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RESEARCH ARTICLE

# Ameliorative efficacy of aqueous extract of clove bud (AEC) against smokeless tobacco product induced antioxidative damages: An experimental study on male albino rat

**Merina Yasmin, Chaitali Kundu, Monalisha Paul, Sandip Kumar Sinha***

## Abstract

The effects of STP (zarda) on several metrics, such as antioxidant and histological indices, were investigated. Such chemicals, together with active likely components, were found in the specific STP (zarda) and clove bud extract (AEC) using LCMS and FTIR analysis. Furthermore, phytocompounds that effectively treat STP-induced changes (antioxidant defense state and histological alteration) were identified in order to evaluate the efficacy of clove bud aqueous extract (AEC). This study also examined the comparative efficacy of vitamin C and AEC. Gr-I (saline control), Gr-II (STP treated only, 85 mg/kg bw), Gr-III (AEC treatment only, 200 mg/kg bw), Gr-IV (STP+Vit C, 100 mg/kg bw), and Gr-V (STP+AEC, 200 mg/kg bw) were the four groups of animals (100–130 gm body weight). Each dosage was administered for a maximum of four weeks. After the animals were killed, blood samples were taken, and serum was made for several investigations. Gr-III (only AEC treated) did not exhibit any toxicity in either of the two parameters, although Gr-V (STP+AEC treated) recovered better than Gr-IV (STP+vit C, 100 mg/kg bw) in all parameters. Furthermore, this study showed that, in contrast to the control group (Gr-I), SOD and Catalase levels dramatically ($p$ <0.05) reduced in Gr-II (only STP) and elevated again in Gr-IV (STP+vit C, 100 mg/kg bw). Histological analyses showed a significant change in tissue morphology. According to the results of the entire study, using STP causes major G.I. health issues because it contains addictive chemicals, but the presence of bioactive compounds in the AEC has greatly reduced the harm caused by STP.

**Keywords:** Smokeless tobacco, Antioxidant enzymes, Clove bud aqueous extract, GI tract organs.

## Introduction

Smokeless tobacco (SLT) use is expanding quickly in India despite little awareness of the harmful impact it has on our physiological systems. The two most popular types of tobacco products are smokeless and smoking; both contain nicotine, which is present in all commercially available forms of traditional STP, including chewing, snuffing, and inhaling. Through this study, a common chewing form of smokeless tobacco (zarda) was used for experiment on animal model. Like other tobacco component, zarda contain also Nicotine. Mainly nicotine is two form either protonated or unprotonated (Saxena *et al*., 2022). An alkaloid that is extremely addictive and dangerous is nicotine. The rate of nicotine absorption actually dictates the degree of addiction, based on the PH of that particular type of STP (Wilhelm *et al*., 2022). STP comes in two primary forms: chewing tobacco and snuff or dipping tobacco. In reality, snuff or dipping tobacco is a dry or moist substance that is put in the mouth or nose. Essentially chewing tobacco is composed of loose or plug-twisted cured tobacco leaves that are chewed in the mouth (Banerjee *et al*., 2014). Gutkha, zarda, khaini, paan, betel quid, areca nut, mawa naswar, snus, snuff, and other STP forms are available in our market (Henningfield *et al*., 1997). These types of SLT are actually designed to make consumers more comfortable and help them keep that particular tobacco product in their mouths for longer. More lately, there have been split-less oral ST items that do not require spitting, like little pouches. To

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improve their test and make them more appealing, flavors of fruit or mint have been added (Tricker & Preussmann, 1989). As a result, the user's physiological system is more susceptible to addiction. The STP (zarda) in this investigation was initially described by LCMS and FTIR study. A wide range of commercially available products and mixes that employ tobacco as the main ingredient and are administered orally or nasally without burning are referred to as smokeless tobacco (Samman *et al*., 1998)**.** Because of its widespread marketing and the potential for nicotine addiction, tobacco smoking is well-known around the world. Legislation and warnings on tobacco use and cheap prices are either nonexistent or inadequate (WHO, 2015). The usage of smokeless tobacco presents several health risks. According to the World Health Organization's 2015 report on the global tobacco epidemic, tobacco-related diseases claim the lives of over 6 million people annually. According to Callahan-Lyon (2014), smokeless tobacco contains 28 non-carcinogenic and nitrogen-specific compounds. Traditional medicine is the most readily available, reasonably priced, and side-effect-free therapy option for these STP-related impairments in the primary health care system. Medicinal plants or plant parts have long played an amazing role in both preventing and treating disease. Many phytocompounds, including flavonoids, alkaloids, tannins, phenolics, and terpenoids, were identified using phytochemical screens of the aqueous extract of plant parts. The combination that intensifies the treatment of illness is the phytocompounds found in medicinal plants. In this study, try to establish the efficacy of clove bud aqueous extract (AEC) against STP related gastrointestinal tract damages. Through this study we also compare between vit C and AEC. Ascorbic acid, or vitamin C, is an essential antioxidant that dissolves in water in human serum. It reduces the oxidative characteristics of deadly chemicals by acting as a biological antioxidant. Compared to non-smokers, smokers have lower vitamin C concentrations (Gunes *et al*., 2008). According to Gallo *et al*. (2010), vitamin C can scavenge free radicals and create a powerful defense against reactive oxygen species (ROS), which cause cellular damage. Malon-di-aldehyde, liver enzymes, and elevated glutathione levels in kidney and liver tissue were all markedly reduced when vitamin C and nicotine were administered together. Furthermore, co-administration of vitamin C markedly enhanced the capacity of hepatic and renal tissue to proliferate (Mahmoud & Amer**,** 2014). Prior research examined the potential benefits of vitamin C in mitigating the metabolic oxidative damage caused by nicotine use. However, the potential protective benefits of vitamin C in combination with nicotine treatment on histological and ultra structure levels have received little attention (Hassan *et al*., 2016). Hence, this present study was evaluated the protective effect of AEC regarding the STP induced changes of oxidative stress of serum and histological alteration of gastrointestinal tissues.

