International Journal of Drug Development & Research
| April-June 2012 | Vol. 4 | Issue 2 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03 & H index 2

Safety and Efficacy Study of Submicronized Aerosols of Sodium Nitrite Respiratory Fluid Against Smoke Induced Oxidative Stress and Lung Injuries

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Abstract

The present study was performed to assess the sub-acute safety and efficacy study of sub-micronized aerosols of sodium nitrite respiratory fluid. Nebulization of sodium nitrite respiratory fluid (1, 3 and 5 %) was carried out in Sprague Dawley rats as per regulatory guidelines using nebulization assembly. Animals were sacrificed and analyzed for hematological, biochemical and organ histopathology parameters. Bronchoalveolar lavage (BAL) fluid was collected and different biochemical markers were measured. No drug related toxicity on morphological or biochemical parameters was seen at any of the drug concentration studied. Histopathological analysis of lung tissues revealed no inflammatory responses, except for vasodilatation of alveoli, which is the desired effect of the drug. For efficacy study sodium nitrite respiratory fluid was inhaled to animals in which oxidative stress and lung injuries were induced by smoke inhalation. There was significant increase in protein concentration, neutrophils, airway epithelia in the BAL, and lung microvasculature changes were evident in experimental animals post smoke inhalation. Pre-treatment with sodium nitrite respiratory fluid decreased airway necrosis, lung edema and alveolar protein leak induced by smoke inhalation. The study highlights the prophylactic potential of sodium nitrite nebulization against oxidative stress and lung injury caused due to smoke inhalation, and could be significant in developing a novel prophylactic/ therapeutic option for victims as well as members of the rescue team, including fire-fighters.

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Key words:
Sodium nitrite; nebulization; subacute safety; bronchoalveolar lavage fluid (BAL); histopathology.

***How to Cite this Paper*:**
Ali Rashid, Mittal Gaurav, Soni Nakse Lal, Bhavna, Sawroop Krishan, Sultana Sarwat and Bhatnagar Aseem* "Safety and Efficacy Study of Submicronized Aerosols of Sodium Nitrite Respiratory Fluid Against Smoke Induced Oxidative Stress and Lung Injuries", Int. J. Drug Dev. & Res., April-June 2012, 4(2): 358-368

Article History:----------
Date of Submission: 07-05-2012
Date of Acceptance: 22-05-2012
Conflict of Interest: NIL
Source of Support: NONE

1. Introduction

Pulmonary drug delivery being a non-invasive delivery system is increasingly used for

administration of many therapeutic drugs. [1 & 2] The large absorptive surface area of the lungs, extensive vasculature and thin alveolar epithelial barrier allow drugs to be delivered directly to tracheobronchial tree and alveoli of lungs making pulmonary route an alternative option for systemic drug delivery. [3] Drug delivery to the lungs can be achieved by means of various devices such as nebulizers, metered dose inhalers and dry powder inhalers. However, pulmonary drug administration imposes stringent requirement on the delivery device; since the particle size of inhaled drug greatly influences its localization and thus the degree of its absorption from the lungs. Physicochemical properties and toxicological profile of a sub-micronic particle differ considerably as a result of its smaller size and unique form as compared to larger micronized particles composed of same materials. [4 & 5]

It has recently been reported that inhalation of vasodilators, including nebulized NO donors can selectively reduce various pulmonary inflammatory responses such as those seen in asthma, chronic pulmonary obstructive disorder (COPD) and pulmonary hypertension (PHT). [6 & 7] The advantages of using inhalation route for treatment of pulmonary disorders include a) reduced dose since only a local effect is needed, and systemic distribution of the drug in therapeutic doses is not required, b) direct and fast action, and c) reduction in side effects because of virtually no therapeutic drug concentration in blood. However, routine inhalation through nebulization may not be useful because the drug gets deposited in pharynx and stomach, followed by trachea-bronchial system, and not alveoli, which receives only 5-10% of the inhaled dose. Thus 90% of delivered dose has no therapeutic value and contributes to side-effects only. The mean diameter of drug aerosols produced this way is in the range of 5-10 microns which is not suited for alveolar drug deposition. [1]

