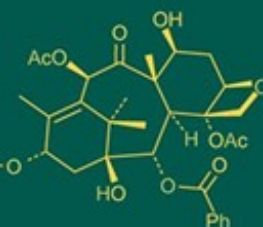
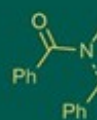
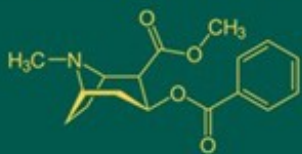


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The names and affiliations of all authors are provided below, following the references.

Tobacco smoke-induced oxidative stress in indoor dogs

Amit Kumar¹, Anil Gattani², Sanju Mandal³, Aafreen Khan⁴, Ravinder Malhan⁵,
 Shashank Vishvakarma⁶, Eshani Dixit⁷, Sankalp Sahu⁸, Shivani Dhurve⁹

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ABSTRACT

Numerous health risks for non-smokers have been linked to passive smoking or ambient tobacco smoke exposure. Companion dogs, which are kept in close physical proximity to their owners, are exposed to tobacco smoke both directly through inhalation (second-hand smoke) and indirectly by ingestion (third-hand smoke) of smoke residues that have been contaminated on skin, clothes and other surfaces. The purpose of this study was to assess how exposure to ambient tobacco smoke influenced healthy pet dogs' oxidative status.

In this investigation, twenty healthy canines were used. Dogs were divided into two groups: ETS (exposed to tobacco smoke; $n = 10$) and NETS (not exposed to tobacco smoke; $n = 10$) groups. Catalase, ferric-bipyridine reducing capacity, superoxide dismutase, Thio barbituric acid-reactive substances (TBARS), reduce glutathione, iron, and copper levels were all assessed in the blood. A significant ($p < 0.01$) increment in the TBARS, GSH and Copper was observed in ETS group whereas the SOD and catalase activity and Iron concentration was significantly lower ($p < 0.05$) in ETS than NETS group. Other serum biochemistry was altered non significantly except glucose that was observed significantly ($p < 0.05$) lower in ETS group. The oxidant/antioxidant balance in companion dogs was shown to be disrupted by tobacco smoke exposure.

Keywords: Passive smoking, Oxidative stress, Companion dogs, Antioxidant defenses, Lipid peroxidation

Background

The harmful effects of smoking on human health have been recognized for decades. Worldwide Tobacco kills more than 7 million people each year including an estimated 1.6 million non-exposed who are exposed to second-hand smoke [1]. This smoke is dangerous to human as well as to animals. Dogs living in smoking households are involuntary "passive smokers" and may suffer long-term consequences from second hand smoke exposure. This passive smoking poses serious health issue to companion animals including oxidative stress, respiratory issue and behavioural changes and after inhalation combustible cigarettes emit a complex mixture of about thousands of chemical compounds including toxins, oxidants and carcinogens that can start inflammatory pathways that result in smoking-related illnesses. When things like superoxide radical anion, hydroxyl radical and hydrogen peroxide are made in metabolic and physiological processes they are called reactive oxygen species (ROS). Excessive generation of ROS can lead to Oxidative stress. Oxidative stress is defined as a situation where the production of the free radicals exceeds the antioxidant defenses which is necessary to detoxify toxic molecules, resulting in molecular disruption and tissue damage (Kumar *et al.*, 2015) [2]. Passive cigarette smoke induces cellular oxidative stress through the generation of ROS, which subsequently damage DNA, lipids, and proteins. (Pryor *et al.*, 1993) [3].

Materials and Methods

This investigation was done in the Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur (M.P.). The experiment was conducted as per the guidelines of Institutional Animal Ethical Committee (IAEC). Twenty healthy companion dogs were used. Dogs were divided into two groups: - ETS (exposed to tobacco smoke; $n = 10$) Passive smokers and NETS (not exposed to tobacco smoke; $n = 10$) non-exposed. The minimum duration of reported tobacco smoke exposure was one year in ETS group. The various antioxidant activities are assessed in which Superoxide dismutase (SOD) activity in plasma was determined according to the method of Madesh and Balasubramanian (1997) [4], with minor modifications. Catalase (CAT) activity was determined spectrophotometrically using a UV-Vis double beam spectrophotometer (Model LI-2800, La Sany, India) as per the method described by Goth (1991) [5]. Serum glutathione (GSH) levels were estimated following the method described by Prins and Loos (1969) [6]. Lipid peroxidation (LPO) was assessed by quantifying malondialdehyde (MDA) formation using the Thio barbituric acid

Corresponding Author:

Amit Kumar

Assistant Professor,
 Department of Veterinary
 Physiology & Biochemistry,
 Mahala Veterinary College,
 Reengus, Sikar, Rajasthan,
 India

(TBA) assay. Total antioxidant capacity was evaluated using the ferri-bipyridine reducing capacity (FBRC) assay performed according to Naji *et al.* (2020) [7] with minor modifications. Serum biochemical examination was also done for the estimation of Glucose, protein, albumin, BUN, Creatinine using Semi-automatic biochemistry analyser (Star 21 plus).