## Materials and Methods

### Materials

Tris bBuffer, DAPA, pyrogallol, ethylenediamine tetrachloroacetic acid (EDTA), $H_2O_2$, potassium phosphate buffer (PBS), Tris-HCl buffer, ter-butyl hydro peroxide (BHP), ethanol, sodium dodecyl sulfate (SDS), acetic acid, Thio barbituric acid (TBA), formalin, hematoxylin-eosin were purchased from Himedia chemicals (Kolkata, west Bengal).

### *Methods*

*Animal maintenance*

These experiments were carried on with male albino rats (100–130 gm body weight), which were obtained from a standard animal supplier (Regn no: 2018/GO/Re/S/18/CPCSEA No.) and those were kept in CPCSEA approved animal house of the institution. The animals were housed in a clean polypropylene cage in a standard temperature (20 ± 2° C) with relative humidity (45–60%) less than 12 hr light dark cycles during the whole study period. Then animals were acclimatized in the animal house for 15 days before starting the experiment.

*Preparation of STP extract*

We collected the readymade STP (zarda), which are available in our local market. Then it was sundried and grinded for powder form by the help of mixture. After that, we combined 50 ml distilled water with 10 g of STP dust for extract preparation. Then it was kept in shaking incubator (37°C) up to 48 hours for well mixed Subsequently the well mixture was filtered by using whatmann filter paper and concentrated by rotary evaporator to dryness of solid residue was obtained. Then it transferred to an airtight bottle and stored in refrigerator at 4°C and further dose preparation and administered orally (Avti *et al*., 2006).

*Preparation of aqueous extract of clove bud (AEC)*

10 gm crude powder was mixed well in 50 ml distilled water. Mixture was left overnight in a conical flask. Then the mixture was extracted again the following day filtered through whatmann filter paper and stored in container and concentrated by rotary evaporator to dryness of solid residue was obtained. Extract was used within two days of preparation. (Ahmed *et al*., 2012).

*Animal sacrifice and sample collection*

At the time of the experiment, the rats were fasted for 24 hours, weighed and then killed by total exsanguinations. The rats were anesthetized with an intraperitoneal injection of serum pentobarbital (8 mg/100 g body weight) and blood will be removing from the inferior vena cava in a syringe containing EDTA (1-mg/mL blood) and chilled on ice throughout processing whole blood was centrifuged at 4°C for 20 minute at 1200x g to obtain plasma. Then serum will be prepared for experiment (Nishina *et al*., 1990).

**Table 1:** Animal group allocation and dose administration

| *Animal (N=6)* | *Group division* | *Treatment protocol* |
|---|---|---|
| Male albino rat (100–130 gm bw) | Group-I (Saline control) | Supply normal diet and water ad libitum with normal saline orally administered. |
| | Group -II (STP only*)* | Supply normal diet and water with STP applied (AEZ) orally at single dose: established on $ED_{50}$. (85 mg/kg bw). |
| | Group-III (AEC only*)* | 200 mg/kg bw AEC administered orally at a single dose. |
| | Group-IV (STP + Vit C*)* | 85 mg/kg bw STP and 100 mg/kg bw Vit C administered orally at 6 hour gap for 4 weeks (repeat dose). |
| | Group-V (STP + AEC*)* | 85 mg/kg bw STP and 200 mg/kg bw AEC administered orally at 6 hour gap for 4 weeks (repeat dose). |

*Characterization of STP extract and AEC*

- *LCMS analysis*

Through LCMS analysis, help to separation and determine the molecular mass of the several biomolecules and synthetic compounds with their diode array detection pattern and structural information through fragmentation study. The high sensitive LC-MS/MS are routinely used for mass measurement of flavonoids, alkaloids, phenolics, peptides, drugs, pesticides, terpenoids, and some synthetic compounds with the mass range of 10-2000 Dalton (Sommer *et al*., 2006).

*FTIR analysis of STP extract and AEC*

FT-IR analysis was carried out by using Fourier Transform Infrared Spectrometer (FTIR Spectrometer) model no-NICOLET 6700, Manufacturer - M/S THERMO FISHER SCIENTIFIC INSTRUMENTS. FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range measured as wave numbers is typically over the range 4000 to 600 $cm^{-1}$ (Berthomieu *et al*., 2009).