Keeping this concept in mind, a novel inhalation formulation of 3% sodium nitrite in 30% ethanol-saline has been developed by our laboratory in Defence Research and Development Organisation (DRDO), India for medical management of respiratory disorders mentioned above. In addition to these conventional disorders, it was considered worthwhile to study the effect of this formulation against lung injuries caused due to other factors such as smoke inhalation. The rationale was based on the fact that like for other respiratory conditions, acute airway inflammation and subsequent pulmonary edema caused by smoke inhalation is partially mediated by activated leukocytes and various other inflammatory cytokines, ultimately enhancing susceptibility to pulmonary infection. [8] As a result any formulation found effective against conventional pulmonary disorders may also have a role in reducing ill-effects of smoke induced lung toxicity.

The formulation is inhaled through specially designed spacer devices *('Anukool'),* also developed by DRDO, which produces drug aerosols less than 1 micron size using commercial nebulizer systems. Further, ethanol in the respiratory solution also results in size reduction of the inhaled aerosol and we were able to achieve an aerosol size in the range of 400-600 nm for sodium nitrite respiratory fluid with the combination of above-mentioned two factors.

Nitrites, including sodium nitrite, are known to have vasodilator property due to on-site production of nitric oxide. Considering that sodium nitrite's ability to relax microvasculature and ethanol's safety through the inhalation route are well known [9], the combination sub-micronic formulation therefore in our view may have additional beneficial effects for treating pulmonary disorders by way of increased drug deposition in lungs. However, since the formulation is novel, the present study was undertaken to investigate safety and vasodilatory responses of sodium nitrite aerosols after nebulization of sodium nitrite formulation in 30%

ethanol-saline over a range of sodium nitrite concentrations in intact rat lungs and to determine if submicronic particles of sodium nitrite are inherently toxic. Efficacy of developed formulation against smoke inhalation induced oxidative stress and lung injury has also been studied in experimental animals.

2. Materials and Methods

2.1. Chemicals and reagents

Sodium nitrite (SN), Thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2) and bovine serum albumin (BSA) was procured from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were of analytical grade and were purchased from Merck, India.

2.2. Animals

Male Sprague Dawley rats weighing 180–200g from the animal house facility of the institute were used for the study. All animal experiments were approved by the Institutional Animal Ethical Committee duly constituted for the purpose and confirmed to general national guidelines on the care and use of laboratory animals. The animals were housed in polypropylene cages in groups of six rats per cage and were kept in a room maintained at 25±2 ^{0}C with a 12 hr light/dark cycle. They were allowed to acclimatize for one week before the experiments and were given free access to standard laboratory animal feed (**Golden Feed Laboratory, Delhi, India**) and water ad libitum.

2.3. Formulation preparation and characterization

Sodium nitrite nebulization formulation (in the conc. of 1, 3 and 5%) was prepared in 30% ethanol and 70% normal saline. The preparations were inhaled to different groups of animals and were freshly prepared before animal exposure.

2.4. Aerosol generation and delivery

A whole body exposure chamber for aerosol inhalation was used in conjunction with a large spacer *('Anukool')* indigenously developed by DRDO, India. The spacer attached to commercially available nebulization assembly (**Acorn II; Marquest Medical, Englewood, CO**) produced aerosols of SN nebulization formulation. The aerosol particle size was measured with the help of particle size analyzer (**Model 310 A, LASAIR II, USA**).

3. Sub-acute safety evaluation

3.1. Treatment regimen

Twenty-four Sprague Dawley male rats were randomly divided into four groups of six rats each. The groups were designated as group I, II, III and IV. The animals of group I served as control and received no treatment. Group II, III and IV animals received aerosols of SN nebulization fluid in concentration of 1, 3 and 5% respectively twice a day for 30 minutes up to seven weeks through an whole body inhalation assembly mentioned above. All animals (groups I-IV) were observed twice daily for any morbidity and mortality. On initiation of dose administration, clinical examinations were performed daily prior to and approximately 1 hour following dose administration and detailed physical examinations were performed weekly. All the animals were sacrificed and dissected after 24 hours of last exposure. Tissue samples of different vital organs (lungs, liver, kidney, heart and spleen) were collected and preserved in 10% formalin solution for histopathology analysis to observe any microscopic changes.

