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Protective role of (Bronco-T) against formaldehyde induced antioxidant, oxidative and histopathological changes in lung of male Wistar rats

Sholapuri Payani[a,b], Cherlopalli Mamatha[a,b], Chinta Chandraprakash[a,b], Matcha Bhaskar[a,b,*]

[a] Department of Zoology, Sri Venkateswara University, Tirupati, 517502, Andhra Pradesh, India
[b] Division of Animal Biotechnology, Department of Zoology, Sri Venkateswara University, Tirupati, 517502, Andhra Pradesh, India

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ABSTRACT

The present study was sought to evaluate the oxidative, antioxidant status and histopathological changes by the acute chronic exposure of formaldehyde. Bronco-T a poly-herbal formulation treatment, changes the oxidative, antioxidant status and histopathology of rat lungs with antioxidant and regenerative property. In this experiment thirty adult male albino Wister rats were used for the study and subdivided in to five groups consist of 6 rats for each group. Group-I served as control and the other 4 groups such as II, III, IV and V are considered as experimental. The control and treatment rats are maintained for 21 days of experimental period. Experimental rats are exposed to 40 percent formaldehyde for 1 h treated with Bronco-T and salbutamol. In the present investigation, the formaldehyde exposed rats a series of free radical chain reactions were grimly provoked, the evaluation of antioxidant enzymes (SOD, CAT), other enzymes oxidative enzymes (G-6-PDH, SDH) and (ALT, ALAT and LDH) were measured. A clear assertive imbalance between oxidation and anti-oxidation status was critically observed, and oxidative stress was clearly exacerbated in lung tissue leading to altrations in architecture of lung histopathology. Oral gavage Bronco-T exhibits a beneficial action by bringing normal architecture in lung tissue of formaldehyde inhaled rats with antioxidant properties. Bronco-T treatment may be a suitable remedy for formalin occupational diseases.

1. Introduction

At the present moment people habitually exposed 70–80% to polluted environment. Formaldehyde (FA) is one of the majority common aldehyde in the environment, a number of studies reported that formaldehyde is an imperative indoor irritant chemical associated with respiratory illness [18,28]. It is recognized as a toxic substance and the chances of destructive effects are amplified at room temperature due to its volatility [37]. Formaldehyde is one of the imprudent pollutants, and has an extensive range of household interiors (flooring materials, wood-based panels, paper products, etc.) and industrial (as a feedstock for numerous industrial processes) sources [34,21]. FA exposure aggravates asthma symptoms [3]. Respiratory system is the major target of formaldehyde. The toxicity of formaldehyde is distress to all who work directly with it such as embalmers, anatomists, histology technicians and medical students are among the people who have high disclosure to formaldehyde [5,24]. After breathing of formaldehyde in rats the volume of formaldehyde was prominent in the lungs than in the liver, kidney and other organs [7]. Formaldehyde inhalation has long been assumed to causing asthma and hyperresponsivity, In anticipation of decisive indication for such effects and clear mechanistic understanding remains mysterious [1,19,31,4,11]. Additional studies suggest a stronger influence of formaldehyde on respiratory function, predominantly increased risk of asthma [22,33,13]. After inhalation of high concentrated FA causes effect on lungs epithelial cells are smash up and loss of their function. A number of metabolic reactions alter particularly those involving amino acids and the demethylation of N^-, O^- and S^- methyl compounds. The destiny of formaldehyde depending on the molecule it comes into makes contact with amalgamation to react with nucleophilic molecules in the cell. As previous reports stated that approximately all cells are able to metabolize formaldehyde via formaldehyde dehydrogenase (FDH) and aldehyde dehydrogenase (ADH) in metabolic condition. The presence of formaldehyde with FDH and ADH enzymes in erythrocytes may causes systemic effects associated with formaldehyde [23]. After inhalation exposure, these enzymes may oxidize the formaldehyde to fumarate (or) carbon dioxide and water. In this reaction also involves a glutathione hemiacetal intermediate (hydraxy methyl glutathione) an NAD^+ cofactor and the presence of S-formyl glutathione hydrolase, which frees the glutathione molecule from the intermediate [36]. Fumarate can also be directly

* Corresponding author at: BOS Chairman, Dept. of Zoology, S.V.U. College of Sciences, S.V. University, Tirupati, 517502, India.
E-mail address: matchabhaskarcnf2015@gmail.com (M. Bhaskar).

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incorporated into macromolecules by a tetra hydrofolate dependent pathway in the one carbon pool. Peraxisomal catalase is capable of oxidizing formaldehyde to fumarate in the presence of hydrogen peroxide. Creation of hydrogen peroxide is the rate limiting step in this reaction, which does not account for a great deal of formaldehyde metabolism [39]. Formaldehyde is considered a hallmark of lung injury. There have been a number of reported cases of work-related asthmatic symptoms in individuals exposed to inhaled formaldehyde [17,6,29,2,27]. Nowadays salbutamol is used to treat wheezing and shortness of breath caused by breathing problems (such as asthma, chronic obstructive pulmonary disease). Salbutamol belongs to a class of drugs recognized as bronchodilators. It was mainly works for opening breathing passages and relaxing muscles in the airways.

The negative effects and the connotation of synthetic drugs and the mounting side effects of their treatment make the usage of natural drugs more accurate and steadfast yet again. The Indian holy books, particularly Vedas and the Ayurveda are very good compilation of the exploitation of the trees and the herbs for the treatment of the diseases. Bronco-T was polyherbal plant product which is consists of five herbal plants *Cinnamomumtamala, Solanumxanthocarpum, Albizialebbeck, Justiciaadhatoda, Yastimadhu.* These plants are extensively used to treat respiratory diseases in Ayurveda. Bronco-T has strong antioxidant and oxidative function to protect the lungs against formaldehyde inhalation in polluted environment. The present study has predictable polyherbal remedy with the potential to improve pulmonary function, to reduce symptoms or to decrease exacerbation severity and frequency on treatment with Bronco - T during lung injury. The elemental analysis in Bronco-T was carried out by EDAX (Energy Dispersive X-ray) Analysis.