*Measurements of oxidative stress (through ROS estimation) of serum*

- *Measurement of Superoxide dismutase (SOD)*

Superoxide dismutase (SOD) was estimated according to the method of Kar Mahapatra *et al*., 2009. Briefly, at first serum sample was taken 50 µL. Then, 50 mM Tris Buffer, 1-µM DAPA and 0.2 µM pyrogallol was taken and well mixed. After that, it was incubated up to 3 minutes at 25°C temperature. Then, after 3 minutes OD value was recorded at 420 nm. SOD was expressed in terms of IU/mg protein.

- *Measurement of Catalase (CAT)*

Catalase activity was measured by the method of Beer & Sizer, 1952. The final reaction volume of 3 mL contained 0.05 M Tris-buffer, 5 mM EDTA (pH 7.0), and 10 mM H2O2 (in 0.1 M potassium phosphate buffer, pH 7.0). About 100 µL aliquots of the serum sample were added to the above mixture. The rate of change of absorbance per min at 240 nm was recorded. Catalase activity was calculated by using the molar extinction coefficient of 43.6 M1 $cm^{1}$ for $H_2O_2$. The level of CAT was expressed in terms of mill moles H2O2 consumed/min/mg of protein.

- *Measurement of Malon-di-aldehyde (MDA)*

Lipid per oxidation was estimated by the method of Ohkawa *et al*. (1979) in serum sample. Briefly, the reaction mixture contained Tris-HCl buffer (50 mM, pH 7.4), ter-butyl hydro peroxide (BHP) (500 lM in ethanol) and 1 mM FeSO4. After incubating the samples at 37°C for 90 minutes, the reaction was stopped by adding 0.2 mL of 8% sodium dodecyl sulfate (SDS) followed by 1.5 mL of 20% acetic acid (pH 3.5). The amount of malondialdehyde (MDA) formed during incubation was estimated by adding 1.5 mL of 0.8% TBA and further heating the mixture at 95°C for 45 minutes. After cooling, samples were centrifuged, and the TBA reactive substances (TBARS) were measured in supernatants at 532 nm by using 1.53 $3\times10^5$ $M^{-1}cm^{-1}$ as extinction coefficient. The levels of lipid per oxidation were expressed in terms of nmoles of TBARS per 90 min/mg protein.

*Histological study*

After animal sacrifice, tissues of the GI tract esophagus, liver, stomach, small intestine and large intestine were used for histological examinations. Tissues were fixed in 10% buffered formalin (pH 7.2) and dehydrated through a series of ethanol solutions, embedded in paraffin, and routinely processed for histological analysis. Sections of 2 µm thickness were cut and stained with hematoxylin-eosin for examination. The stained tissues were observed through a Carl-Zeiss microscope (Axio 1) and photographed by a chare-couple device (CCD) camera (Titford, 2009).

*Data analysis*

All the data were expressed as mean ± SEM. Statistical analysis was calculated by one way ANOVA by origin 6.0 professional software (SAS Institute Inc.,1985).

## Results

### *LCMS analysis of STP (Zarda) and clove bud aqueous extract*

In this study, aqueous extract of STP and clove bud were prepared for liquid chromatography mass spectrometry (LCMS) for identification and seperation of probable bioactive compounds presents in the sample. From LCMS study, STP aquoeus extract contain numbers of chemical compounds, among them nicotine is the major compound and aqueous extract of clove bud contain a probable compound of hydroxybenzoic acid derivatives ,i.e., chlorogenic acid.

From LCMS analysis of this STP (zarda), main compound is nicotine (162.64 g/mol). Above chromatogram represent the highest peak is 162.84, which match with the exact molar mass of nicotine. another compounds are benzo (b) fluoranthene (BBF), benzo (k), fluoranthene (BKF), etc.

From LCMS analysis of this AEC, main compound is chlorogenic acid (354.31 g/mol). Above chromatogram represent the highest peak is 354.33, which match with the exact molar mass of chlorogenic acid. Another expected bioactive compounds are gallic acid, ferulic acid, salicylic acid and ellagic acid.

