**Research Article**

**Protective effect of Aloe vera and Bryophyllum pinnatum extracts against Smokeless tobacco induced nephrotoxicity in albino mice**

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**Abstract**

**Objective:** The present study was carried out with an objective to evaluate the protective effect of methanolic extract of *Aloe vera* leaf gel and *Bryophyllum pinnatum* leaf against smokeless tobacco Gutkha (STG) induced toxicity in the kidney of Albino mice. **Materials and Methods:** The Swiss albino mice were divided into six (6) groups, among which two groups were treated with 100mg/kg and 200mg/kg body weight doses of STG orally for 90 days. Another three groups of mice were given STG along with *A. vera* and *B. pinnatum* leaf extracts singly (300 mg/kg) b. wt and in combination (mixture of both plant in 1:1 proportion) at an interval of 10 hours. Changes in kidney were evaluated at histological (LM and SEM level) and biochemical (SOD, CAT and MDA) level by following standard procedures. **Results:** LM analysis of histological structure of kidney revealed swelling and individualization of tubular epithelial cells, glomerular proliferation along with atrophied and enlarged glomerulus in STG treated mice. SEM analysis of surface structure of kidney of STG treated animals showed heterogenous surface morphology with reduced, necrosed and individualized tubules of kidney. Significant (p<0.01) alterations were also observed in selected enzyme parameters of STG treated mice. However, such histological and biochemical changes were not observed in *A. vera* and *B. pinnatum* extract treated animals. **Conclusion:** Result of the present study suggested the ameliorative potential of *A. vera* and *B. pinnatum* methanolic extract against STG induced nephrotoxicity with more efficacy of combined mixture than their individual application.

**Keywords:** Smokeless tobacco, *Aloe vera*, *Bryophyllum pinnatum*, nephrotoxicity

**Introduction**

The smokeless tobacco (ST) includes a large variety of products that contain tobacco as their main component and are used without burning through oral or nasal route. Unlike smoking, the ST consumers never come into direct exposure of the toxic combustion product of tobacco and thereby carry low risk of smoking related health issues. Such belief along with the easy availability and cheaper price make smokeless tobacco popular among all strata of society day by day. However, these ST products have been found to be associated with a number of health effects due to its harmful chemical composition and a large proportion of the society may become victim of it.
process and thereby it becomes a target organ for most of the toxic substances enter into the systemic circulation of our body. Renal impairment may be associated with many other health ailments and thereby the adverse effects of lifestyle factors on renal function have gained special interest of researcher. Tobacco is also considered as one of the particular risk factor for renal disorders. The adverse effects of smokeless tobacco on kidney structure and functions are well evident from several studies (Kumari and Dutta, 2012; Kumari et al., 2013; Cheekuramelli et al., 2014). Although, a few report is available regarding the protective role of plant based remedies against smokeless tobacco induced toxicity.

At present, plant based traditional medicines gain keen attraction in the field of therapeutic researches. Due to presence of various beneficial phytochemicals, plants are effectively used to treat numerous diseases. Besides possessing wide range of pharmacogenic properties, plant based medicines also shows less or no side effects unlike other synthetic drugs. Owing to its beneficial properties, plants may be considered as remedial or protective measure for several health ailments. Amidst the large variety of useful plants, Aloe vera (Aloe barbadensis Miller.) and Bryophyllum pinnatum are selected for the present experimental study, by reviewing their ethno-botanical importance and role in traditional medicinal system.

Aloe barbadensis Miller, commonly Aloe vera (A. vera) is a xerophytic succulent plant, belongs to family Liliaceae. The plant is enriched with more than 200 different bioactive phyto-components including polyphenols, sterols, acemannan, anthraquinones, aloins, barbaloin, isobarbaloin, emodin, lipids, amino acids, enzymes etc. (Chauhan et al., 2007). The long history of A. vera as medicinal plant revealed it as a plant with wound healing, anti-inflammatory, antiviral, immunostimulating, blood glucose lowering, gastro protective and lipid lowering activity (Shikarwar et al., 2010).

Bryophyllum pinnatum (B. pinnatum), a perennial herb, belonging to family Crassulaceae, is used in traditional medicinal system since antiquity. The leaves of this plant have been reported to possess antimicrobial, anti-fungal, anti-ulcer, anti-inflammatory, analgesic and antihypertensive activities (Patel et al., 2011; Ojewole, 2002). Chemically, a number of bioactive compounds are identified in B. pinnatum leaf including flavonoids, steroids, organic acids, glucosides, phenols, tannins, saponins etc., which enriches the plant with medicinal values.