EDAX systems are attachments to electron microscopy instruments like scanning electron microscopy (SEM) or transmission electron microscopy (TEM)) instruments where the imaging capability of the microscope identifies the specimen of interest. The data generated by EDAX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analyzed. Elemental mapping of a sample and image analysis are also possible.

In a multi-technique approach EDAX becomes very powerful, particularly in contamination analysis and industrial forensic science investigations. The technique can be qualitative, semi-quantitative, and quantitative and also provide spatial distribution of elements through mapping. The EDAX technique is non-destructive and specimens of interest can be examined *in situ* with little or no sample preparation.

2. Materials and methods

2.1. Animals

A healthy adult (3 months old) male wistar rats of 30 in number weighing between180 −250 g obtained from animal house of University of Bangalore, Bangalore were used for this study. They were housed in each polycarbonate cage under standard laboratory conditions at a room temperature of 24 ± 2°c and humidity 45–64% with 12 h Light/dark cycle and fed with standard diet obtained from Hindustan lever ltd, Bangalore, India and water was supplied through plastic bottle provided with nipples.

2.2. Experimental design

The animals were assigned into five groups (I, II, III, IV, and V) containing 6 rats in each group. Group I serves as the control and Group - II to V treated as the experimental groups. The rats were maintained at controlled temperature (21–25 °C) on a 12 h light/dark cycle. commercial diet were used as feed for rats and water was available *ad libitum*.The control group (Group - I; n = 6) was not exposed to forty percent (40%) formaldehyde as they were kept at separate environment and Group II treated with Bronco-T while the other test animals (Group - III to Group - V) were exposed to 40% formaldehyde vapor environment at a single period of time (1 h) daily (40%formaldehyde at room temperature). The exposures of 40% formaldehyde was conducted by soaking it in cotton wool and placed in enclosed (designed) wire gauze within the animal cage, thus exposing the animals to the vapor. It was exposed for 21 days in Group - III, IV and V respectively. But Group - IV with bronco-T and Group - V with salbutamol were treated after 1 h exposure of Formaldehyde by oral gavage.

2.3. Sample collection and analysis

The animals were sacrificed at the expiration of the exposure period and the thoracic cage was cut open and the lungs were collected. It was fixed with 10% formalin by immersing in high volume of the fixative and injects the fixative in the lungs with syringe. It was used for histopathological studies and processed within two days after sacrificing.

2.4. Anti-oxidative enzymes

The 5% homogenate (w/v) of lung tissues were prepared in 50 mM iced up phosphate buffer (pH 7.0) contain 0.1 mM EDTA. Centrifugation was carried out at 10,000 rpm for 10 min at 4 °C. The supernatant was separated and used for the enzyme assays of SOD and CAT.

2.5. Superoxide dismutase (SOD - EC: 1.15.1.1)

Superoxide dismutase activity was calculated by measuring the optical density at 480 nm for 4 min in Hitachi U-2000 spectrophotometer according to the process of Misra and Fridovich, (1972). The oxidation of epinephrine was inhibited by the enzyme was equal to one unit of SOD activity. The assay mixture contains supernatant, carbonate buffer (0.05 M, pH 10.2, containing 0.1 mM EDTA) and to it 30 mM epinephrine (in 0.05% acetic acid) was added.

2.6. Catalase (CAT - EC: 1.11.1.6)

The catalase activity was considered by decreasing the velocity at 240 nm in UV -spectrophotometer according to the method of Aebi (1984). Ethyl alcohol was added to supernatant and kept in an ice bath for half an hour. Then Triton X-100 RS was added. The reaction mixture containing 200 µl of phosphate buffer and 50 µl of supernatant to it 250 µl of 0.066 M H_2O_2 (in phosphate buffer) was added. One unit of activity is equal to the moles of H_2O_2 degraded / mg protein / min.

2.7. Aspartate aminotransferase (AAT) (E.C.2.6.1.1)

Aspartate aminotransferase activity in the mitochondrial fraction of rat lung was assayed by using the method adopted as described by Bergmayer and Bruns (1965). The reaction mixture contained100 µM phosphate buffer (pH : 7.4), α-ketoglutaric acid (20 µM), L - aspartic acid (20µM), Enzyme source & distilled water were added and incubated at 37^0C for 30 min and then 2,4-dinitrophenyl hydrazine (0.01 M) added and allowed to stand at 20 min and then yellow colour complex formed on addition of NaOH (0.4 N). The reaction was measured at 545 nm in a UV-VIS spectrophotometer against NaOH blank. The enzyme activity was expressed as µ moles of pyruvate formed / mg protein /min.

2.8. Alanine aminotransferase activity (ALAT) (E.C.2.6.1.2)

Alanine aminotransferase activity in the mitochondrial fraction of rat lung was assayed at 545 nm in a UV- spectrophotometer by the method of Reitman and frankel (1957) as described by Bergmayer and Bruns (1965). The reaction mixture contains phosphate buffer (100 µM) pH : 7.4 α-ketoglutaric acid (20 µM), Alanine (100µM). Enzyme and distilled water were added and allowed it to stand for 20 min and then

Table 1
Showing the levels of Antioxidant and Oxidative Enzymes in lung tissue of Control and Experimental rats.

