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Onion Juice Supplementation Increases Antioxidant Enzyme Status and Maintains Cellular Integrity of the Broncho-Alveolar Epithelium of the Lung in Adult Male Wistar Rats

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ABSTRACT

Many scientific researchers have reported on onion extracts' potent antioxidant property linked to its rich flavonoid compound - quercetin. This study was designed to investigate how raw onion juice supplementation can boost the antioxidant status and improves broncho-alveolar epithelium in adult male Wistar rats. Fifteen healthy adult male Wistar rats were divided into three groups: Group A - Control; Group B was given 2.5ml/kg of raw onion juice supplementation while Group C received 5ml/kg of raw onion juice supplementation orally for 14days. Results obtained shows superoxide dismutase activity increased significantly in group B (673±1.50) vs Control (671±11.50) and C (587±3.50) at $p<0.05$ while Glutathione peroxidase (GPx) increased significantly in group B (6921±11.10) vs control group (6321±16.80) and Group C (6355±35.50). The histological demonstration shows a well proliferative bronchoalveolar epithelium. Raw onion juice supplementation helps protect broncho-alveolar epithelium by its proliferative and antioxidant increasing ability linked to its rich flavonoid compound quercetin.

Keywords: Quercetin, broncho-alveolar epithelium, antioxidant, proliferative and superoxide dismutase

INTRODUCTION

Ethnopharmacological relevance of plants and herbs is a universal phenomenon; Modern drug was derived from plant extracts with documented medicinal properties and recently there has been a growing interest in alternative therapies, every culture all over the world has a relay on a vast variety of natural compound in the herbal plant due to their therapeutic properties [1]. Recently, a wide number of plant-derived pharmaceutical products are being used in traditional medicine because of their beneficial properties [2].Onion (*Allium cepa*) is of the family Liliaceae, it is a common pungent medicinal spice all over the world used both as a food and for medicinal application [3]. Onion contains certain chemical substances that are beneficial to health such as phenolics and flavonoids reported to have potential anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties [2]. Chemical components of fresh Onions contain 89% water, Chromium, 1.5% protein, Selenium, Vitamins, potassium Peptides, Polysaccharides such as fructans, saccharose, essential oil [4], Cepaenes, Thiosulfinates, Flavonoids e.gQuercetin[5],Kaempferol, and pigments such as anthocyanins[4]. Yang *et al.,* listed the following as dietary constituents of onion juice: Protein, Fat, Carbohydrate, Fiber, Water, Sugars, vitamins such as Vitamin C,E,B6, B5(Pantothenic acid), B9 (Folate), B3 (Niacin), B2 (Riboflavin),minerals such as Potassium, Calcium, Manganese, Zinc, Magnesium, Phosphorus, Sulfur, Iron and Fluoride. Onion expectorant properties in treating cold and respiratory disorders such as bronchitis and nasal congestion are kinked to its volatile oils containing sulfurous constituents like ally-propyldisulfide, allicin and alliin reported to acts as a mucolytic agent by liquefaction of mucus and inhibits its formation [3].

Uzum et al., reported that lung is a primary target for oxygen toxicity in everyday human life, because of its constant exposure to high oxygen levels and environmental oxidants that are linked to respiratory tract infection. Many scientific researchers showed that onion extract has significant antioxidant activity because of its high amount of flavonoids such as quercetin, strong antioxidant flavonoids which play a crucial role in the cell defense system against oxidative stress [6,1]. Quercetin's promising role in the alleviation of oxidative epithelial cell injury in lung inflammation was reported by[7,8]. Glutathione peroxidase is an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage and lipid peroxidation. Quercetin is the chief flavonoid compound in onions and it has been reported to protect DNA and other important molecules from oxidation and probably protects pulmonary epithelial cells against oxidation and improves alveolar tissue [9,10]it also helps to alleviate oxidative cell damage induced by stress, drugs or chemical and suggesting onion

therapeutics in homeopathic medicine [11]. Hence, the mechanism of action needs to be documented. Hence our present research aims to investigate the effects of fresh onion juice supplementation on the pulmonary tissue by assessing antioxidant defense enzyme markers Superoxide Dismutase [SOD], Glutathione Peroxidase [GPx] and histological appearance of the pulmonary tissue following ingestion of varying doses of raw onion juice supplement.