By considering the presence of valuable phyto-components and their inevitable medicinal benefits, A. vera and B. pinnatum were selected for the present study. It was expected that supplementation of these plant extracts either singly or in combination may impart a protective role against STG induced detrimental effects. This experimental project was aimed at studying the ameliorative potential of methanolic extracts of A. vera leaf gel and B. pinnatum leaf individually or in combination against STG induced toxic stress in kidney of albino mice.

Materials and methods

Plant extract preparation

A. vera leaf gel extract was prepared by the method of Pawar et al. (2005) with modification. For that, mature and healthy leaves of A. vera were collected freshly, authenticated and washed with fresh water. The thick epidermis was selectively removed. The solid leaf gel was dried in the oven at 80°C for 48 hour and then grinded to powdered form by using Mortar and Paste. Then, 20 grams of this powder was soaked in 200 ml of methanol for 24 hours with occasional shaking. The contents were then filtered and the filtrate was evaporated to dryness. This dried extract was further powdered and then dissolved in distilled water.

B. pinnatum leaf extraction was done by following the method of Joshi and Chauhan (2013). Freshly collected, mature leaves of Bryophyllum pinnatum were washed and shade dried. About 10 grams of dried powdered leaves of B. pinnatum was uniformly packed into a thimble and extracted in Soxhlet extractor with 260 ml of methanol. The extraction process was continued for 24 hour (6-7 cycles). After that the extract was taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was further powdered and mixed with distilled water.

Animal maintenance and experimental design

Healthy Swiss albino mice of age group 8±1weeks, weighing 20-25 g were procured from King Adward Pasteur Institute, Shillong (Meghalaya), India. The animals were maintained in standard environmental conditions (25±2°C temperature, 12 hours light/dark cycle and relative humidity 52±2 %) throughout the experimental period. The animals had access to commercial pellet diet and water ad libitum. The experimental works were carried out with due permission from institutional ethical committee (vide letter no. IAEC/PER//2016/2017-1017) and all the guidelines for care and use of laboratory animals were followed accordingly (CPCSEA guidelines-Committee for the purpose of control and supervision on experiments of animal, India).

Animals were randomly divided into six groups (housed as 5 mice/cage) for oral exposure study.

Control: Fed on standard rodent diet and water ad libitum.
STG-1: Orally fed STG in dose level 100 mg/kg b. wt daily
STG-2: Orally fed STG in dose level 200 mg/kg b. wt daily
STG-AV: Orally fed STG 200 mg/kg b. wt + 300 mg/kg b. wt. *A. vera* extract daily at an interval of 10 hours
STG-BP: Orally fed STG 200 mg/kg b. wt + 300 mg/kg b. wt. *B. pinnatum* extract daily at an interval of 10 hours
STG-C: Orally fed STG 200 mg/kg b. wt + mixture of *A. vera* and *B. pinnatum* (1:1 proportion) at 300 mg/kg b. wt dose level daily at an interval of 10 hours.

Animals were sacrificed after 30 and 90 days of treatment. During treatment period, animals were monitored and weighed regularly, where any abnormal signs were recorded carefully.

**Histopathology**

Three animals were sacrificed from control and treated groups at 30 and 90 days by cervical dislocation under anesthesia. The kidneys were excised for gross morphological and histological study. For light microscopic study, tissues were fixed in Carnoy's fixative, dehydrated through graded ethanol series and embedded in paraffin wax by following standard procedure. About 4µm thick sections of tissue were stained with Eosin and Haematoxyline method, (Luna 1968) and microphotographs were taken with light microscope (Olympus CX-23).

For scanning electron microscopy, kidney tissues were fixed immediately in primary fixative (2.5-3% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer, pH 7.3) and kept for 4-5 hours at 4°C (Sabatini et al., 1963). The tissues were then cut into small pieces followed by post fixation in secondary fixative (1% osmium tetroxide buffered with cacodylate buffer, pH=7.3) for 1 hour at 4° C (Palade, 1952). After that, tissues were dehydrated through ascending grades of acetone and dried by using Samdri drier PVT3 critical point drier. Dried tissues were underwent thin conductive gold coating by using fine coat ion sputter (Jeol, JFC-1100). The coated tissues were observed and photographs were taken with the help of a scanning electron microscope (Model no- JSM 6390LV) at an accelerating voltage of 1-30 KV.