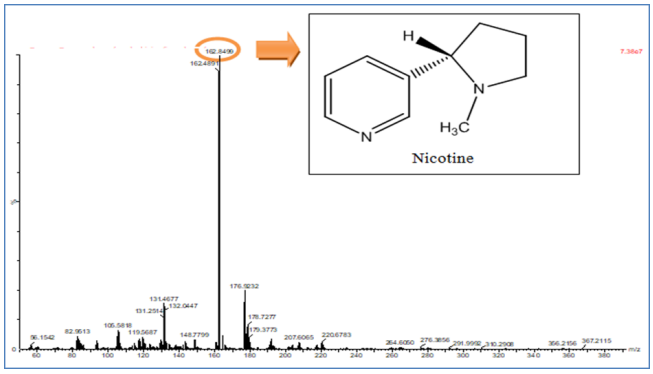


**Figure 1:** Identified chemical compound from STP (Zarda extract) formulation using LC-MS/MS

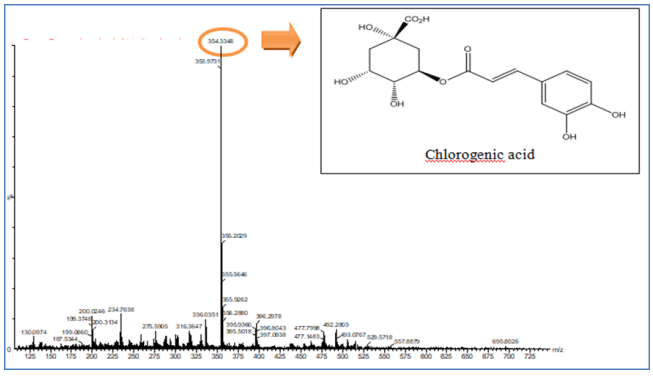


**Figure 2:** Identified phytocompound from aqueous extract of clove bud formulation using LC-MS/MS

### *FTIR analysis of the sample*

The FTIR spectroscopy was carried out to ascertain fuctional groups. The FTIR spectrum of IR absorption bands in the reigeon 3200 to 3500 $cm^{-1}$ corresponds to O-H streching , intermolecular strong H-bond group present.The observed band at 2500 to 3300 $cm^{-1}$ can be assigned to C-H streching strong H- bond. The band at 1715 to 1730 $cm^{-1}$ can be due to C=O streching , strong bond(α,β unsaturated esters) present. The band at 1600 to 1650 $cm^{-1}$ can be due to C=C ,streching medium (conjugated alkene respectively) bond is present. IR absorption bands in the reigeon 1000 to 1400 $cm^{-1}$ can be due to C-N streching strong bond is present.

The FTIR spectroscopy was carried out to ascertain fuctional groups. The FTIR spectrum of IR absorption bands in the reigeon 3200 to 3500 $cm^{-1}$ corresponds to O-H streching , intermolecular strong H-bond group present.The observed band at 2500 to 3300 $cm^{-1}$ can be assigned to C=O streching strong H- bond. The band at 2840 to 3000 $cm^{-1}$ can be due to C-H streching , strong bond present.The band at 1600 to 1620 $cm^{-1}$ can be due to C=C ,streching medium bond is present. IR absorption bands in the reigeon of 1365 to 1370 $cm^{-1}$ can be due to C-O bending bond is present.

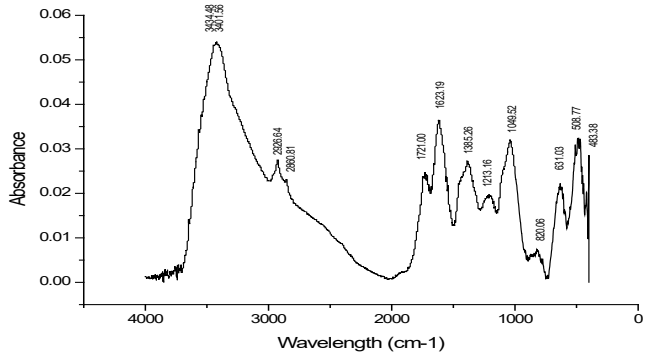


**Figure 3:** Analysis of IR spectra of STP

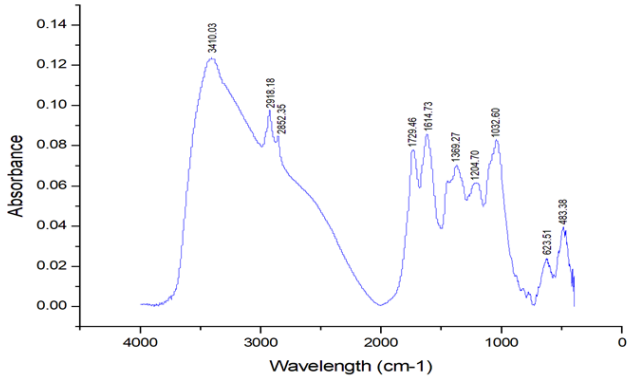


**Figure 4:** Analysis of IR spectra of AEC

**Table 2:** FTIR analysis of STP

| *Sl. No.* | *Frequency range ($cm^{-1}$)* | *Expected functional group* |
|---|---|---|
| 1 | 3200-3500 | O-H, Stretching |
| | | N-H, Stretching |
| 2 | 2500-3300 | C-H, Stretching |
| 3 | 1715-1730 | C=O, Stretching |
| 4 | 1600-1650 | C=N, Stretching |
| | | C=C, Stretching |
| 5 | 1000-1400 | C-N, Stretching |
| | | C-F, Stretching |

**Table 3:** FTIR analysis of AEC

| *Sl. No.* | *Frequency range ($cm^{-1}$)* | *Expected functional group* |
|---|---|---|
| 1 | 3200-3550 | O-H, Stretching |
| 2 | 2500-3300 | C=O, Stretching |
| 3 | 2840-3000 | C-H, Stretching |
| 4 | 1720-1740 | C=O, Stretching |
| 5 | 1610-1620 | C=C, Stretching |
| 6 | 1365-1370 | C-O, Bending |
| 7 | 1200-1260 | =C-O, Stretching |
| 8 | 1040-1050 | CO-O-CO, Stretching |

The band at 1200-1260 $cm^{-1}$ can be due to =C-O streching present.At last, the observed band 1040 to 1050 $cm^{-1}$ can be due to CO-O-CO, streching.

