International Journal of Scientific and Innovative Research 2022; 10(1) : 16 - 19
P-ISSN 2347-2189, E- ISSN 2347-4971

# AMELIORATION OF CARBON TETRACHLORIDE INDUCED DAMAGES BY HERBAL PREPARATION KOFLET IN RATS

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## ABSTRACT

*Herbal preparation koflet caused significant recovery in damaged lung membranes of rats which was evident by toxicological, biochemical and histopathological parameters.*

***Keyword*:** Koflet ; Pulmonary dysfunctions ; Flyash; $CCl_4$; Physiological functions of the cell

## INTRODUCTION

Ayurveda is the science of life which was derived from spiritual visions. Since time immemorial, it still continues as an important system of medicine and drug therapy in India. Many therapeutic properties of a large number of herbs in pulmonary dysfunctions have been attributed in "Sushruta Samhita" which is the most representative work of Ayurveda. Mammalian respiratory system is structurally complex arrangement of organs primarily for intake of oxygen and the elimination of carbondioxide. The respiratory tract is divided into two portions based on gross anatomy and physiology i.e., proximal conducting airways and distal respiratory region. Proximal conducting airways include the nose, pharynx, larynx, trachea, bronchi, nonalveolarized bronchioles and the distal bronchioles, alveolar ducts and alveolar air sacs. Gaseous exchange between air and blood is restricted to the distal respiratory region which is located in the lung parenchyma. Nasal airway is divided into two passages by nasal septum. Each nasal passage extends from the nostrils to nasopharynx. Pharynx connects the nasal and oral airway with laryngeal airway during breathing. Right and left portion of nasopharynx join at the bend and it is an important site for inhaled particles and gas deposition. We have seen the experimental beneficiary effect of herbal preparation koflet on damaged lung membranes of rats and results are presented in detail in this research paper.

## MATERIALS AND METHODS

**A. Preparation of flyash dust samples**

Flyash was obtained from a thermal power station in India. It was passed through a 400 mesh sieve to remove coarse particles and was briefly suspended in distilled water. The supernatant containing fine particles of flyash was siphoned off, then centrifuged at 800g for 10 minutes and the sediment was dried at $100^{0C}$. The flyash particles thus obtained contained more than 90% of the particles less than five 5um in diameter.

**B. Inoculation of animals**

The animals were divided into four groups and 20 rats were kept in each group and animals for intratracheal instillation were lightly anaesthetized. The trachea was exposed ventrally by blunt dissection at throat and 1.0 ml of the dust (flyash) suspension (10.0 mg flyash in 1.0 ml physiological saline) kept constantly agitated to prevent sedimentation within syringe forcibly and using 50% air usually helped to ease dispersion of the dust into the lung alveoli. The open wound was ligated by a single suture.

**C. Administration of carbon tetra chloride**

Animals were administered $CCl_4$ in combination with mineral oil orally gastric intubation. Albino rats of 150-175gm body weight obtained from animal house colony were reared under normal conditions of

husbandry and fed *ad libitun* water and pellet diet supllied by Lipton India. Rats were divided into four groups and 20 rats were kept in each group as follows:-

Group I: Controls rats were inoculated intratracheally with 1.0ml physiological saline only, under light ether anaesthesia.

Group II: Rats were gavaged with preparation koflet 1.0ml daily to each rat.

Group III: Rats were inoculated intratracheally 10.0mg flyash in 1.0ml physiological saline to individual rat.

Group IV: 1.0ml koflet was gavaged to individual rat as group II started. After one week of pretreatment of preparation koflet orally, the animals were inoculated 10.0mg flyash intratracheally in 1.0ml of physiological saline to each rat.

Ten rats from each group were sacrificed after one week and remaining rats were sacrificed after 30 days while the animals of II, III and IV groups received 1.0ml daily doses of preparation koflet though oral route up to 30 days. After the animals were sacrificed, liver, trachea, lung and blood were collected. Liver, lung and trachea were washed properly with cold physiological saline to remove the sticked blood and then minced fine with scissor. Homogenization of tissue was carried out in 2.5mM EDTA buffer, pH7.4 as 10% w/v. Homogenate was centrifuged at 10,000g for 30 minutes and supernatant was used for enzymatic analysis and other constituent estimations.