MATERIALS AND METHODS

Experimental Animals: Fifteen health male adult Wistar rats were obtained and bred in the Animal House of Anatomy Department of Bingham University, Karu Nigeria. They were kept in well-ventilated rats cages in ambient laboratory conditions [Temperature: 37˚C; Humidity: 50-55% and 12hr light/dark cycles]. All animals were allowed to acclimatize and fed standard rat pellets and water *ad libitum.* Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (2001). The ethics regulations were followed by Institutional ethics committee guidelines in the use of Animals during experiments.

Plant material procurement and extraction: Fresh red onions used for this study were purchased in the Masaka market, Nasarawa State. Specks of skin or dirt were removed and the Onion rinsed in water. They were then cut into small pieces and blend using a blender. The fine onion blend pulp was poured into a fine mesh strainer and a spoon used to push the pulp into the strainer, separating the juice from the pulp. The remaining pulp was into the center of cheesecloth and the juice squeezed out. A sterile filter [filtered using a sterile muslin] was used to filter the pure onion juice to rid it off any pulp. This procedure was followed routinely to obtained fresh onion juice during the experiment.

Onion extract dosage: The acute toxicity test was performed by administration of ripe onion juice using oral gastric gavages at doses of 2ml/kg daily and 5ml/kg daily; administered orally for 14days.

Experimental Duration: Two Weeks (14 days)
Experimental design:**Group A**- Control group were fed and given water for 14 days.
Group B- Were administered with onion juice orally at 2.5ml/kg (equivalence of 2.5g/kg) for 14 days
Group C- Were administered with onion juice orally at 5ml/kg (equivalence of 5g/kg) for 14 days

Animal Euthanasia: At the end of the study, final body weight was taken and animals euthanized by cervical dislocation. An incision made in the thoracic region and the lungs excised and wet weights taken.

Collection of Lung tissue for Histological stains: The right lung specimen was fixed in a specimen bottle containing 10% formal saline, after which it was prepared for histological analysis and staining for Hematoxylin and Eosin [H and E]. The samples were processed using an open automated tissue processor through graded alcohol, cleared in xylene, impregnated and embedded in paraffin wax. Thin sections were cut with a Leica rotary microtome set at 5um. Slides were stained using hematoxylin and eosin (H and E) stain and viewed under an Olympus Light Microscope to analyzehistoarchitectural changes in the pulmonary tissue.

Lung tissue collection for antioxidant enzyme assay: The left lung was homogenized in 0.25% sucrose and kept in a refrigerator with about -40c temperature. It was used to check for the activities of some antioxidant enzymes such as Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx).

SOD analysis: SOD detecting kit was purchased from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom. The role of SOD is to accelerate the dismutation of the toxic superoxide radical (O2), produced during the oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye detectable at 505 nm.

Glutathione peroxidase analysis: The commercial kit was obtained from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom). According to this method, GPxcatalyzes the oxidation of glutathione and in presence of glutathione reductase and NADPH, oxide glutathione converts to reduced form by changes in the oxidation of NADPH to NADP+ in absorbance at 340 nm.

Statistical Analysis: The statistical analyses were performed on a Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed using a one-way analysis of variance (ANOVA) followed by the least significant difference of P values set at $P<0.05$ was considered statistical significance. Data were expressed as Mean±SEM.

RESULTS

Bodyweight: There was a general increase in body weight gain in all experimental groups as shown in Figure 1. There was a statistically significant increase in body weight gain in Group B and C treated rats as compared with the control A ($P<0.05$). Group C treated rats show a significant increase in body weight gain at $P<0.05$)

