Research Article

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

Nabanita Medhi[1]*, Uma Dutta[2]

[1]*Post-Graduate Department of Zoology, Darrang College, Tezpur, Assam, India*

[2]*Department of Zoology, Cotton University, Guwahati, Assam, India*

Received: 1 February 2019 Revised: 19 March 2019 Accepted: 23 March 2019

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.

Address for Corresponding Author:
Nabanita Medhi
Address: Post Graduate Department of Zoology,
Darrang College, Tezpur, District-Sonitpur, Assam, India, Pin-784001
Email : nabanitamedhi97@gmail.com

The present scenario reflects availability of a number of ST products in market, among which 'Gutkha' is most commonly used and broadly marketed product. Smokeless tobacco Gutkha (STG) is a dried powdered mixture of tobacco, areca nut, lime, catechu, spices, flavorings and sweeteners which is commercially manufactured and marketed in small attractive sachets. Tobacco, the major ingredient of gutkha exerts toxicity due to the presence of its prime component-nicotine [3-(1-Methyl-2-pyrrolidinyl) pyridine]. Nicotine and its metabolites are found to be responsible for DNA damage, formation of DNA adducts and causing cancer in oral cavity and other parts of the body. Likewise, areca nut is another ingredient of STG, which possess mutagenic, cytotoxic and genotoxic properties (IARC, 1985). Generation of Reactive oxygen species (ROS) in the oral cavity of the STG chewer is favored by slaked lime and it can promote DNA damage, activation of pro-carcinogens and alteration of body's antioxidant defense mechanism (Kumari et al, 2011).

Kidney functions as blood filtering organ during excretion

DOI: https://doi.org/10.31024/ajpp.2019.5.5.7

process and thereby it become 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, immuno-stimulating, 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 Pastle. 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*

without exposing to any other test substance

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 Haemotoxylene 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 Huge 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 1. Body weight and Kidney weight (in g) and relative ratio between Kidney weight to body weight in control and treated groups of albino mice (Values shown as Mean*±S.E. of 5 animals)

Groups	Effects on parameters during time duration (in days)					
	30			90		
	Body wt.	Kidney wt.	Ratio	Body wt.	Kidney wt.	Ratio
Control	28.48±0.23	0.226^{a}±0.31	0.007^{a}	37.62±0.13	0.380^{a}±0.07	0.010^{a}
STG-1 treated	26.42±0.33	0.241^{a}±0.05	0.009^{a}	35.36±0.27	0.462^{a}±0.08	0.013^{a}
STG-2 treated	24.09±0.07	0.309^{a}±0.01	0.012^{a}	16.69±0.19	0.718^{b}±0.17	0.043^{b}
STG-AV treated	26.42±0.13	0.219^{a}±0.05	0.008^{a}	36.85±0.12	0.488^{a}±0.12	0.013^{a}
STG-BP treated	25.84±0.26	0.234^{a}±0.27	0.009^{a}	36.14±0.48	0.458^{a}±0.61	0.012^{a}
STG-C treated	27.6±0.25	0.284^{a}±0.15	0.010^{a}	38.0±0.14	0.487^{a}±0.38	0.012^{a}

*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.