### *Measurements of oxidative stress (through ROS estimation) of serum*

Serum antioxidant enzymes level among different groups was shown in Figure 5(A, B, C). The level of SOD and catalase of Gr-II (only STP treated) were lower than (Gr-I) control group ($p$ <0.05).Whereas, the MDA level of serum were significantly higher than (Gr-I) control group. The result of serum SOD, catalase and MDA level of Gr-III (only AEC treated) were not significant changes for absence of its own toxicity. SOD, catalase & MDA level of serum of Gr-IV (STP+VitC treated) changes significantly compare to Gr-II (Only STP treated) and level towards control group, but Gr-V (STP+AEC treated) showed more significant ($p$ <0.05) changes compare to Gr-IV.

### *Histological images of esophagus, liver, stomach and small & large intestine*

Because smokeless tobacco contains hazardous chemicals like nicotine, it can change the histological properties of G.I. tract tissues. Thus, in the STP treated group (Group-II), histological changes were observed in the esophagus, liver, stomach, and small and large intestine. In every tissue, Gr-III

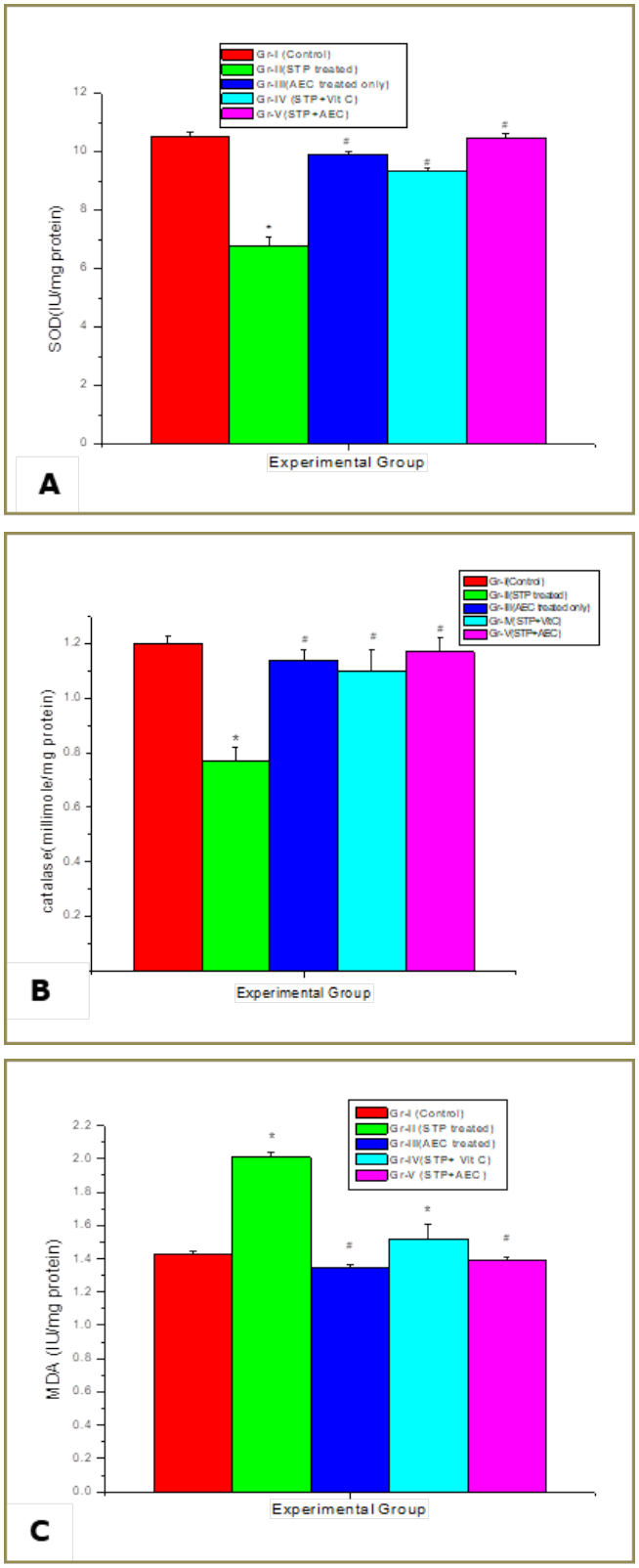


**Figure 5:** (A, B, C) shows changes of serum SOD, Catalase and MDA level respectively. Here * indicates the level of significant (at p<0.05) difference by one way ANOVA in comparison to the group-I with other groups. # indicates the significant changes of group-II with other groups.