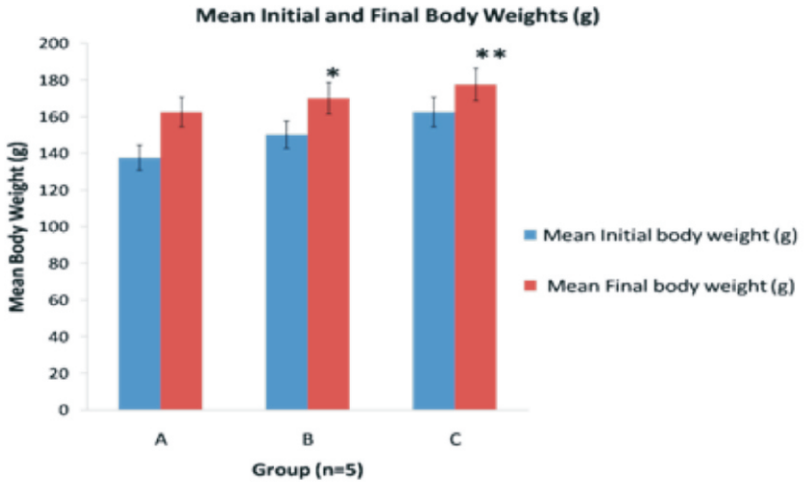

Figure 1: Effect of Onion juice on body weight of Adult Male Wistar Rats. Each value is expressed as Mean±SEM Statistical significance (*) taken at p<0.05. Legend; A= Control, B=5ml/kg body weight of Onion Juice and C= 10ml/kg body weight of Onion Juice. * Significance of B vs A, (**) Significance of C vs B [P<0.05]

Wet weight of the Lung: Figure 2 shows the changes in wet of the lung in experimental animals following onion juice ingestion. There was an overall increase in body weight gain in all groups. There was a statistically significant increase in weight gain of the lung in onion juice treatment groups as compared to the controls at P<0.05.

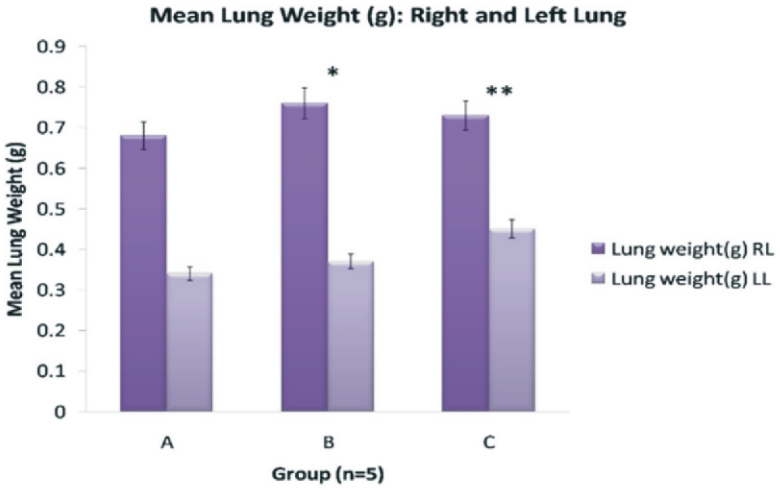

Figure 2: Effect of Onion juice on body weight of Adult Male Wistar Rats. Each value is expressed as Mean±SEM

Statistical significance (*) taken at p<0.05. Legend; A= Control, B=5ml/kg body weight of Onion Juice and C= 10ml/kg body weight of Onion Juice. * Significance of B vs A, (**) Significance of C vs B [P<0.05].

Glutathione peroxidase Enzyme Activity: Figure 3 shows the changes in glutathione peroxidase antioxidant enzyme in the experimental animals. Glutathione peroxidase activity increased significantly as compared to the control group –A in 5ml/kg Onion juice treatment at P<0.05. But the higher dose group [C-10mg/kg] had a significant decrease as compared to B [5ml/kg body weight] as compared to B and A groups at P<0.05.