Parameter	Group I	Group II	Group III	Group IV	Group V
SOD superoxide anion reduced/ mg protein/min.	9.52 ± 1.01	10.01 ± 1.06(+5.14) N.S	5.01 ± 0.59 (-47.26) P < 0.001	8.54 ± 0.71 (-10.29) P < 0.001	7.42 ± 0.41 (-22.05) P < 0.001
CAT (μmoles of H_2O_2 metabolized/mg protein/min)	68.56 ± 7.34	69.63 ± 7.26 (+1.56) N.S	48.25 ± 5.74 (-29.62) P < 0.001	59.86 ± 5.16 (-12.69) P < 0.001	52.21 ± 4.47 (-23.84) P < 0.001
AAT (μ moles of pyruvate formed/ mg protein / min)	8.44 ± 0.81	8.41 ± 0.85 (-0.35) N.S	5.82 ± 0.69 (-31.04) P < 0.001	7.10 ± 0.72 (-15.87) P < 0.001	6.91 ± 0.48 (-18.12) P < 0.001
ALAT (μ moles of pyruvate formed/ mg protein / min)	0.98 ± 0.29	0.79 ± 0.06 (-19.38) P < 0.001	0.23 ± 0.022 (-76.53) P < 0.001	0.92 ± 0.08 (-6.12) N.S	0.66 ± 0.14 (-32.65) P < 0.001

Values are mean ± S.D. of 6 individual rats.
Values are significantly different from control at P < 0.001; NS indicates no significant change.

NaOH (0.4 N) was added. Yellow colour was observed. The enzyme activity was expressed as μ moles of pyruvate formed / mg protein /min.

2.9. Oxidative enzyme activity

10% (W/V) homogenates of the lung tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at l000 *g* for 15 min at 4 °C. The supernatant fraction was used for enzyme assays of LDH, SDH and G-6-PDH.

2.10. Lactate dehydrogenase (LDH) (l - lactate: NAD+ oxidoreductase - E.C: 1.1.1.27)

The activity of Lactate dehydrogenase was determined at 495 nm in a spectrophotometer by measuring the formazone using the protocol as described by Nachlas *et. al.,* (1960) as advised by Prameelamma and Swami (1975) with little changes and expressed in moles of formazone formed/mg protein/min. The enzyme utilizes NAD and INT was reduces to form formazone then it was extracted overnight into 5 ml of toluene at 5^0C.

2.11. Succinate dehydrogenase (SDH) (Succinate acceptor Oxidoreductase - E.C: 1.3.99.1)

Nachlas et al., (1960) developed a method as suggested by Prameelamma and Swami (1975) to check the specific activity of SDH by the measuring the absorbance at 495 nm in a spectrophotometer as enzyme utilize FAD and INT reduces to form formazone and it was expressed in μ moles of formazone formed / mg protein / min.

2.12. Glucose-6-Phosphate dehydrogenase (G-6-PDH) (E.C: 1.1.1.49)

Glucose-6-posphate dehydrogenase activity was calculated by measuring the intensity of colour formed when enzyme reacts with INT and NADPH and it was read at 495 nm against the toluene blank in spectrophotometer and expressed in μ moles of formazone formed / mg protein / min. This method was adapted from Lohr and Waller (1965), as modified by Mastanaiah.et al., (1978).

2.13. Energy dispersive X-ray analysis

Dispersive X-ray analysis (EDAX) was done to identify the elemental mapping of a sample in Bronco-T for electron dispersive X-ray analysis (EDAX - System, Sathyabhama University, TN, India). The EDAX-system was coupled with SEM using mixed BSE (Back scatter electron) + LSE (Lateral secondary electron) signal detectors.

2.14. Histological changes

After cervical dislocation of rats, Lungs were collected from all 4 groups. The tissues were fixed in neutralized formalin, dehydrated with ethanol and embedded in paraffin wax (56 °C). Some tissue were made into thin sections and stained with haematoxylin and eosin. The stained sections were viewed under microscope and the histological changes were recorded with the help of a pathologist.

2.15. Statistical analysis

Data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one-way analysis of variance (ANOVA). A value of p < 0.001 was considered to indicate a significant difference between groups. All the data were expressed as the Mean ± Standard Error of Mean (SEM).

3. Results

In (Table 1) studies in animal the activity of SOD in control rats was found to be (9.52 mg protein / min) in lungs. In the Group-II, where rats were control but treated with Bronco-T extract the activity was showed no significant change in lungs when compared to control (10.01 mg protein/min). In Group-III, formaldehyde inhaled rats the activity was found significantly decrease (5.01 mg protein/min) over control, Group-IV and V had showed increased activity when compared to Group-III

Catalase (CAT) activity levels in the lung tissues were found to be significantly decreased (48.25 mg protein/min) in the FA inhaled group. In control rats was found to be (68.56 mg protein/min). In Group-II, the activity was slightly increased with NS change. Group-IV and V also Catalase the activity showed decreased levels when compared to control rats whereas they had showed increased activity when compared to group-III rats showed in Table 1. But treatment with Bronco - T completely abolished all these changes.

Our results showed that previous exposure to FA in Group-III rats significantly reduced activity of AAT when compared to FA/Bronco-T treated rats (Group-IV). FA inhalation per se reduced AAT relative to the FA/Bronco-T. The AAT from the FA/Bronco-T, and FA/Salbutamol groups were similar. In addition, the Bronco-T challenge (Group-IV) increased the AAT relative to the Group-V.