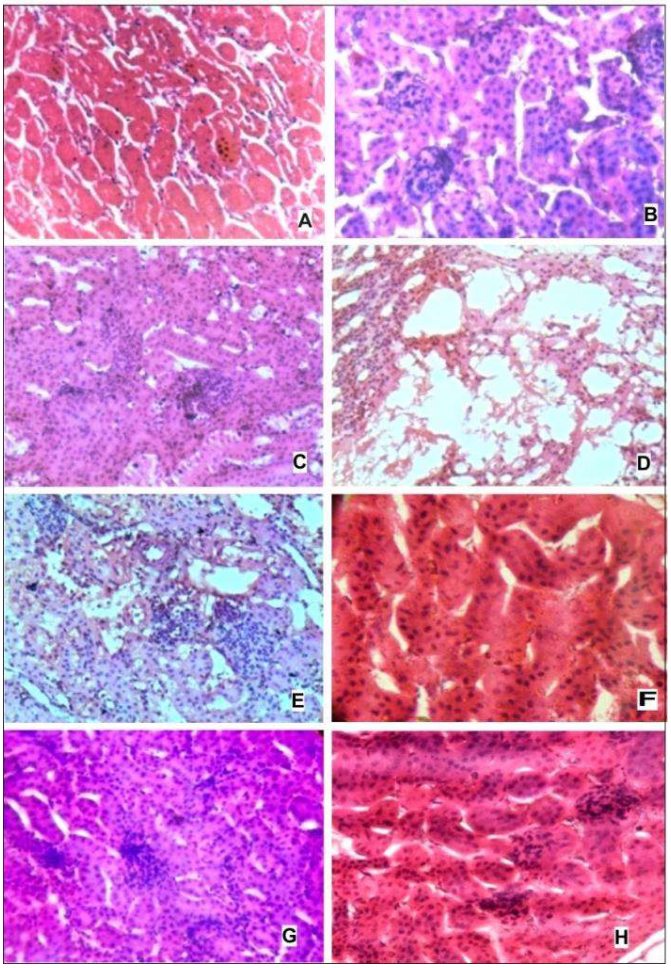

Figure 1. Microphotographs of kidney section from control, STG treated and plant extract supplemented animal groups. (40x); **A**: Kidney from control group showing normal histological architecture; **B:** Individualization and reduction of tubular epithelium and glomerular proliferation after 30 days 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.

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).

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).

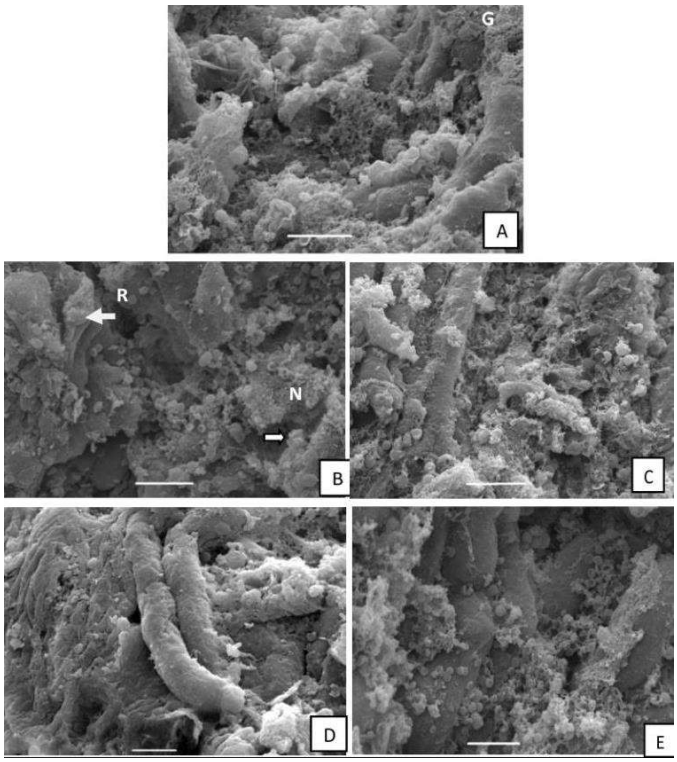

Figure 2. A: SEM image of cut surface view of kidney from control group showing glomerulus (G) and homogenous membrane fold X900 (bar=20 µm); **B:** Scanning electron microphotograph of cut surface view of kidney from STG treated group after 90 days of treatment showing necrosed (N) tubules with ruffled (R) membrane and cell fusion; **C: Kidney** from STG-AV group showing intact tubular cells with cytopodial projections; **D:** STG-BP supplementeded kidney showing homogenous tubules after 90 days of exposure**; E:** Cut surface view of kidney from STG-C supplemented animals showed uniform tubular arrangement with presence of normal RBC -- X900 (bar=20 µm**)**.