The methods for enzymatic analysis and constituents estimations are as follows:-

i. Alkaline phosphatase : The method of Weiser, 1973[1] was followed.
ii. $Ca^{2+}$- $Mg^{2+}$ - ATPase: The $Ca^{2+}$- $Mg^{2+}$ - ATPase activity was determined according to Hidelgo *etal*., 1983[2] method.
iii. Glutamic oxaloacetic transaminase (GOT): The enzyme activity was measured by the method of Wooten, 1964. [3]
iv. Glutamic pyruvic transaminase(GPT): The enzyme activity was measured by the method of Wooten, 1964. [3]
v. Protein estimation: protein of liver, lung, trachea and serum was estimated by the method of Lowry *etal.*, 1951[4]
vi. Estimation of carbohydrate: Total hexoses were estimated by anthrone reagent using the method of Roe, 1955. [5]
vii. Estimation of sialic acid: The method of Warren, 1959 [6] was followed.
viii. Serum cholesterol: the content of cholesterol was measured by the method of Zlatkis *etal*., 1953. [7]

## OBSERVATIONS AND RESULTS

Effects of physical and chemical and irritants (flyash and $CCl_4$) on physiological functions of the cell are as follows:-

**A. Effect of preparation koflet on body weight and food consumption:** $CCl_4$ treated animals did not gain body weight during 7 days treatment where as herbal preparation koflet receiving animals showed comparatively better weight gain in comparison to the respective untreated controls. Simultaneously, preparation koflet receiving animals along with $CCl_4$ treatment showed a consistent body weight with no significant fall during the time period. This suggests that preparation koflet protects the loss in weight due to $CCl_4$ treatment. There was tremendous decline in the food consumption of $CCl_4$ treated animals. Preparation koflet also provide significant protection against the loss of appetite caused by $CCl_4$. The loss of body weight caused by $CCl_4$ was due to less consumption of food. The ingredients of preparation koflet were able to check the loss of appetite which provided the protection against the loss of body weight.

**B. Effect of preparation koflet on rat lung and liver enzymes:** There was no significant change in lung enzymes activities even after 30 days following the treatment of herbal preparation koflet. Flyash instillation showed a time dependent increase in lung enzymes indicating the lung damage. Flyash instilled animals receiving pre and post treatment of preparation koflet showed more or less similar enzyme activities as that of controls. This suggests that preparation koflet checks the effect of flyash influenced enzyme increase. $CCl_4$ treated animals also showed significant increase in GOT, GPT and $Ca^{2+}$- $Mg^{2+}$- ATPase activities at the end of one week. $CCl_4$ treated animals also receiving preparation koflet revealed enzyme activities more or less similar to the controls. Almost similar

enzyme patterns were also observed in case of liver tissue of flyash, $CCl_4$ and preparation koflet receiving groups of animals.

**Effect of preparation koflet on rat liver, lung and tracheal contents:** There was no significant alteration in total protein, carbohydrate and sialic acid contents of liver, and lung of herbal preparation koflet receiving animals and control animal in both short and long term treatments. A slight decline in tracheal carbohydrate and sialic acid contents were observed following preparation koflet treatment after 1 week, and 1 month. However, the alteration caused by flash and $CCl_4$ treatments in total protein, carbohydrate and sialic acid contents of all the tissues examined showed the normal control values in the groups pre and post treated with preparation koflet.

## DISCUSSION

Preparation koflet has a specific role to play in cough amelioration and cure. This observation has been substantiated in our experiments by data in terms of the irritant $CCl_4$ & flyash. We can safely say that herbal preparation koflet is a miracle drug for cough and certainly the synergistic effect of the ingredients goes a long way in proving its efficacy as an indigenous herbal drug in India. Significant ameliorative effect of koflet formulation was observed against pyridine induced pharyngitis in rats by Vishwanath *et al.,* 2014. [8] Preparation koflet facilitates easy expectoration of sputum due to its mucolytic action and soothens central depressant effect or drying action on respiratory.

Several workers Wehner et al., 1979 [9] , 1980[10] ; Kaw and Waseem, 1992[11] ; Srivastava et al., 1984 [12] have demonstrated that the membrane enzymes and transport systems are associated with specific lipid. Any change in the lipid composition of membrane during various physiological disorders lead to changes in the activity of membrane. Infection of respiratory tract is a common ailment as it is directly exposed to external atmosphere. Bacteria or viruses enter the respiratory tract through inhalation of contaminated air and diseases are prevalent. Mucosal cells of the respiratory tract are the first to get adversely effected by the infection and the physiological functions of cell membrane and cells are deranged. As a result of phagocytosis, exudation and pus formation takes place which are expectorated out in the form of purulent sputum. Similar reaction is also seen in response to physical or chemical irritants when they come in contact of cells of respiratory tract. For treatment of such damages to respiratory tract and related complications, preparation koflet is best treatment.

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