**Biochemical Assays**

Estimation of enzymatic activities of Superoxide Dismutase (SOD), Catalase (CAT) and levels of Malondialdehyde (MDA) were estimated by preparing kidney tissue extract after animal sacrifice. The activities SOD level was assayed by method of Asada et al., 1974 using SOD assay kit (Abcam Ltd, No. ab65354), where absorbance was measured at 543 nm and results were expressed in µg/g Protein. Activity of enzyme Catalase was estimated by using Catalase assay kit (Cayman chemicals, No. 707002) through colorimetric method (Sinha, 1972), where OD was measured at 610 nm and results were expressed in kU/g Protein. Again, MDA levels in tissue extracts were assayed by standard method of Huse and Aust, 1978 using MDA assay kit (Abcam Ltd., no. 118970) and colour reaction measured at 540 nm.

**Results**

**Gross Morphology**

The STG-1 and STG-2 treated animals showed decreased body weight along with increased size and weight of kidneys as compared to the control group, which was statistically significant (p<0.01) in STG-2 group (Table-1). Besides, swollen and patchy haemorrhagic areas were also appeared in the kidney surface of STG-2 treated animals. The ratio of kidney weight relative to body weight were also increased gradually, showing statistical significance in

<table>
<thead>
<tr>
<th>Groups</th>
<th>Effects on parameters during time duration (in days)</th>
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<tr>
<td></td>
<td>30</td>
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<tr>
<td>Control</td>
<td>28.48±0.23</td>
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<tr>
<td>STG-1 treated</td>
<td>26.42±0.33</td>
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<tr>
<td>STG-2 treated</td>
<td>24.09±0.07</td>
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<tr>
<td>STG-AV treated</td>
<td>26.42±0.13</td>
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<tr>
<td>STG-BP treated</td>
<td>25.84±0.26</td>
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<tr>
<td>STG-C treated</td>
<td>27.6±0.25</td>
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*Values with different superscripts (a,b) differ significantly between groups (p<0.01)
STG-2 treated group. However, kidneys from STG-AV, STG-BP and STG-C supplemented groups, revealed almost normal morphological features. The kidney weights were found to be increased with the increased body weight at a gradual pace in all three plant extract supplemented groups. Ratio of kidney weight relative to body weight in all three plant extract supplemented groups appeared to be increased in a slow and insignificant manner.

Histopathology

Light microscopic observation of histopathological changes of kidney from STG-1 and STG-2 treated animal revealed severe alteration in comparison to control (Figure 1.A) group as well as plant extract supplemented groups. Individualization and reduction of tubular epithelium, glomerular proliferation, loss of cytoplasm as well as tubular fusion and marked calcination were observed in STG-1 treated group (Figure 1.B). In STG-2 treated group, atrophied and enlarged glomerular tuft with focal coagulative necrosis and fibrinous exudates were observed after 30 days of treatment (Figure 1.C). Complete degradation of parenchymal cells and loss of nucleus along with cytoplasmolysis in several areas inside the capsule gave rise to a networking appearance in kidney section of STG-2 treated animal (Figure 1.D). Besides, the basement membranes of capillaries were infiltrated by some eosinophilic protein cast indicating the presence of eosinophilic amyloid in lumen of the vessels. Lumens of the collecting tubules were blocked by hyaline cast causing cellular atrophy (Figure 1.E). Occurrence of lymphocyte infiltration, hypercalcinemia and presence of macrophages indicated tissue degeneration as well as nephrocalcinosis in certain areas.

In both STG-AV and STG-BP supplemented groups, some degree of glomerular proliferation were observed along with normal cellular architecture (Figure 1.F and 1.G). In STG-C supplemented group, homogenously packed structure of kidney cells were observed with minimal proliferative glomerulus structure (Figure 1.H).