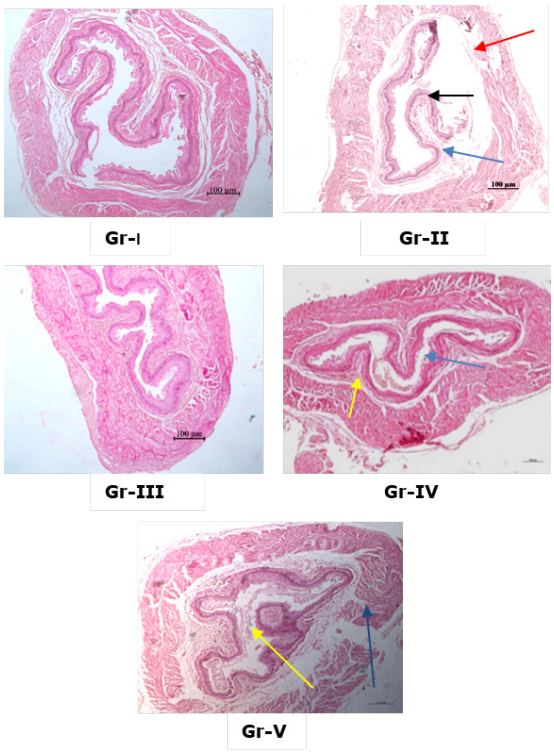


**Figure 6:** Histological changes in esophagus. In Group I, the esophagus of the control group showed normal tissue architectures. In Group II, the esophagus tissue showed lesion of the mucosal layer (black arrow), necrosis of sub mucosa layer (blue arrow) and discontinuous muscularis mucosa (red arrow). In Group III does not show any significant changes in esophagus. In Group IV, congested luminal space (blue arrow) and discontinuous lamina propia (yellow arrow) In Group V, esophageal tissue showed better muscularis mucosa and proper luminal space (blue arrow & yellow arrow) and mucosal layer comparatively towards Gr-II and Gr-IV (black arrow). (Bar = 100µm). (H&E).

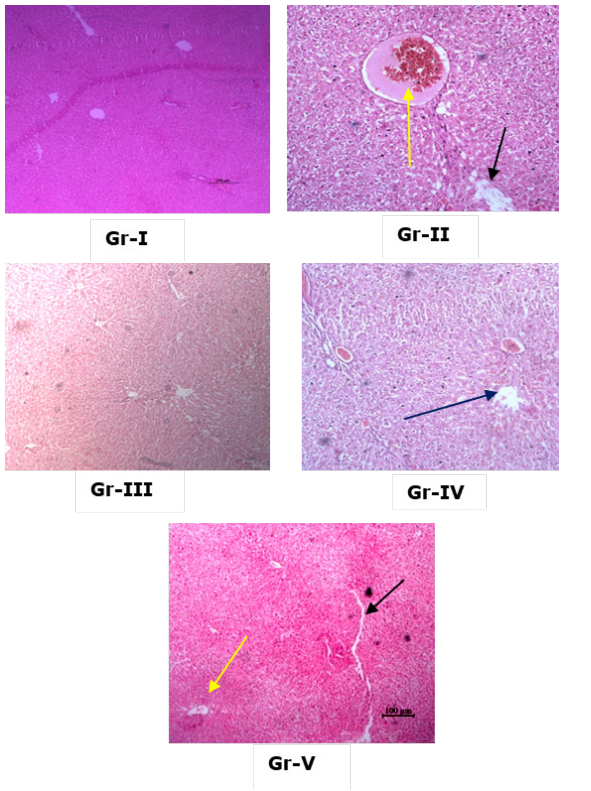


**Figure 7:** Histological changes in liver. In Group I, the liver of the control group showed normal tissue architectures. In Group II, black arrow indicates disorganization of hepatic cords, the yellow arrow shows enlarged central vein. In Group III does not show any significant changes in liver. In Group IV, reduce the lesions of hepatic sinusoids and central vein (blue arrow). In Group V, liver tissue showed less disorganization of hepatic cords (black arrow) and comparatively less enlarged central vein (yellow arrow) (Bar = 100µm). (H&E)

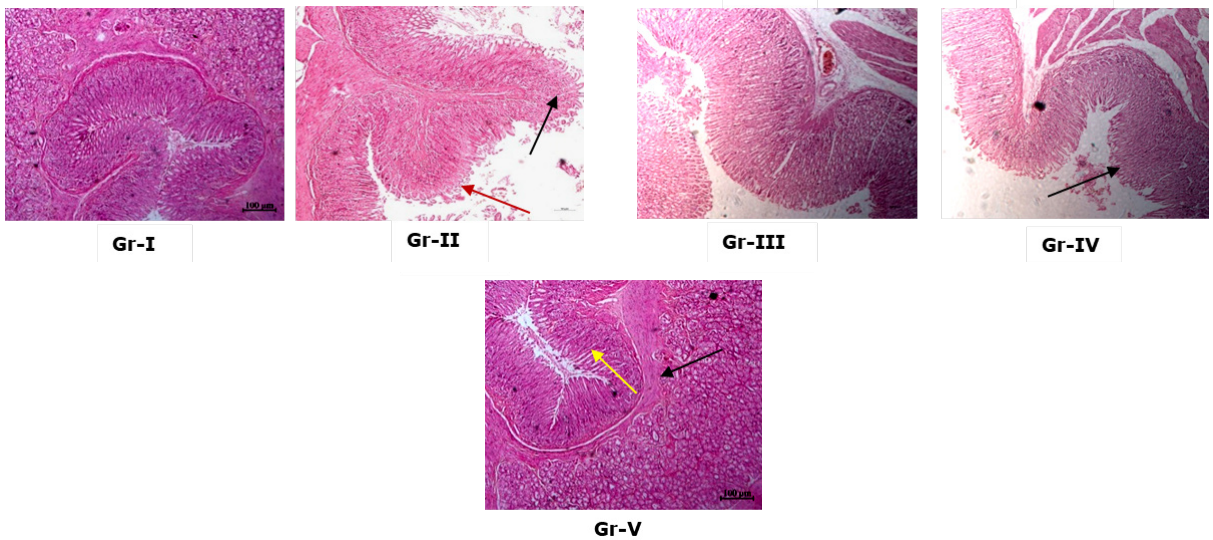


**Figure 8:** Histological changes in stomach. In Group I, the stomach of the control group showed normal tissue architectures. In Group II, the cell damages (Black arrow) and gastric gland swelling (red arrow) were observed. In Group III does not show any significant changes in stomach. In Group IV, few lesions seen in epithelium brush border (black arrow). In Group V stomach tissue showed less cell damage (Black arrow) and less swelling in gland (yellow arrow)(Bar = 100µm). (H&E)