The activity of ALAT in control rats was found to be (0.98 mg protein/min) in lungs. In Group-II, where rats were control but treated with Bronco-T extract and the activity was slightly decreased (0.79 mg protein/min) in Lungs, in Group - III, formaldehyde exposure rats the activity was found significantly decreased in lungs (0.23 mg protein/minute). Group-IV and Group-V rats also showed increased levels of activity when compared to formaldehyde exposed rats.

Finally Group-IV (formaldehyde exposed and treated with Bronco-T) rats are great improvements to clear the anti oxidant disturbances showed in Table 1 when compared to Group-V.

Table 2
Showing the alterations in Oxidative Enzymes activities in Lung tissue of Control and Experimental rats.

Parameter	Group I	Group II	Group III	Group IV	Group V
G-6-PDH (μ moles of formazon formed/ mg protein /min)	4.65 ± 0.48	4.47 ± 0.36 (-3.87) NS	3.00 ± 0.32 (-35.48) P < 0.001	4.35 ± 0.33 (-6.45) P < 0.001	3.63 ± 0.36 (-21.93) P < 0.001
LDH (μ moles of formazon formed/ mg protein /min)	4.14 ± 0.42	4.06 ± 0.33 (-1.93) NS	0.49 ± 0.052 (-88.16) P < 0.001	3.48 ± 0.21 (-15.94) P < 0.001	2.22 ± 0.21 (-46.37) P < 0.001
SDH (μ moles of formazon formed/ mg protein / min)	12.12 ± 0.91	11.55 ± 0.81 (4.70) NS	1.20 ± 0.12 (-90.09) P < 0.001	11.45 ± 1.02 (-5.52) NS	9.41 ± 0.93 (-22.21) P < 0.001

Values are mean ± S.D. of 6 individual rats.
Values are significantly different from control at P < 0.001; NS indicates no significant change.

3.1. Oxidative enzymes activity

In (Table 2) a decreased change in succinate dehydrogenase activity was observed only in the group exposed to FA. No changes in comparison with the control were observed in the two remaining groups of animals. In groups which were treated with Bronco-T and salbutamol (Group - IV and V) slight changes in the reaction intensity and pattern were detected. In the FA inhaled group without treatment, a marked succinate dehydrogenase activity decrease was found in lungs. The activity of SDH in control rats was found to be (12.12 μ mol/mg protein/min). In Group-II, rats were control but treated with Bronco-T extract and the activity was very slightly decreased over control rats. In Group - III, the activity was found significantly decreased to (1.20 μ mol/mg protein/ min). Group - IV and V rats also showed decreased levels of activity when compared to control rats. But, the activity in them was increased when compared to Group - III rats.

In lung control rats of the Lactate dehydrogenase activity were found to be (4.14μ mol/mg protein/min). The control but treated with Bronco-T extract, the activity was very slightly decreased. In Group - III, the activity was found to be significantly decreased (0.49 μ mol/mg protein/ min) over control Group - IV and V rats also showed decreased levels of activity when compared to control rats (Table 2). But, the activity in them was increased when compared to Group - III rats.

G-6-PDH activities in control rats were found to be (4.65μ mol/mg/ protein/min). In Group-II, the activity was slightly decreased. In Group - III it was found significantly decreased (3.00 μ mol/mg protein/min). Group - IV and V also showed decreased levels of activity when compared to control rats, whereas they showed increased activity when compared to group - III rats (Table 2).

3.2. Element analysis

In (Table 3**)** Bronco-T had O, Cl, Ca, K, elementswere identified in (EDAX) Energy Dispersive X-ray analysis.

4. Discussion

Pro-oxidant and antioxidant balance is requisite for normal biological functioning of the cells and tissues. The present study discovered SOD is an enzymatic antioxidant which catalyzes the transformation of super oxide radical to hydrogen peroxide (not a free radical itself, but a reactive molecule) and molecular oxygen. Decreased activities of enzymatic antioxidants such as SOD have been well stated in formaldehyde inhaled rats [38]. In normal conditions its activity would be parallel to the amount of super oxide radicals produced. The increase with a non-significant change in its activity in Group-II, where rats were normal and treated with Bronco-T extract was due to the neutralization of reduction potential developed by the over produced super oxide radicals. In case of Group-III the significant decrease in its activity was due to the formaldehyde inhalation (Fig. 1A). It induces lung injury through the generation of free radicals in high concentration. The decreased activity of SOD in lung injury rats, as reported previously, which could be leading to increased production of free radicals' and decreased mechanism of detoxification. Treatment with Bronco-T extract and salbutamol has raised the activities of SOD, which could be a result of decreased lipid Peroxidation and/or improved detoxification mechanism.

Similarly in lung injury rats the activity of CAT was reduced and this showed a number of deleterious effects due to accumulation of hydrogen peroxide [35] and some condition may be responsible for 21 days of formaldehyde exposure in the present study might induced lung injury leading to reduced CAT activity [15]. The decreased activities of SOD and CAT may be due to the concomitant increase in the generation of free radicals, when formaldehyde exposure rats were treated with Bronco-T extract the activity is increased over Group III rats or otherwise stress is relieved because of the neutralization of free radicals leading to increased Catalase activity [14,10]. Catalase and non-enzymatic scavengers of hydroxyl radicals were found to protect against formaldehyde toxicity in rats [15,20]. CAT, which is present virtually in all mammalian cells of mitochondria, is responsible for the removal of H_2O_2 [8].