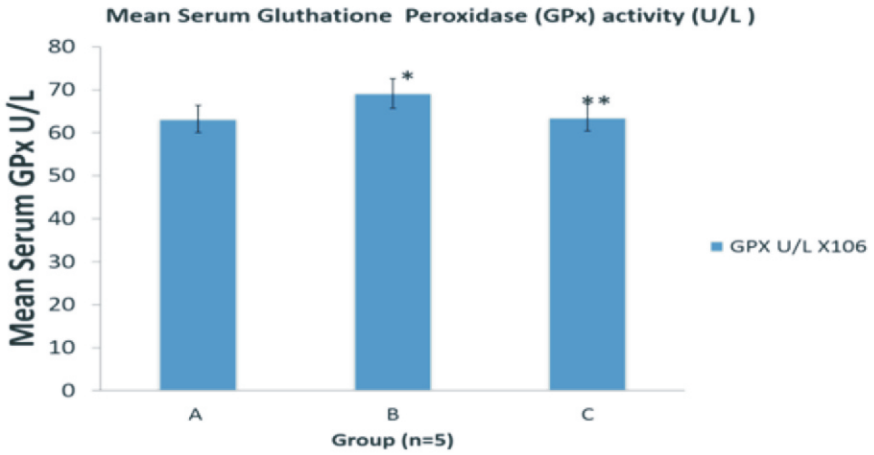

Figure 3: Effect of Onion juice on Glutathione Peroxidase Antioxidant enzyme activity in Adult Male Wistar Rats. Each value is expressed as Mean±SEM Statistical significance (*) taken at p<0.05. Legend; A= Control, B=5ml/kg body weight of Onion Juice and C= 10ml/kg body weight of Onion Juice. * Significance of B vs A, (**) Significance of C vs B [P<0.05]

Superoxide Dismutase activity:
Figure 4 shows the antioxidant enzyme activity of superoxide dismutase (SOD) activity in the experimental animals. B had a significant increase in SPD activity as compared to A at P<0.05. C had a statistically significant decrease in SOD activity as compared to B and C at P<0.05.

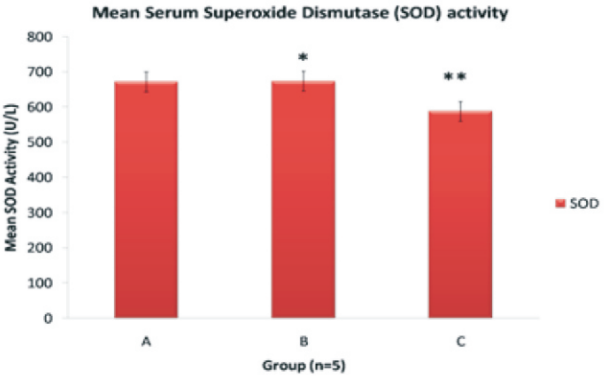

Figure 4: Effect of Onion juice on Superoxide Dismutase Antioxidant enzyme activity in Adult Male Wistar Rats. Each value is expressed as Mean±SEM. Statistical significance (*) taken at p<0.05. Legend; A= Control, B=5ml/kg body weight of Onion Juice and C= 10ml/kg body weight of Onion Juice. * significance of B vs A, (**) Significance of C vs B [P<0.05]

Histological Result: Figure 5: Micrograph showing histological staining of the pulmonary tissue viewed under a light microscope of control and onion juiced treated rats. H & E staining (400x, H & E). (A) Control animals showing normal architecture. (B) and (C) Onion juice treated shows the normal alveolar epithelial arrangement and bronchiole epithelium with no distortion in pulmonary tissue histoarchitectural arrangement.

DISCUSSION

This present study demonstrates the antioxidant improving status and mechanism through which raw onion juice supplementation mediates broncho-alveolar protection in adult male Wistar rats. There was an overall increase in the mean body weight of all experimental groups as displayed in Figure 1. Onion juice supplementation resulted in a significant increase in body weight gain (P<0.05) as compared to the control group. Contrary to notable finding that onion juice supplementation causes a reduction in body weight gain [12] but in this study Onion juice caused a dosage-dependent increase in body weight gain hence its anti-obesity property was not exhibited in this study. Though quercetin-rich onion juice supplementation had been reported to significantly reduced body weight and percentage body fat in rats; it was not notable in our present study. Contrary to [13] reports that Onion juice lowers oral glucose consumption, our animal carbohydrate food intake increased. The wet weight of