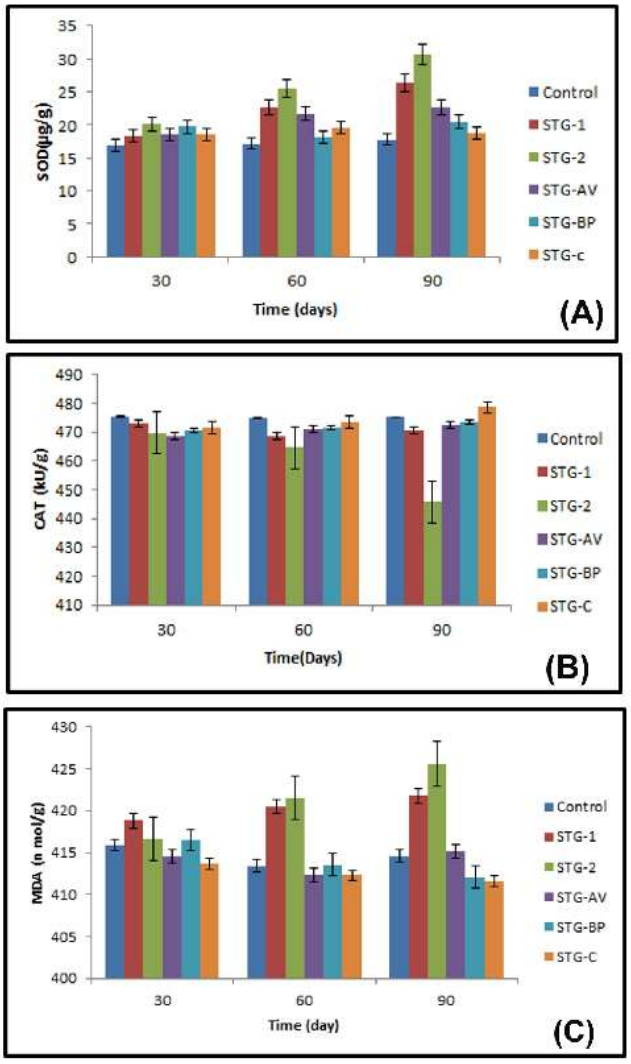

Figure 3. (A) Superoxide Dismutase (SOD) (µg/g prot.) activity; (B) Catalase (CAT) (kU/g prot.) activity; **(C)** Malondialdehyde (MDA) (N mol/g prot.) content (LPO), in Kidney tissue extract in control, STG treated and Plant extract supplemented groups

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 pinpointed 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.

Acknowledgement

The Authors like to offer thanks to department of Zoology, Darrang college, Tezpur and Cotton University, Guwahati for providing laboratory facilities. Authors are also thankful to the Sophisticated Analytical and Instrumentation Facility (SAIF), North Eastern Hill University, Meghalaya and Tezpur University, Assam for providing SEM facility.

Conflicts of Interest

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

References

Arakawa M, Tokunaga J. 1972. A scanning electron microscope study of the glomerulus- Further consideration of the mechanism of the fusion of podocyte terminal processes in nephritic rats. Laboratory Investigation: A Journal of Technical Methods and Pathology. 27(4):366.

Asada K, Kiso K. 1973. Initiation of aerobic oxidation of sulphite by illuminated spinach chloroplasts. European Journal of Biochemistry 33**:** 253–257.

Ashakumary L, Vijayammal PL. 1996. Effect of nicotine on antioxoidant defense mechanisms in rats fed a high fat diet. Pharmacology 52: 153-158.

Bagchi M, Bagchi D, Adickes E, Stohs SJ. 1996. Chronic effects of smokeless tobacco extract on liver histopathology and production of HSP-90. Journal of Environmental Pathology, Toxicology and Oncology 14(2): 61-68.

Benjamin N, Kushwah A, Sarma RK, Katiyar AK. 2005. Histopathological changes in liver, kidney and muscles of pesticides exposed malnourished and diabetic rats. Indian Journal of Experimental Biology 44: 228-232.

Chauhan OP, Raju PS, Khanum F, Bawa AS. 2007. *Aloe vera* – Therapeutic and Food applications. Indian Food Industry 26(3): 43-51.