Amino transferases are extremely susceptible to pollutants, hence considered as one of the best indicators for monitoring and assessing the necrosis and cell inflammation in the lungs [8]. In the current study, the activities of AAT and ALAT decreased which might be associated with the release of the enzymes due to the injury to epithelial cells and impaired function of the lung cells. This has mainly observed in Group III rats. There is an increment in the activities of AAT and ALAT in the lungs of the Bronco-T and salbutamol treated animals. When compared to Bronco-T with salbutamol treated animals the changes in AAT and ALAT was high. Parallel to other studies the biochemical studies presented in our study finally showed that SOD, CAT, AAT and ALAT enzymes are significantly reduced in the lung tissue of the exposed to FA. Activities are better to increase in Bronco –T treated rats. The poly herbal compound Bronco-T extract play much more effective role in curing the lung injury rats compared to salbutamol treated rats.

SDH activity was drastically reduced, suggesting that lung injury compromised the respiratory chain function in lung of rats. It is well known that mitochondrial oxidative phosphorylation system generates free radicals [32] and that the complexes of electron transport chain are vulnerable to damage by free radicals [9]. It is reasonable to suggest that the inflammatory cells in the airway increased the production of reactive species [26,25] which could induce the inhibition of mitochondrial enzyme activities presented in our study. In the current study, we notified that Bronco-T was able to put a stop to the reduction of SDH and complex II activities caused by formaldehyde inhaled lung

Table 3
Showing the electrolytes weight and atomic percentage in *Broncho – T*.

S. No.	Element	Weight%	Atomic %
1	O K	85.18	93.24
2	Cl K	3.10	1.53
3	K K	9.88	4.42
4	Ca K	1.84	0.81
	Totals	100	100

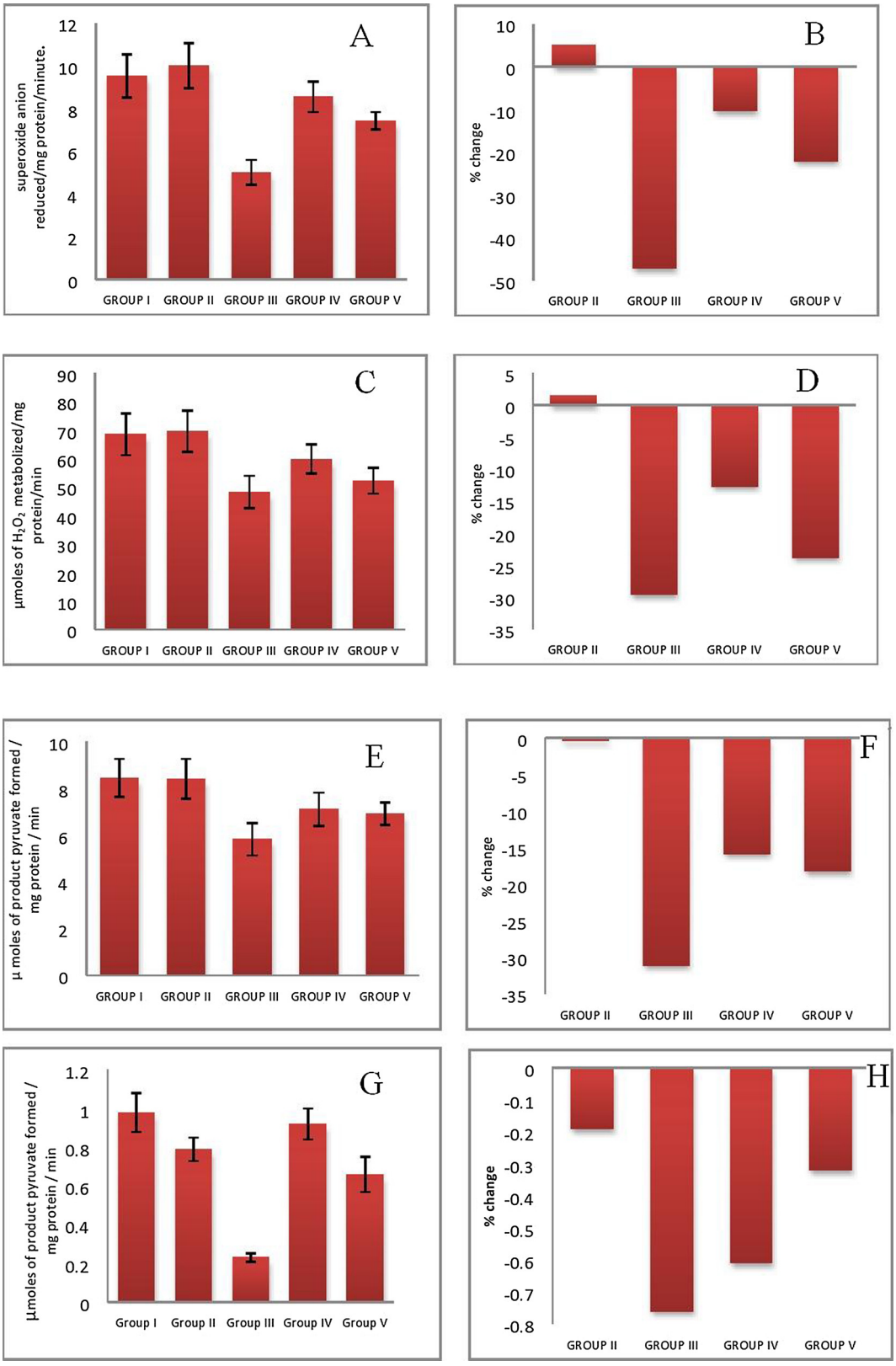

Fig. 1. A) Showing SOD levels in lung tissue of control and experimental animals. B) Showing % change of SOD activity in lung tissue of experimental animals. C) Showing catalase levels in lung tissue of control and experimental animals. D) Showing % change of Catalase activity in lung tissue of experimental animals. E) Showing AAT levels in lung tissue of control and experimental animals. F) Showing % change of AAT activity in lung tissue of experimental animals. G) Showing ALAT levels in lung tissue of control and experimental animals. H) Showing % change of ALAT activity in lung tissue of experimental animals.