the lung of onion treated animals increased significantly as compared to the control groups. Reactive oxygen species [ROS] in vascular tissues like lung tissue is linked to NAD (P) H oxidases in the vascular tissue membrane. ROS induce the expression of different molecules in the endothelial cell surface such as vascular cell adhesion molecule-1 (VCAM-1), which stimulates monocyte binding and subsequent macrophage differentiation in the pulmonary tissue. An Increase in ROS generation is linked to endothelial dysfunction which will affect gaseous exchange in the alveolar sac. In our study, we evaluated the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) as a marker to investigate onion antioxidant potential. Glutathione peroxidase is related to the balance between the GSSG (reduced form of glutathione) and GSH (oxidized form of glutathione) through the interaction with GPx. Reduced glutathione can neutralize hydroxyl radicals and detoxify the peroxides. Glutathione peroxidase (GPx) activity as shown in Figure 3, increased in Group B onion supplementation as compared to Group A- The control group as $P<0.05$. SOD activity reduces in a dose-dependent order as shown in Figure 4. The group feed with 2.5ml/kg of onion juice supplementation had a significant increase in SOD activity as compared to the control groups at $p<0.05$, while those feed with 5ml/kg of Onion Juice had a reduction in SOD activity as compared to group B feed 2.5ml/kg. This reduction is linked to onion juice increasing activity of ubiquitous SOD to mob off ROS and reduced SOD activity is linked to a reduction in SOD activity due to a reduction in oxidative stress generation of ROD in the Lung cellular environment. SOD protects the cells against superoxide and hydrogen peroxide generated by a stressor. The source of hydrogen peroxide is mainly SOD-mediated dismutation of superoxide radicals, which is generated by various enzyme systems and hence reduces SOD activities. These aforementioned results on Onion juice supplementation ability to reduce antioxidant enzyme status are linked to its potential to mob off free radicals capable of causing irritating in the respiratory tracts especially inflammation to alveolar epithelium [14]. It has been reported that consumption of raw onion juice helps to prevent allergies and this property is linked to quercetin with a strong ability to inhibit histamine and cellular secretion linked to allergic reaction pathways. Hence, our study supports [15]report that consumption of raw onion juice can reduce oxidative stress due to its strong antioxidant status which was in agreement with [16] whose study revealed significant reduction in oxidative stress caused by hyperglycemia and diabetes, their study revealed that quercetin found in onion extract has high antioxidant activity against hyperglycemia and diabetes. [17]from their study revealed that *Echispyramidum* venom which exerts its toxic influence on the liver and kidneys was significantly reduced through the administration of quercetin preventing oxidative stress on the organs. It is worthy to know that that antioxidant activity displayed in onion juice supplementation is linked to quercetin, a flavonoid with strong antioxidant activity capable of increasing activities of superoxide dismutase and glutathione peroxidase in the stress-induced lung-mode as reported by [18,19]. Histological appearance of the lung tissue was displayed using Haematoxylin and Eosin stain as shown in Figure 5; Plate A is the control showing the alveolar and bronchiole epithelial cells. Group B displayed some red blood cells within the lung tissue, less proliferative alveolar and bronchial epithelium as compared to Group B showing no red blood cells within the pulmonary tissue and well proliferative epithelial cells of the alveolar and bronchioles. The observed cellular proliferation in high dose onion juice supplementation group correlates with [20]reports that Quercetine in raw onion juice aid cellular proliferation while increasing antioxidant enzyme status within the pulmonary tissue. Quercetin'scytoprotective function against hydrogen peroxide (H_2O_2)-induced cytotoxicity in lung epithelial cell lines where it suppressed H_2O_2-induced apoptotic events hence attenuating oxidative epithelial cell injury in the lungs[21]. Alpsoy et al., (2011) further buttress quercetin roles in interacting with free radicals such as hydroxyl, superoxide, alkoxyl, and peroxyl radical; subsequently scavenging them thereby protecting lung tissue against their cytotoxic effects while showing its anti-inflammatory role. This showcase on the mechanism of onion juice use in homeopathic medicine in treating various pulmonary infections.Also, quercetin's anti-inflammatory and antioxidant properties are linked to the activation of lymphocytes activity [3]. Consumption of onion juice helps to prevent allergies and asthma due to the presence of the compound quercetin, which inhibits histamine and other cell secretions that cause allergic reactions, while sulfur present in onion is effective as an anti-inflammatory agent. This finding was in agreement with [22]study which revealed that inhalation treatment with quercetin mitigates pneumonitis induced by radiation through the reduction of inflammatory cell number accompanied by downgrading inflammatory response and pathological changes. Quercetin relaxes the airway and provides relief from asthma symptoms. Raw onion juice helps to treat cold and other respiratory problems due to the presence of sulfur-containing compounds. Volatile oil-dislodges mucus and prevents the formation of new mucus. Onion juice helps to heal cough, bronchitis, and congestion due to its anti-inflammatory properties [14]. [23] reported that onion extracts increase GPx and SOD level indicating its antioxidant prevention of oxidation in blood this was also in consonance with [24] revealing that quercetin has therapeutic benefit by down-regulating cellular activities associated with agedness and up-regulating the expression of antioxidant enzyme genes on dermal fibroblasts of older people.