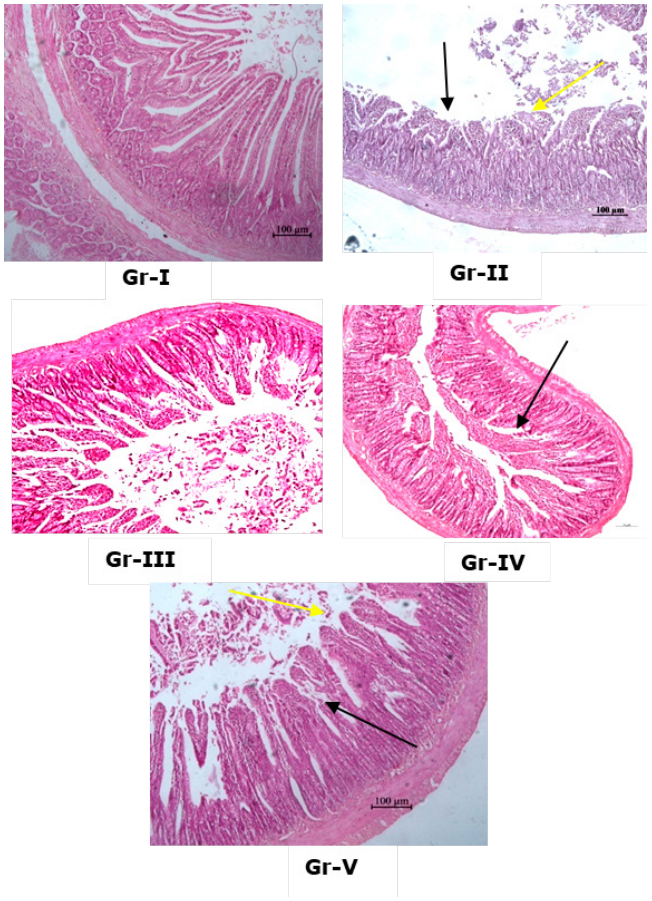


**Figure 9:** Histological changes in small intestine. In Group I, the small intestine of the control group showed normal tissue architectures. In Group II, the degeneration of apical surface epithelium (black arrow), congested cells in the intestinal glands (red arrow) and irregularities in microvillus (Yellow arrow) were observed. In Group III does not show any significant changes in intestine. In Group IV, intestinal luminal space such congested and few lesions seen among microvillus (black arrow). In Group V, intestinal tissue showed less congested cell in the intestinal gland (black arrow) and less irregularities in microvillus (yellow arrow)(Bar = 100µm). (H&E).

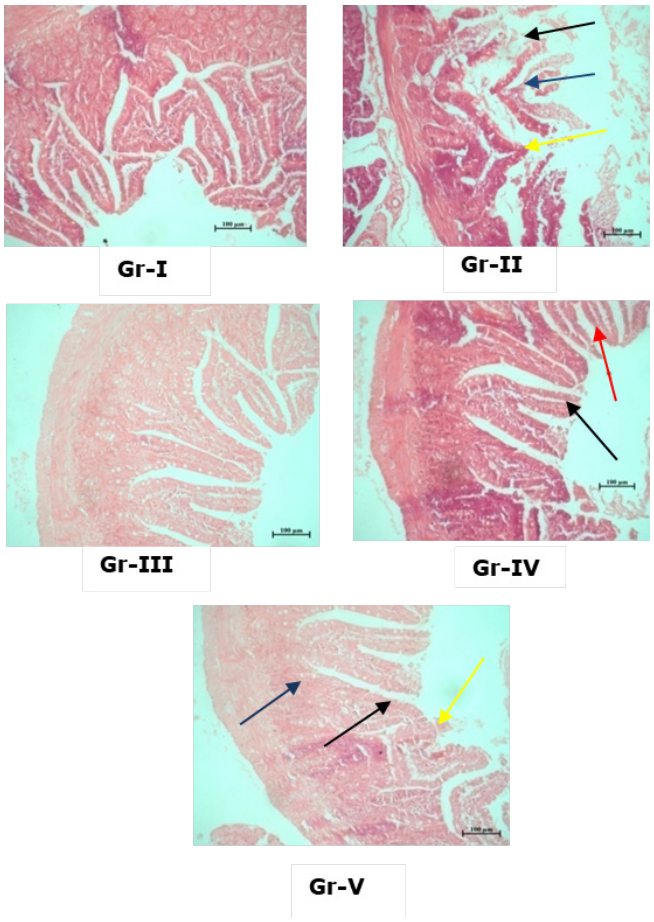


**Figure 10:** Histological changes in Large intestine. In Group-I, the large intestine of the control group showed normal tissue architectures. In Group II, the degeneration of apical surface epithelium (yellow arrow), and absence of prominent columnar epithelial cell (black arrow) and Crypts of Liberkuhn (blue arrow). In Group –III (only AEC treated) does not show any significant changes in large intestine. In Group-IV, less discontinuous of surface epithelium (black arrow) and crypts of Liberkhun (red arrow) compare to group-II. In Group-V, intestinal tissue showed less degeneration of epithelium cell in the intestinal gland (black arrow) and less irregularities in surface epithelium (yellow arrow) less shrinkage of muscularis mucosa (blue arrow) comparative to Group-IV (Bar = 100µm) (H&E).