3.2. Observations

3.2.1. Morphological assessment

Morphological parameters of toxicity i.e., mucous membrane (nasal secretion), eye irritation, tear secretion, excessive blinking, salivation, cyanosis, lethargy, pyloerection (ruffled fur), paralysis, skin irritation, edema, erythema, respiratory rate,

sleeping habits as well directed and non-directed movements within the cage were observed throughout the experiment.

3.2.2. Body weight, food and water intake

Changes in body weight gain, food and water intake were recorded and local injuries were studied during treatment of animals. The changes in total body weight of the animal were recorded weekly.

3.2.3. Clinical pathology: Haematology and clinical biochemistry

The rats were fasted overnight prior to blood collection. Blood was collected from the vena cava at the time of necropsy into tubes containing EDTA (haematology) and no anticoagulant (serum chemistry). The values of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and platelets (PLT) counts were determined and compared with controls. Hematological parameters were assayed using an auto-analyzer (**Roche Integra, 400 Plus, Diagnostic Systems**). Clinical chemistry parameters were determined using automated analyzer (**Hitachi Model 912**) and included blood levels of albumin, total cholesterol, total protein, total bilirubin, glucose, urea, creatinine, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase.

3.3. Bronchoalveolar lavage fluid (BALF) analysis

Bronchoalveolar lavage (BAL) fluid was collected as per previously reported method. [10 & 11] Briefly, rats were anesthetized and then euthanized by exsanguinations. Their trachea and lungs were exposed by thoracotomy after which a cannula was inserted into the trachea and was ligated using a thread. Twenty-five mL/kg body weight of warm phosphate-buffered saline (0.15M NaCl–50mM phosphate, pH 7.4; 37 ^{0}C) was instilled into the animal's lungs via a syringe fitted with tracheal cannula. Phosphate-buffered saline was allowed to stay in lungs for 30 seconds, then retrieved and re-instilled with the help of syringe. The process was repeated three times with the same solution. Volume of recovered BAL fluid from each rat was 22.47 ± 0.38 ml/kg body weight. BAL fluid was centrifuged (300g, 10 min) to collect acellular supernatant which was stored at –20 ^{0}C. The supernatant was used for the estimation of total protein concentration and other enzymatic parameters.

3.3.1. Total cell counts

The total cell counts were observed using an aliquot of BAL fluid by the method as reported earlier. [12]

3.3.2. Total protein concentration

Increase in total protein concentration is suggestive of transudation of plasma proteins (i.e., alterations in the alveoli: capillary barrier). Total protein in BAL fluid was analyzed by the method described by Bradford, 1976. [13] Sample protein concentrations were determined from a standard curve using bovine serum albumin standards. The assay was based on the absorbance shift from 465 to 595 nm that occurs when Coomassie Blue G-250 binds to proteins in an acidic solution.

3.3.3. Alkaline phosphatase

Alkaline phosphatase (AP) is a lysosomal enzyme indicative of tissue damage. Activity of AP was determined by the method [14], where AP catalyzes the reduction of p-nitrophenol phosphate to p-nitrophenol and phosphoric acid in the presence of magnesium ions and adenosine monophosphate. The rate of change in absorbance at 400 nm is directly proportional to AP activity in the sample.

3.3.4. Lactate dehydrogenase

An increase in the cytosolic enzyme lactate dehydrogenase (LDH) in BAL fluid is indicative of cell damage and lysis. It is a very sensitive indicator for any lung injury. LDH activity was estimated by previously described method [15]. In brief, the reaction mixture consisted of NADH (0.02 mM), sodium pyruvate (0.01M), sodium phosphate buffer (0.1M, pH 7.4) and distilled water in a total volume of 3ml. Formation of reduced NAD (NADH) results in an increase in absorbance at 340 nm and enzyme activity was calculated as nmol NADH oxidized/ min/ml of BAL fluid.