Scanning Electron Microscopic analysis

Scanning electron microscopic observation of surface structure of kidney from control animal revealed a compact uniform surface topography with closely packed tubules and uniform arrangement of microvilli along the membrane. Flattened tubular cells showed homogeneity with identical membrane folds and Bowmen's capsule (Figure 2.A). Kidney surface of STG-2 treated animals showed necrosed tubules with presence of ruffled membrane, grooves, microvilli as well as cytopodial structures that got anastomosized with lateral folds giving impression of cell fusion (Figure 2.B) after 90 days of treatment. However, kidney structure from all three plant extract supplemented groups showed closely packed, intact and homogeneous kidney tubular arrangements among which STG-C group showed more integrity than STG-AV and STG-BP treated groups after 90 days of exposure (Figure 2.C; 2.D and 2.E).

Figure 1. Microphotographs of kidney section from control, STG treated and plant extract supplemented animal groups. (40x); A: Kidney from control group showing normal histological architecture; B: Individualization and reduction of tubular epithelium and glomerular proliferation after 30 days of STG-1 treatment; C: Atrophied and enlarged glomerular tuft with focal coagulative necrotic areas and eosinophilic protein cast after 30 days of STG-2 treatment; D: Kidney section showing complete tubular degradation with lymphoidal hyperplasia after 90 days of STG-2 exposure; E: Kidney after 90 days of STG-2 treatment showing disrupted tubular wall with presence of hyaline sheet, macrophages (MP) and calcinated areas; F: kidney after 90 days of STG-AV treatment; G: STG-BP supplemented kidney showing normal tubular cell with glomerular proliferation at some areas after 90 days; H: Kidney section from STG-C treated group showing uniform tubule structures after 90 days of exposure.
Biochemical Assays

The activity of Superoxide dismutase (SOD) and Catalase (CAT) as well as lipid peroxidation level through estimation of Malondialdehyde (MDA) in kidney tissue extract of control, STG treated and plant extract supplemented groups are represented in figure 3.A; 3.B and 3.C. There observed a significant (p<0.01) elevation of SOD values in both the STG treated groups as compared to the control group. A slow, gradual but non-significant incline in SOD values were observed in all three plant extract supplemented groups, among which STC group showed lowest SOD activity (Figure 3.A).

The values of CAT activity showed a gradual and significant (p<0.01) decline in STG-2 treated group as compared to the control group. In STG-AV supplemented group, fluctuated values of CAT were observed. Whereas, the STG-BP and STG-C supplemented groups showed an increased CAT activity after 90 days of exposure (Figure 3.B).

MDA content in kidney extract of STG treated groups showed a gradual and significant (p<0.01) elevation as compared to control group; whereas fluctuated but non-significant values were shown in all three plant extract supplemented groups (Figure 3.C).

Discussion

The kidney is one of the most vital body organs which perform blood filtering activity to eliminate nitrogenous wastes from the body. It also regulates extracellular fluid volumes and blood pressure level as well as maintains homeostasis. On account of its blood filtering activity, the kidney is very much prone to encounter a variety of metabolites and toxic substances produced during metabolic activities of the body. Such factors may cause different grades
of structural and functional alterations of the organ.

The central mechanism of STG intoxication is lying in the conversion of major component of tobacco (nicotine) into various nitrosamines. STG, when entered into body's metabolic route, immediately converted to its metabolites and bind with plasma proteins that eventually deposited in highly vascularised organs like kidney, which is evident in the present study. Various vital body organs like liver, kidney, lung, nasal mucosa and brain may be involved in tobacco metabolism process as reported by Hukkanen et al. (2005). After get metabolized, tobacco nicotine is excreted through glomerular filtration and tubular secretion in the kidney. Due to such active role of kidney in tobacco metabolism process, there arises utmost possibility of deposition of tobacco metabolites in this organ and consequent degenerative changes.

In the present study, several destructive changes were appeared in kidney section under LM and SEM observation as a result of STG treatment. Such destructive changes like parenchymal degeneration, swelling and individualization of tubular epithelial cells, glomerular proliferation, increased cytoplasmic granularity, focal necrosis as well as nuclear degeneration might be resulted from the inability of kidney cells to cope up with toxicant induced functional imbalances as were reported earlier (Benjamin et al., 2005; Rekha et al., 2013). Towards the end of STG exposure, the lumens of collecting tubules were appeared to be precipitated by hyaline cast causing atrophy of tubular cells. Presence of macrophages in renal tissues indicated tissue degeneration, which was supported by the fact that multinucleated foreign bodies and macrophages usually appears near to the exposed hyaline cast and degenerated epithelium. The precipitates of the excreted metabolites were gradually accumulated in the form of eosinophilic protein cast, which might be the probable cause of proliferative glomerular nephropathy. SEM observation of kidney surface structure in STG treated animals showed reduced, necrosed and individualized tubules with extensive membrane ruffles as well as proliferated glomerulus after 90 days of exposure. The adverse effects of ST products on kidney structure and functions are well evident from several studies (Arakawa and Tokunaga 1972, Kumari and Dutta, 2012; Kumari et al., 2013; Cheekuramelli et al., 2014).

