in inhibiting free radical reactions and consequently protect the human body against damage by reactive oxygen species, it also owns a protective effect against paracetamol, rifampicin, alcohol and carbon tetrachloride-induced hepatic toxicity, and a protective effect against kidney damage.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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