3.4. Macroscopic examination and organ weights

A complete necropsy was conducted on all animals. Necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Organ weights of vital organs (lungs, liver, spleen and kidneys) were recorded and appropriate organ weight ratios were calculated in relation to total body weight.

3.5. Histopathological analysis of lung

Tissues were processed by standard histopathological techniques. [16] Histopathological changes in lungs and respiratory route were specifically observed. The lung tissues were fixed in 10% formalin and embedded in paraffin. 5 µm size sections were cut from the lung tissue of each group. The sections were deparaffinized using xylene and ethanol. The slides were washed with phosphate buffer saline (PBS) and permeabilized with permeabilization solution (0.1M citrate, 0.1% Triton X-100). The deparaffinized sections were stained with haematoxylin and eosin. Tissue histology was evaluated and microscopic changes were analyzed. Tissue sections were observed under a light microscope (**Olympus BX 60**), at a magnification of 40X and compared with those of control animals.

4. In vivo efficacy evaluation

Eighteen Sprague Dawley male rats were randomly divided into three groups (n=6). The groups were designated as group I, II and III. The animals of group I served as control and received no treatment. Group II animals were exposed to cigarette smoke for 30 minutes daily for five days (two cigarettes daily up to five days) using whole body inhalation assembly. The cigarettes were fitted at one end of the inhalation assembly housing the animals leading to their direct exposure. Group III animals on the other hand received pre-treatment with 3% sub micronized SN nebulization fluid (3 ml drug nebulized once a day up to five days) followed by cigarette smoke exposure similar to Group II.

All the animals were sacrificed and dissected after 24 hours of last exposure. Various morphological, haematological and biochemical parameters were studied. Blood samples were collected from cardiac puncture in a heparinized syringe for haematological and serum biochemical analysis. Histopathology analysis of the lung tissue was done to observe any microscopic changes in the cigarette smoke exposed animals with and without SN inhalation.

5. Statistical analysis

The data are expressed as mean ± S.D. changes in body weight, food consumption, clinical pathology, and organ weight data were analyzed using student-t test to determine inter group differences and minimum criterion for statistical significance was set at $p < 0.05$ for all comparisons. Clinical pathology values for white blood cell types that occur at a low incidence (i.e., monocytes, eosinophils, and basophils) were not subjected to statistical analysis.

6. Results

6.1. Formulation preparation and characterization

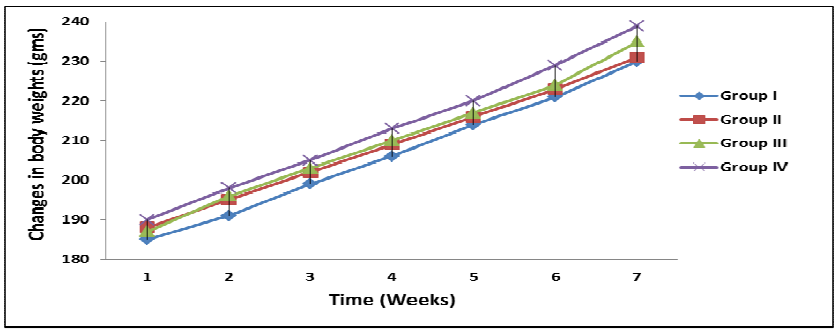
The size of aerosols for SN nebulization formulation delivered to experimental animals through nebulizer-spacer assembly was found to be in the range of 400-600 nm.

6.2. Sub-acute safety evaluation

6.2.1. Gross pathology

No mortality was observed during the experimental procedure and period of the study. The animals were at normal nutritional status and healthy in the three test groups and no differences were noted with respect to control groups. There were also no significant changes in pre- and post-treated weight of test groups as compared to control (**Figure 1**).