Cheekuramelli SN, Deepa RN, Janarthan M, Durraivel S. 2014. Protective effect of *Vigna aconitifolia* methanolic seed extract against smokeless tobacco (Gutkha) chewing and on chemically induced hepatotoxicity and nephrotoxicity in rats. International Journal of Pharmacy and Pharmaceutical Sciences 6(4): 88-95.

Harlalka GV, Patil CR. 2007. Protective effect of *Kalanchoe pinnata* pers. (Crassulaceae) on gentamicine induced nephrotoxicity. Indian Journal of Pharmacology 39(4): 201-205.

Huege JU, Aust SD. 1978. Microsomal lipid peroxidation. Methods in Enzymology, 5: 302-310.

Hukkanen J, Jacob P, Benowitz NL. 2005. Metabolism and disposition kinetics of nicotine. Pharmacology Review 57:79-115

International Agency for Research on Cancer (IARC). 1985. Tobacco habits other than smoking. IARC Monographs on Betel-quid and Areca-nut Chewing and Some Related Nitrosamines 37: 141–200.

Joshi A, Chauhan RS. 2013. Phytochemical analysis and cytotoxicity studies of *Bryophyllum calycium* in BHK-21 cells A. Scholars Academic Journal of Pharmacology 2(3): 190-194.

Kumari S, Dutta A. 2012. Histochemical studies on the induced toxicity of pan masala on various organs of swiss mice and the protective effect of *Elettaria cardamomum (L.) maton*. Annals of Biological Research 3(4): 1919-1922.

Kumari S, Dutta A, Lal N. 2013. Long-term toxicity of pan masala in female swiss mice. European Journal of Experimental Biology 3(2):271-274.

Kumari S, Mojidra BN, Gautam AK, Verma Y, Kumar S. 2011. Reproductive toxic potential of Pan Masala in Swiss albino mice. Toxicology and Industrial Health 27(8): 683-690

Luna LG. 1968. Manual of Histologic Staining. Methods of Armed Forces Institute of Pathology.3 rd Edn. American Registry of Pathology, pp. 12-39. New York, McGraw Hill.

Miladi S, Damak M. 2008. In vitro antioxidant activities of *Aloe vera* leaf skin extracts. Journal de la Société Chimique de Tunisie 10:101-109

Ojewole JAO. 2002. Antihypertensive properties of Bryophyllum pinnatum (Clam) Oken) leaf extracts. American Journal of Hypertension 15(4): A34-A39.

Okwu DE, Josiah C. 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology 5(4): 357-361.

Ozsoy N, Candoken E, Akev N. 2009. Implications for degenerative disorders: Antioxidative activity, total phenols, flavonoids, ascorbic acid, β-carotene and β-tocopherol in *Aloe vera*. Oxidative Medicine and Cellular Longevity *2*: 99-106.

Palade GE. 1952. A study of fixation for electron microscopy. Journal of Experimental Medicine 95(3):285.

Patel K, Kumar N, Sharma KH. 2011. Pharmacognostic & phytochemical evaluation of *Bryophyllum pinnatum* leaves. Journal of Advanced Scientific Research. 2(1): 42-49.

Pawar VC, Bagatharia SB, Thaker VS. 2005. Antibacterial activity of *Aloe vera* leaf gel extracts against *Staphylococcus aureus*. Indian Journal Microbiology. 45(3): 227-229.

Rekha RS, Hamid S. 2013. Histopathological effects of pesticide-cholopyrifos on kidney in albino rats. International Journal of Research on Medical Sciences 1 (4):465-475.

Sabatini DD, Bensch K, Barnett RJ. 1963. Cytochemistry and electron microscopy: the preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. Journal of Cell Biology 17:19-58.

Shikarwar SM, Patil BM, Sharma S, Bhat V. 2010. *Aloe vera:* Plant of Immortality. International Journal of Pharmaceutical Sciences and Research 1(1): 7-10.

Sinha KA. 1972. Colorimetric assay of Catalase. Annals of Biochemistry. 47: 389-394

Yadav NP, Dixit VK. 2003. Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. Journal of Ethnopharmacy 86: 197-202.

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