(only treated with AEC) displayed no toxicity. All tissues treated with Gr-IV (STP+ Vit C) recovered from STP toxicity, but Gr-V (STP+ AEC) recovered better. Histological analysis once more demonstrated that our chosen medicinal spice extract (AEC) improved more than vitamin C.

## Discussion

Numerous investigations claim that STP is a chemically diverse substance that affects our gastrointestinal health in addition to other physiological systems (Rajasekhar *et al*., 2006). The effects of chewing ST exposure on blood, antioxidant enzyme levels (SOD, Catalase) and MDA vary greatly due to a number of important factors (Avti etal., 2005). The present study was conducted to investigate the possible ameliorative effect of AEC as well as vitamin C also against STP induced alteration of anti oxidative profile and lipid per oxidation (MDA level) in serum. Lower levels of antioxidant enzymes such as SOD, CAT might be due to the overwhelming effects of free radicals, as evidenced by the elevated levels of lipid per oxidation (Kalpana *et al*., 2004). The variable effects of STP extract on different organs could be due to the different metabolites affect in these organs (Hecht, 2007; Hatshukhami *et al*., 2007; Stepanov and Hecht, 2005b). These might have altered the antioxidant defense system in such a way that the oxidative stress had a variable effect on the different G.I. tract organs. Cellular antioxidant enzymes such as SOD, CAT, and free radical scavengers like vitamins C protect cells and tissues against noxious

radicals. An imbalance between cellular pro-oxidant and antioxidant levels results in the oxidative stress that leads to tissue damage (Chattopadhya & chattopadhya, 2008). The antioxidant enzymes react directly with reactive oxygen species (ROS) to yield non-radical products. Superoxide dismutase, a mitochondria as well as cytosolic enzyme, dismutase $O_2$ to $H_2O_2$, which is decomposed by CAT to $H_2O$. In this present study, the activity of serum SOD and catalase was decreased significantly which might have led to the inefficient removal of $O_2$ radicals from the cellular milieu, resulting in the ROS burden. Whereas, MDA level was significantly increased compare to control group with 28 days administration of STP extract. So, Overproduction of these radicals has an inhibitory effect on the enzymes responsible for removal of ROS such as CAT and SOD. It has been reported that superoxide radicals inhibit CAT activity and that H2O2 suppresses SOD activity (Hassan and Fridovich, 1978). Because nicotine is easily absorbed through the mouth cavity of the mammalian system, it is a major chemical that has the ability to cause addiction in general populations, according to LCMS and FTIR study of STP. However, an aqueous extract of clove buds (AEC) from a medicinal spice investigation using LCMS and FTIR revealed the existence of a expected bioactive compound called chlorogenic acid, which may have demonstrated recovery potential against the toxicity of STP. In comparison to the treatment group, a medicinal spice extract (AEC, 200 mg/kg b/w) shown higher efficacy and improved the alternation at a significant level ($p<0.05$). In addition, Gr-V (STP+AEC 200 mg/kg b/w) exhibits superior efficacy against STP-related histological tissue damages (oesophagus, liver, stomach, small intestine and large intestine) in a comparative research between vitamin C and AEC and all parameters, and they differ considerably ($P<0.05$) from the treated group and the control group. Vitamins constitute one of the most important micronutrients as well as well known antioxidant that modulate the defense mechanism. Vitamins such as vitamin A, C, and E are the chief non-enzymatic antioxidants present in the body to scavenge free radicals. Among them, Vitamin C is an important extracellular antioxidant, which disappears faster than other antioxidant when exposed to ROS. It is present in cell membrane and plasma. It is readily exchanged between cells and plasma with a balance in favor of plasma.

## Conclusion

This study attempted to identify the detrimental elements of STP that are genuinely in charge of causing injury or altering a number of physiological parameters, particularly the amount of antioxidant enzymes and the organs of the gastrointestinal system. In light of this perspective, efforts were also made to identify the bioactive compound of clove bud aqueous extract, which was used on male albino rats for a specified amount of time in accordance with references, and which was able to restore the altered or damaged tissues and various parameters. Significant changes in the treated group's parameters when compared to the control group were largely caused by the effective dose. Therefore, based on the overview, it can be concluded that the consumption of smokeless or unburned tobacco may be the cause of changes in the oxidative stress status of biological systems. Regardless of whether it is consumed directly or indirectly. Additionally, plant-based sources are more effective against these damages since they contain a number of bioactive components.

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## Conflict of interest

There are no conflicts of interest.

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