Figure 1: Body weight gain pre and post inhalation of SN respiratory fluid.



6.2.2. Clinical pathology: haematology and clinical biochemistry

The values of RBC, WBC, Hb, HCT, MCV, MCH and platelets were found to be within normal range in treated animal groups and there were no significant changes ($p>0.05$) as compared to the control animals (**Table 1**).

Table 1: Effect of sodium nitrite respiratory fluid on hematological parameters.

Parameters	Groups			
	Group I	Group II	Group III	Group IV
RBC (10^6/µL)	5.447±0.424	5.323±0.538	6.843±0.505	6.280±0.713
WBC (10^6/µL)	10.867±1.41	9.000±1.353	11.600±0.985	11.400±1.11
Hb (g/dL)	12.667±0.80	12.433±1.069	14.333±1.124	14.267±0.961
HCT (%)	36.400±2.900	35.400±2.905	36.767±3.522	37.667±3.557
MCV (fL)	66.400±4.480	68.967±3.350	64.300±5.951	63.600±7.019
MCH (pg)	22.500±1.179	22.467±2.255	22.067±3.099	21.867±2.444
Platelets (10^3/µL)	416.45±15.10	439.66±22.54	434.66±26.95	404.33±28.00

All the values are mean ± SD of six rats in each group.

Further, there was no significant effect on any of the biochemical parameters studied after inhalation of sodium nitrite aerosols (**Table 2**).

Table 2: Effect of inhalation of SN respiratory fluid on serum biochemical parameters.

Parameters	Groups			
	Group I	Group II	Group III	Group IV
ALP (U/L)	212.27±15.8	232.43±17.0	236.33±14.1	247.26±16.9
SGPT (U/L)	64.67±3.41	66.23±4.35	65.60±2.98	67.40±3.11
SGOT (U/L)	115.44±8.42	123.32±7.53	119.84±7.9	121.28±8.7
Protein (g/dL)	6.28±0.56	7.40±0.36	6.36±0.52	7.16±0.68
Glucose (mg/dL)	192.40±7.48	178.96±3.35	187.30±5.58	190.60±4.92
Cholesterol (mg/dL)	62.50±3.17	64.46±2.25	59.06±3.09	66.86±3.44
Urea (mg/dL)	18.54±1.52	17.63±1.68	20.12±2.02	19.32±1.86

All the values are mean ± SD of six rats in each group.

6.2.3. Macroscopic examination and organ weights

No adverse SN aerosol inhalation-related macroscopic findings were noted at the scheduled necropsies. The weights of vital organs (lung, liver, kidney and spleen) were not significantly different in experimental animals as compared to controls (**Table 3**).

Table 3: Effect of inhalation of SN respiratory fluid on organ/ body weight of vital organs.

Organs	Organ/Body weight			
	Control	Group II	Group III	Group IV
Lungs	1.325±0.067	1.313±0.065	1.365±0.055	1.335±0.146
Liver	6.467±0.804	5.600±0.780	5.983±0.564	5.883±0.928
Kidney	0.638±0.030	0.600±0.032	0.615±0.031	0.632±0.018
Spleen	0.482±0.049	0.490±0.052	0.503±0.048	0.517±0.052

All the values are mean ± SD of six rats in each group.

6.2.4. Measurement of lung toxicity parameters in BAL fluid

Total cell counts, total protein, lactate dehydrogenase and alkaline phosphatase observed in BAL fluid of experimental animals of all the test groups showed no significant change with respect to control indicating no adverse effect of SN aerosol inhalation. Moreover, there was no change in LDH activity post inhalation of the drug at all the concentrations studied, further confirming its safety and no lung injury (**Table 4**).

Table 4: Effect of inhalation of SN respiratory fluid on BAL fluid parameters.