The A. vera and B. pinnatum extracts, when supplemented individually, produced mild glomerular proliferation during later days of exposure. However, the combined treatment of A. vera and B. pinnatum did not showed any destructive change on kidney tissue. Therefore, it was evident that combined supplement of A. vera and B. pinnatum exerted beneficial effect on kidney tissue as compared to the individual application of them. Earlier studies also reported some of the beneficial effect of the selected plants against kidney toxicity. Yadav and Dixit (2003), reported nephro-protective activity of the B. pinnatum against gentamicin induced nephrotoxicity in mice, which might be due to the antioxidant and oxidative radical scavenging activities of the plant. Harlalka and Patil, (2007) furthermore reported the nephroprotective activity of B. pinnatum. Joshi and Chauhan (2013) referred B. pinnatum as a widely used medicinal plant against kidney disease due to its protective efficacy.

There is a relationship between the enzymatic activity and the level of damage or degree of protection to any organ of the body. The present study focuses on the level of damage or protection of kidney tissue, selecting two antioxidant enzyme SOD and CAT along with lipid peroxidation activity through determining the MDA content in kidney tissue of STG treated as well as plant extract supplemented groups. Result of the present study showed increased SOD and decreased CAT activity in kidney extracts of both STG treated groups, which indicated higher levels of oxidative stress as a result of large number of ROS generation during STG treatment. Since, plant derived phytochemicals possessed antioxidant activity, it may extend antioxidant defense against STG induced toxicity as observed in the present work. Moreover, a higher level of MDA generation in kidney tissue supported the recurrent oxidative stress in it. MDA, being the end product of lipid peroxidation, regarded as a convenient biomarker for lipid peroxidation. A higher level of MDA content in the tissues pinpoint a higher degree of lipid peroxidation, where free radical induced stressful stimuli leads to damage of cellular architecture of tissues as was manifested in SEM and LM analysis in present study. In contrary to the STG treated groups, the groups with plant extract supplement depicted lower SOD and MDA levels along with higher CAT levels. Earlier reports on antioxidant activity as well as free radical scavenging property possessed by A. vera and B. pinnatum strongly support the present finding (Okwu and Josiah, 2006; Ozsoy et al., 2009; Miladi and Damak, 2008). The crude extracts of these two plants can elicit a greater antioxidant response in the body of experimental animals, when applied in a collective manner, against STG induced toxicity compared to their individual counterparts of plant extract supplements.

Several reports from prior studies clearly suggested that STG can induce toxic stress due to its varied chemical composition that is responsible for free radical generation (Ashakumary and Vijayammal, 1996; Bagchi et al., 1996). The free radical and ROS generated by means of STG treatment can also interact with genetic transcription machinery of the cell leading to carcinogenic changes. Such degenerative change in cytomembrane or other cellular
components might be subjected to repair or ameliorate by the aid of plant derived phytocomponents. Plants selected in this experimental study i.e., *A. vera* and *B. pinnatum* have been reported to be enriched with several beneficial phytochemicals, which might enhance their protective ability against stressful situation produced by STG in animal model.

From the analysis of present findings, it can be concluded that *A. vera* and *B. pinnatum* has protective efficacy against STG induced nephrotoxicity, where the combined mixture of two plants exhibit more ameliorative potential than their individual counterparts.

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**Conflicts of Interest**

The author declares that there is no source of funding and no conflict of interest among the authors.

**References**


**Abbreviations**

*A vera* : *Aloe vera*

*B pinnatum* : *Bryophyllum pinnatum*

b. wt. : Body weight

CAT : Catalase

LM : Light Microscope

MDA : Malondialdehyde

SEM : Scanning Electron Microscope

SOD : Super Oxide Dismutase

ST : Smokeless Tobacco

STG : Smokeless Tobacco Gutkha