Various studies involving onion extracts or quercetinin its active compound has shown that it helps to increase

GPx and SOD activities. An Increased GPx activity mediated by quercetin has been reported to help ameliorate Chlorpyrifos induced lung toxicity by improving histological of the lung tissue [21]. [25]from their study reported that quercetin has free anti-oxidant and scavenging potency through which PI3K/Aktsignaling pathway upregulates activities of SOD, GPX, CAT, GR, as well as reducing the activities of ROS and MDA associated with oxidative stress incurred in diabetic complications.Quercetin supplementation attenuates histological alterations of the lung while increasing SOD and GPx activities, showing chemoprevention and chemotherapeutic effects linked to its antioxidant property. Onion potential use in nutraceuticals is linked to its rich quercetin- flavonoid compound that mobs free radicals- thereby preventing disease such as pulmonary and cardiovascular diseases [26,27]. Also,[28]study on osteoarthritis recorded that quercetin is potent against oxidative stress which is expressed by upregulation of SOD activities and TIMP-1 expression, downregulate MMP-13 expression acting against oxidative stress with a resultant effect seen with the increased extracellular matrix of the cartilage. Other reports its importance in the treatment of oxidative stress-induced influenza vireo infection where quercetin in onion protects against free radicals generated by alveolar macrophages by its ability to induce an increase in superoxide radical peroxidation [7].

CONCLUSIONS

The lung is a primary target of oxygen toxicity because of its exposure to oxygen level and environmental oxidants hence it is significant to have readily accessible supplements to combat these ROS generated. The results obtained indicate that onion juice supplementation daily effectively improves antioxidants status of the lung, ameliorates oxidative stress while improving alveolar epithelium.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENT

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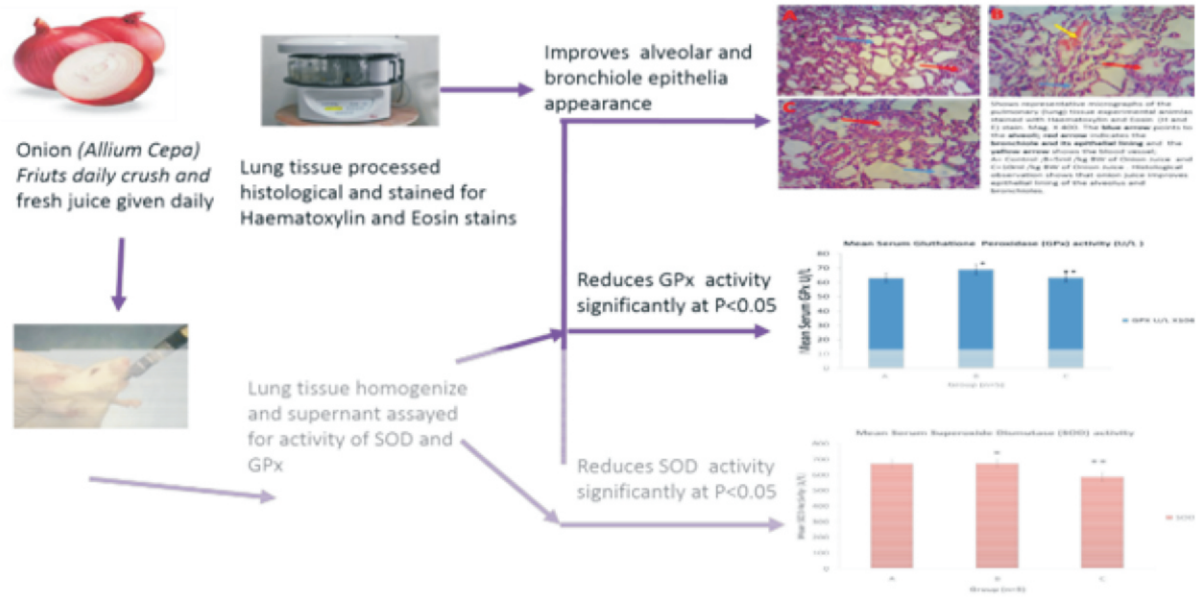
GRAPHICALABSTRACT



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