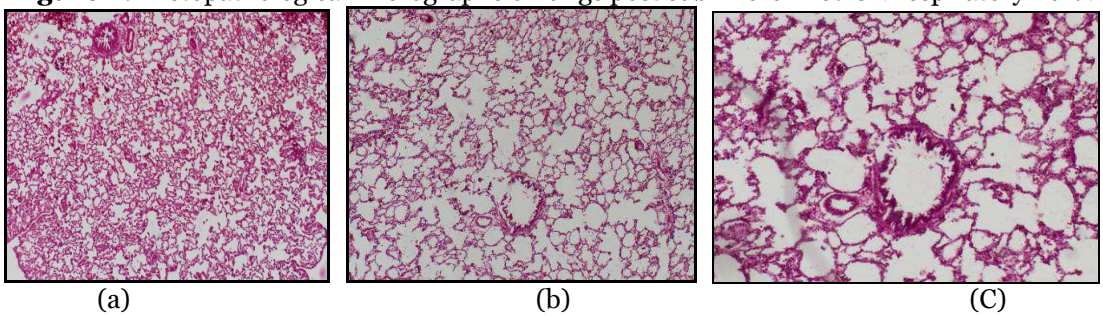
BAL fluid Parameters	Group I	Group II	Group III	Group IV
TCC (x10⁶/mL)	0.0275±0.0053	0.0271±0.0033	0.0316±0.0057	0.0321±0.0048
TP (mg/mL)	0.0164±0.0156	0.0152±0.0168	0.0186±0.0183	0.0185±0.0192
LDH (unit/mL)	63.41±6.79	66.29±6.380	65.93±8.708	67.36±8.982
ALP (U/L)	47.06±2.74	49.08±3.877	48.74±4.799	50.40±5.721

All the values are mean ± SD of six rats in each group.
TCC: Total cell counts
TP: Total protein
LDH: Lactate dehydrogenase
ALP: Alkaline phosphatase

6.2.5. Histopathological analysis of lungs

Sodium nitrite aerosol inhalation-related histopathological findings of lungs of test animals in even higher concentration groups (3 and 5% sodium nitrite respiratory fluid) confirmed that inhalation of the drug caused no inflammatory cell infiltration, interstitial and alveolar edema, vascular congestion or alveolar collapse. However, vasodilation in alveoli of lungs was observed in higher concentration groups (**Figure 2**).

Figure 2: Histopathological micrographs of lungs post sub micronized SN respiratory fluid.



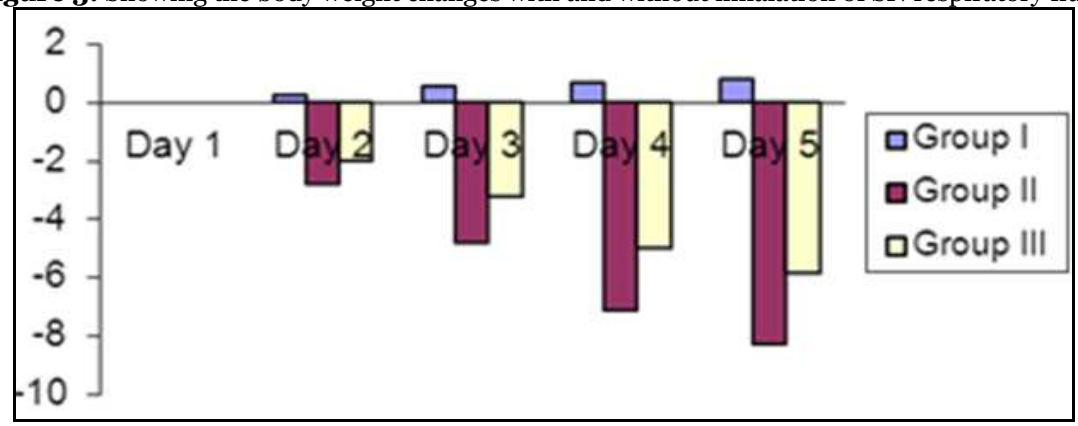
(a) (b) (C)

6.3. In vivo efficacy evaluation

6.3.1. Gross Pathology

No major morphological difference was seen in animals of all the groups. Animals exposed to cigarette smoke were found to have eye irritation; tear secretion and excessive blinking that were expected. Group II animals exposed to cigarette smoke had an increased heart rate as well as respiratory rate, which improved when SN inhalation was given. Body weight of smoke inhaled animals was found to decrease with time. However, the per cent decrease in weight with time was less in animals which were pre-treated with SN inhalation **(Figure 3**).