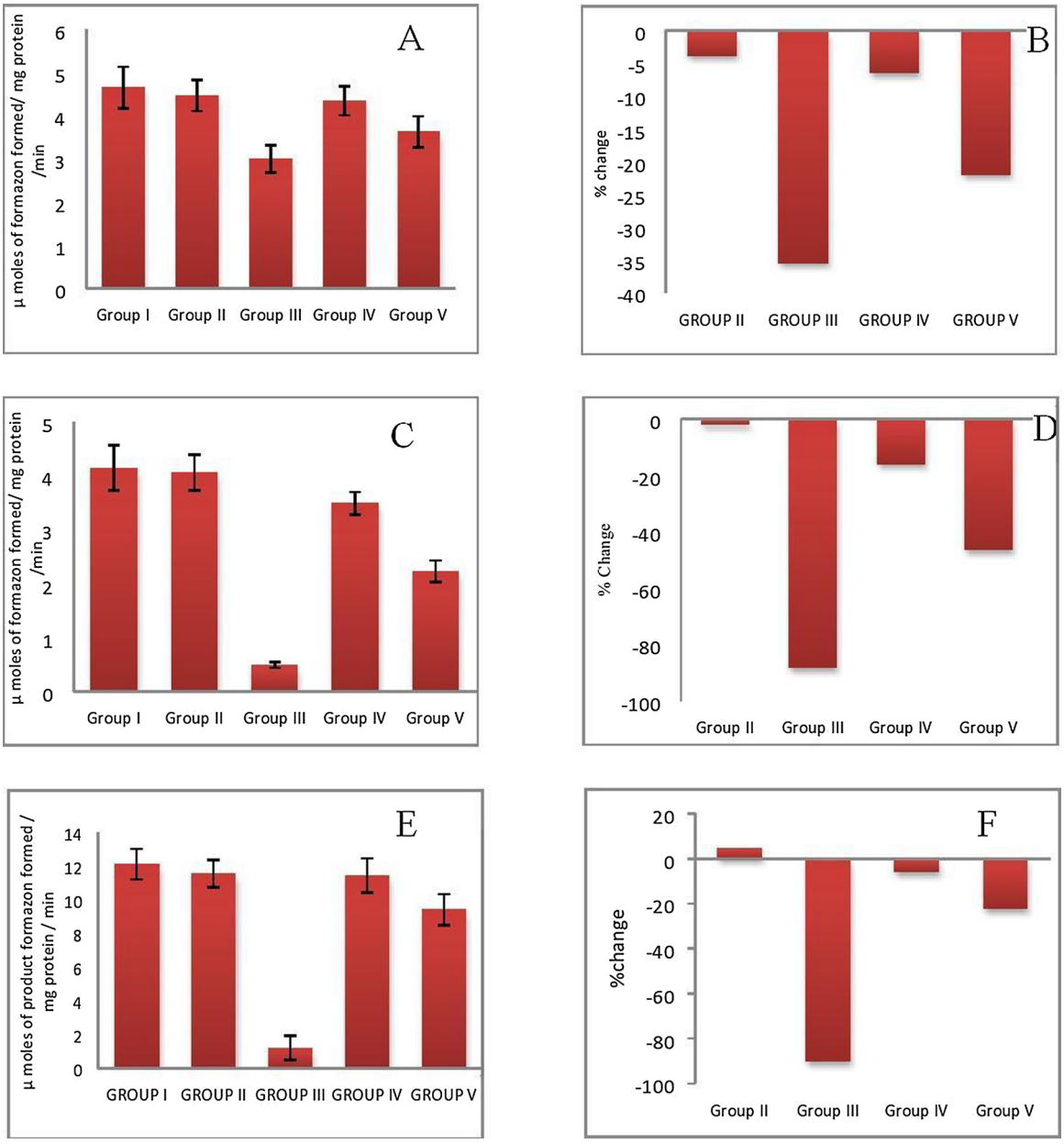

Fig. 2. A) Showing the levels of G6PDH enzyme in lung tissue of control and experimental animals. B) Showing % change of G6PDH activity in lung tissue experimental animals. C) Showing the levels of LDH activity in lung tissue of control. and experimental animals. D) Showing % change of LDH activity in lung tissue of experimental animals. E) Showing the levels of SDH activity in lung tissue of control and experimental animals. F) Showing % change of SDH activity in lung tissue of control and experimental animals.

injury. Since Bronco-T prohibited the increase in reactive oxygen species (ROS), nitrite levels, damage to protein, as well as the decrease in antioxidant defenses such as LDH content, we believe that Bronco-T could protect the SDH activities from oxidative rudeness caused by formaldehyde lung injury. Since lung injury is accompanied by impaired respiratory mechanisms and edema arrangement [12], which provide to the respiratory collapse [15], we observed air flow may be decreased in airways in lungs with interstitial fluid accretion and may be causative to the decrease in compliance and respiratory system resistance observed in this experimental model (Figs. 1, 2 and 3).

Bronco-T might contain rich of antioxidant and anti-acidosis properties which can activate the dehydrogenases activity leading to minimize the lung injury on exposure to formaldehyde. Elevation of total LDH activity in lung tissue was found several days after exposure of Albino rats to high or low levels of O_2, to formaldehyde [8]. But, these studies did not distinguish between LDH of lung tissue and of susceptible cells. A decrease in lactate dehydrogenase activity was observed only in the group exposed to Formaldehyde. In unexposed wistar rats, LDH activity was highest in the bronchial airways [26,30]. The eminent LDH tissue levels remained for more than 2 weeks after exposure, suggesting some outstanding sub acute inflammation.