Figure 3: Showing the body weight changes with and without inhalation of SN respiratory fluid.



6.3.2. Clinical pathology: hematology and serum biochemistry analysis

Hematological and serum biochemical analysis also showed that pre-treatment with SN inhalation was efficacious in terms of improvement in renal and liver functions in smoke exposed animals, as can be seen from improvement in urea, creatinine as well as ALT and AST values (**Table 5**).

Table 5: Showing the serum biochemical parameters on inhalation of SN respiratory fluid.

Parameters	Groups		
	Group I	Group II	Group III
Urea (mg/dL)	26.65 ± 3.30	42.42 ± 8.68	34.09 ± 6.72
Creatinine (mg/dL)	0.52 ± 0.10	0.67 ± 0.15	0.56 ± 0.12
ALT (U/L)	60.43 ± 8.24	145.25 ± 20.45	84.67 ± 12.82
AST (U/L)	121.46 ± 16.35	247.76 ± 30.10	194.57 ± 24.40

All the values are mean ± SD of six rats in each group.

6.3.3. Bronchoalveolar lavage fluid analysis

Total cell counts, total protein and lactate dehydrogenase levels observed in BAL fluid of smoke exposed animals were significantly altered as compared to control animals. Pre-treatment with sub micronized SN inhalation resulted in significant improvement in BAL fluid markers, and tended to approach towards normal, indicating potential efficacy of SN inhalation (**Table 6**).

Table 6: Showing the oxidative stress markers in BAL fluid of with and without SN inhalation.

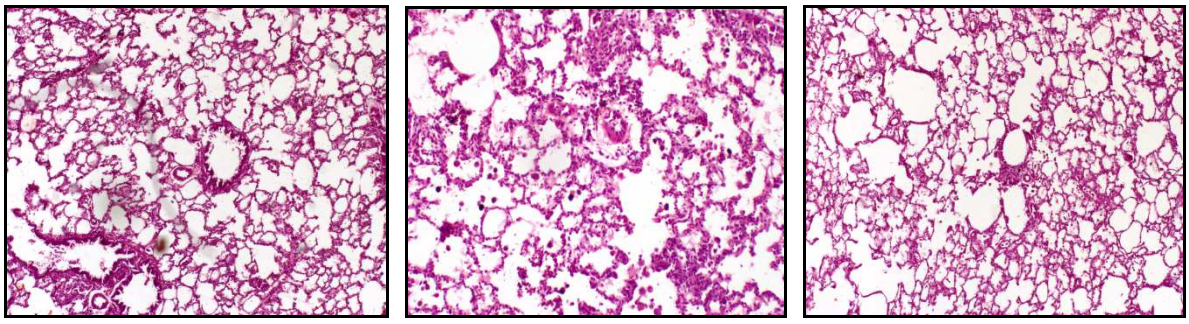
Parameters	Groups		
	Group I	Group II	Group III
Total Cell Counts (x10^6 cells/ mL)	0.061 ± 0.007	0.29 ± 0.030	0.19 ± 0.023
Total Protein (mg/ mL)	17.46 ± 2.37	30.53 ± 4.52	21.93 ± 3.28
Lactate Dehydrogenase (unit/ mL)	5.84 ± 0.71	8.4 ± 0.78	6.58 ± 0.73

All the values are mean ± SD of six rats in each group.