G6PDH plays an important role in the cellular response to oxidant stress is seen in free-living organisms [16]. In the present study can be attributed for the decreased G6PDH activity during formaldehyde exposure and after Bronco-T treatment, the same was recovered which can anticipate that the Bronco-T possess the compounds which can exhibit beneficial recovery of damaged lung cellular organization leading to improve the activity of G6PDH activity as was observed on treatment with salbutamol which is in practice by the medical representatives. But the Bronco-T treatment has some advantage of lacking side effects on treatment. In addition Bronco-T can lead to prevalence of electrolyte disturbances in lung injury.

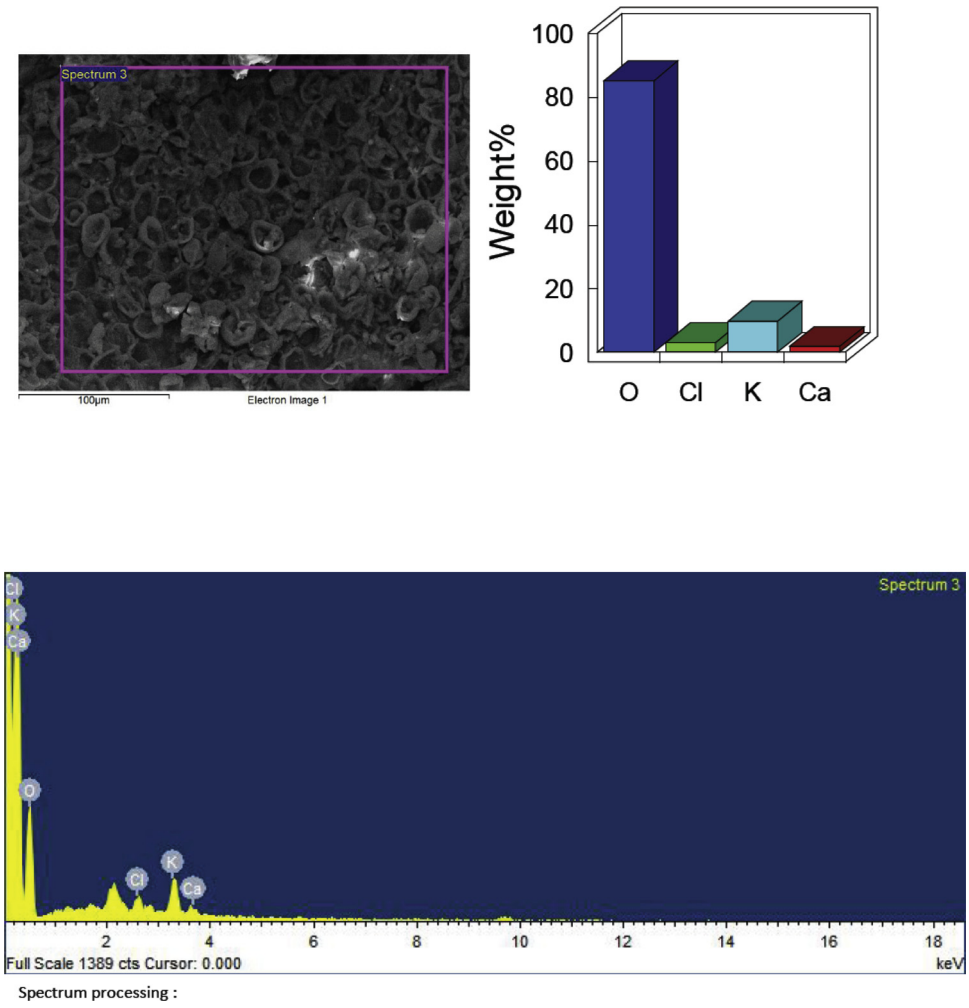

Fig. 3. A) Scanning Electronic Microscopic Image. B) EDX elemental composition Quantitative Analysis. C) Spectrum processing Histological Procedure.

4.1. Histopathology

Lung sections of hematoxylin and eosin stain of control Group - I (Fig. 4) revealed normal pulmonary tissue architecture with clear obvious bronchial passages and alveolar cavities as well as the alveolar sacs, the alveolar ducts and the alveoli. The alveolar wall was thin and lined by two types of epithelial cells (pneumocytes), squamous epithelial cells (pneumocyte Type I) and large cuboidal cells (pneumocyte Type II). The alveolar septa had normal width with no aberration in alveolar septal blood capillaries. Normal construction of bronchiolar epithelium and standardparabronchiolar lymphoid aggregation was also observed.

After FA exposure for 21 days, Group - III (Fig. 4C) lungs showed bronchiolar epithelial hyperplasia characterized by many abnormal epithelial cell layers formed newly that observed inside the bronchiolar lumen. Squamous metaplasia of the goblet cells characterized by abnormal proliferation of the goblet cells, hyperplastic parabronchiolar lymphocytic aggregations also were observed. Then it showed dilatation of the pulmonary blood vessels accompanied by vacuities, this vascular reactivity characterized by marked edema detected in the smooth muscle of pulmonary blood vessels which infiltrated with inflammatory cell aggregations. Some specimens showed signs of epithelial dysplasia which was found in more than one layer of bronchiolar epithelium characterized by appearance of multiple mitotic figures, cytoplasmic and vesicularity.

The Group - IV (Fig. 4D) animals on treatment with Bronco-T to the formaldehyde inhaled rats better improvement in lung epithelial layers and decreased inflammation in the smooth muscle of pulmonary blood vessels was observed. Epithelial dysplasia also was cleared.