6.3.4. Histopathological analysis of lungs

While, no morphological changes were observed in lungs of control animals **(Fig. 4a),** histopathological analysis of the lung tissue in group II animals revealed that cigarette smoke inhalation resulted in massive inflammatory cell infiltration, interstitial and alveolar edema, vascular congestion and alveolar collapse **(Fig. 4b).** On the other hand, SN treatment led to significant reduction in cellular infiltration and pulmonary edema, and intact alveolar architecture could be seen **(Fig. 4c),** confirming the efficacy of developed formulation in preventing/ reducing smoke induced lung injury.

Figure 4: Showing the microscopic changes in the lung micrographs of SN and smoke inhalation.



(a) **(b)** **(c)**

7. Discussion

The present study describes pre-clinical safety and efficacy study with respect to a novel formulation of 3% sodium nitrite in 30% ethanol-saline in experimental animals as per regulatory guidelines of Indian Council for Medical Research. The formulation is meant for treating pulmonary hypertension and other associated respiratory conditions, besides protecting against oxidative stress and lung injuries caused due to other factors such as smoke inhalation. The present study aimed at fulfilling the mandatory task of generating animal safety and efficacy data prior to carrying out phase-1 human trials.

A whole body exposure chamber was used for inhalation of drug aerosols, which provided a few advantages. Firstly, it provided an exposure environment in which the test subjects were unrestrained and could move about freely within cages. The system also provided efficient exposure to a large numbers of animals at the same time, and is very useful for chronic exposure studies where daily exposure duration may be as much as 24 hours. Since the animals left are unrestrained, the experimental procedure does not result is any significant physical stress to the animal, which may otherwise affect the experimental findings to a variable extent. However, disadvantages include that it also leads to exposure of the entire body, resulting in possible oral and dermal exposure of the test compounds. Any such exposure in the present study with no adverse effects may be taken as an added confirmation of the safety of sodium nitrite aerosols.

The assessment of toxicity in inhalation studies is similar to that used for toxicology studies conducted by any other route of administration. Common parameters that are studied include assessment of body weight, food consumption, clinical signs of toxicity, clinical pathology (hematology, clinical chemistry), gross necropsy (except nasal cavity), and histopathology. In the present study no adverse effects on any of the hematological and biochemical parameters were seen following treatment with aerosols of sodium nitrite respiratory fluid in any of

the drug concentration studied. No morphological changes were observed in alveolar macrophages. There were no changes in enzyme activity as well. There were also no indications of nonspecific immune responses, as indicated by normal ALP and LDH activity in BAL fluid. These studies indicate that aerosol delivery of sodium nitrite directly to rodent lungs resulted in no activation of the local cellular immune system. We also investigated the tracheal ultra-structure in rats that received drug exposure. The exposed animals did not exhibit any cellular influx to the site of administration, and the alveolar macrophages exhibited no altered morphology relative to controls.

Collection and analysis of BAL fluid has become an established technique to study cellular and soluble components of the lower respiratory tract. [17] The study of cells and proteinacious substances in lung washings provides an insight into the effects of any particular inhalation agent on lung biochemistry. BAL fluid analysis was therefore done to ascertain any sodium nitrite inhalation induced toxicity. BAL is a minimally invasive procedure that offers an opportunity to investigate intra-alveolar alterations associated with lung diseases [18] and a valuable tool for studying immune and inflammatory mechanisms in pulmonary disorders. [17, 18 & 19] Our data confirms that there are no changes in lung injury markers in BAL fluid post-inhalation of submicronic sodium nitrite aerosols inhalation. Marginally increased total protein concentration of BAL fluid might be due to vasodilatory action of inhaled submicronic sodium nitrite particles in alveoli of lungs.

8. Conclusions

The results show that sodium nitrite does not cause any significant alterations in any of the hematological, biochemical, BAL fluid or microscopic parameter, except pulmonary vasodilation on repeated inhalation. The study highlights the prophylactic potential of sodium nitrite nebulization against oxidative stress and lung injury caused due to smoke inhalation, and could be significant in developing a novel prophylactic/ therapeutic option for victims as well as members of the rescue team, including fire-fighters.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are thankful to Defence Research and Development Organization, Ministry of Defence, Government of India for providing the funds to carry out this work.

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