Group - V (Fig. 4E) rats also showed recover in the alterations observed due to formaldehyde inhalation when treated with salbutamol rats. But these rats are compared with Group-IV (treated with Bronco–T) the rats are better to regain of the damaged cells in lungs. Bronco-T is identified as a very effective poly herbal drug which can be used for bronchitis and respiratory abnormalities.

Our data has therefore suggested that the effects of Bronco - T produced benificial results on injured lung tissues, including proliferation and decreased smooth muscle inflammation. Anti-inflammatory property of a treatment with Bronco - T in order to better appreciate the mechanisms association was investigated. Our results have shown that a treatment with Bronco – T reduced the lung inflammation, decrement in cell influxes in the alveolar space in the better way when compared to salbutamol induced by an FA inhalation.

5. Conclusions

In conclusion our study showed that Formaldehyde inhalation leads to Antioxidant, oxidative imbalance on the albino rat lungs and the histological changes had direct correlation with formaldehyde vapor exposure. The administration of Bronco-T followed by formaldehyde lung injury, mainly clear the antioxidant and oxidative enzyme disturbance and make changes in lung inflammation and development of histological changes. Bronco-T shows protective and anti-inflammatory activity against these harmful effects.

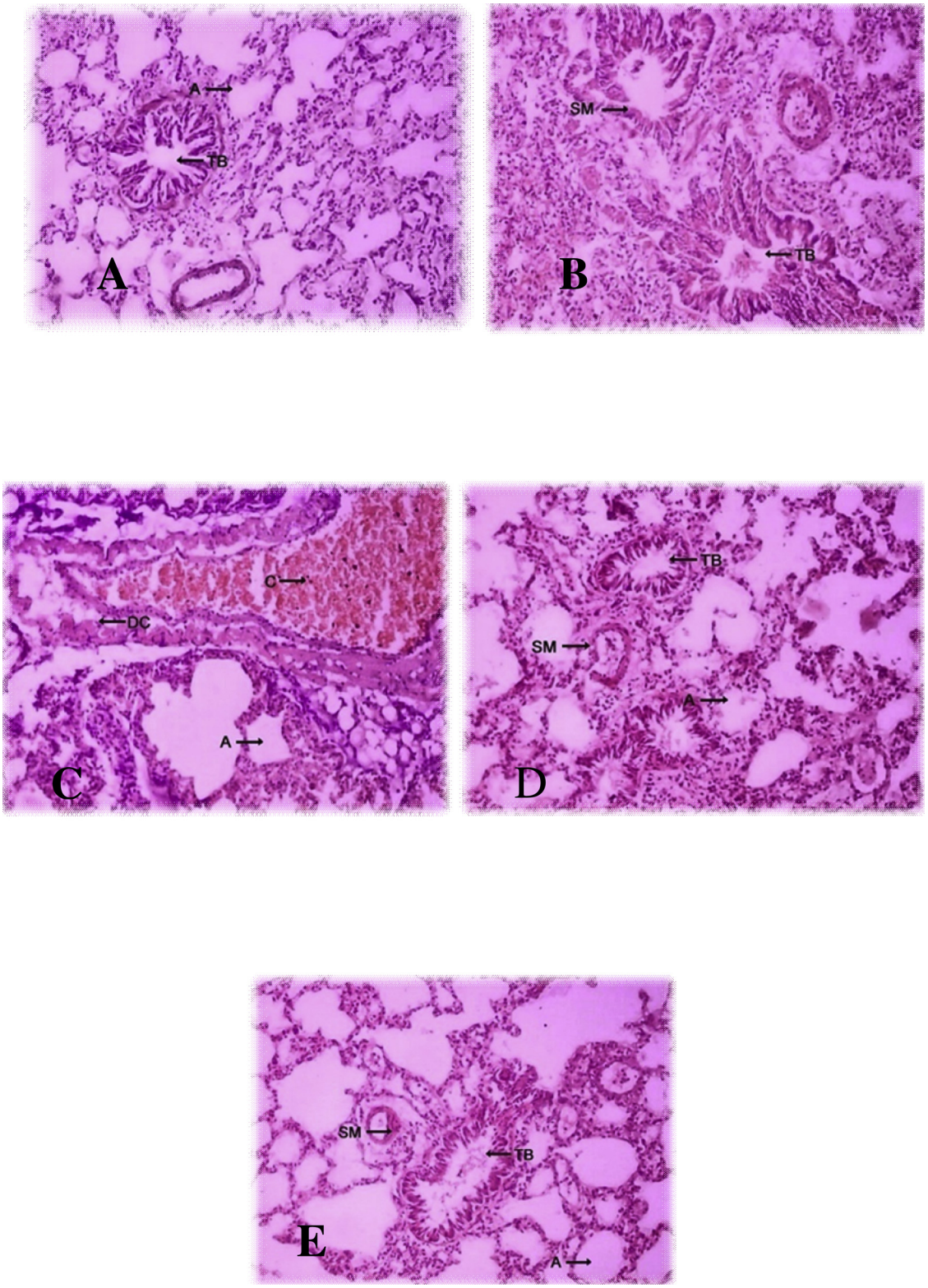

Fig. 4. A) Normal Cyto-architecture of lung tissue in control rats (10X magnification). B) Normal architecture of lung tissue on treatment with Bronco-T (10X magnification). C) Shows hemarages and congestion leading to structural of lung on expose to formaldehyde (10X magnification). D) Shows Regenerative changes in lung was observed similar to normal architecture of lung on treatment with Bronco-T (10X magnification). E) Shows Regeneration of lung tissue similar to normal architecture of lung on treatment with Salbutamol *(*10X magnification).

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

The authors declare no conflict of interest.

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