Results and Discussion

The present study showed clear changes in the oxidant-antioxidant balance of indoor dogs exposed to tobacco smoke. Compared with non-exposed dogs, the exposed group showed lower SOD, CAT and FBRC levels, along with higher TBARS concentrations. This pattern suggests increased oxidative stress in dogs which are regularly exposed to tobacco smoke. The cigarette smoke contains thousands of reactive and toxic compounds that can increase reactive oxygen species (ROS) production and disturb antioxidant defences (Puranik *et al.*, 2003) [8].

SOD activity was significantly lower in exposed dogs (70 ± 1.9 U/ml) than in non-exposed dogs (93 ± 3.5 U/ml). SOD provides an important first-line defence against superoxide radicals therefore this reduced activity may reflect increased oxidative damage due to smoke exposure. Similar finding has also been reported by Valko *et al.*, 2016 [9]. These changes in antioxidant defences have been associated with oxidative stress and free-radical generation. FBRC also decreased markedly from 7.7 ± 0.4 μ mol/ml in non-exposed dogs to 3.0 ± 0.17 μ mol/ml in exposed dogs indicating a reduced overall reducing capacity. The FBRC assay provides an estimate of antioxidant reducing capacity based on the reduction of Fe^{3+} to Fe^{2+} by antioxidant compounds (Naji *et al.*, 2020) [7].

CAT activity showed a similar decline, from 177 ± 3.3 U/ml in non-exposed dogs to 156 ± 2.7 U/ml in exposed dogs. As catalase is involved in the removal of hydrogen peroxide, the reduction in SOD, CAT and FBRC suggests that prolonged tobacco smoke exposure may affect several components of the antioxidant defence system. In contrast,

TBARS increased from 4.3 ± 0.22 to 9.2 ± 0.27 nmol/ml, indicating enhanced lipid peroxidation. This finding is consistent with the ability of cigarette smoke-derived oxidants to promote oxidative damage to cellular lipids (Pryor *et al.*, 1993) [3].

GSH play key role in the antioxidative defence system. GSH reacts with a range of ROS such as H_2O_2 and O_2^- which is the basis of its antioxidant action. GSH concentration was numerically higher in exposed dogs (0.28 ± 0.01 nmol/ml) than in non-exposed dogs (0.20 ± 0.008 nmol/ml). This increase may represent an adaptive response to oxidative challenge rather than improved antioxidant protection. Alterations in glutathione metabolism are known to occur during oxidative stress (Rahman *et al.*, 2000) [10].

Some serum biochemical parameters also differed between groups. Glucose was lower in exposed dogs (56.0 ± 4.0 mg/dl) than in non-exposed dogs (83.0 ± 3.2 mg/dl) similar findings were reported by Meng *et al.*, 2022 [11] in mice which was exposed to cigarette smoke. Albumin was also reported lower in exposed dogs (2.2 ± 0.17) compared to non-exposed dogs (2.8 ± 0.12 g/dl) in agreement with observations reported by Roohi *et al.*, 2017 [12] in human smokers. Total protein showed little difference, while creatinine and BUN were only slightly higher in exposed dogs and should not alone be interpreted as evidence of renal dysfunction.

Serum iron was lower in exposed dogs (1087.21 ± 34.26 μ g/L), whereas copper was higher (669.3 ± 20.06 μ g/L) and Similar alterations have been reported by Demirtas *et al.*, 2023 [13] and Kocyigit *et al.*, 2001 [14]. Since iron and copper participate in redox reactions, these changes may be relevant to the observed oxidative imbalance.

Overall, reduced SOD, CAT and FBRC together with increased TBARS indicate an altered oxidant-antioxidant balance in tobacco smoke-exposed dogs. Further studies involving larger populations, quantified smoke exposure and additional oxidative and inflammatory markers would help clarify the biological significance of these findings.

Table 1. Antioxidants Status and Serum Biochemical parameters in dogs which were exposed to tobacco smoke and not exposed to tobacco smoke

Biochemical parameter	Not exposed to tobacco smoke	exposed to tobacco smoke
SOD U/ml	93 ± 3.5	70 ± 1.9
FBRC μ mole/ml	7.7 ± 0.4	3.0 ± 0.17
Catalase U/ml	177 ± 3.3	156 ± 2.7
GSH nmol/ml	0.2 ± 0.008	0.28 ± 0.01
TBARS nmol/ml	4.3 ± 0.22	9.2 ± 0.27
Glucose mg/dl	83.0 ± 3.2	56.0 ± 4.0
Total Protein g/dl	8.5 ± 0.22	8.6 ± 0.07
Albumin g/dl	2.8 ± 0.12	2.2 ± 0.17
Creatinine mg/dl	0.69 ± 0.02	0.75 ± 0.02
BUN mg/dl	15.08 ± 0.95	15.58 ± 0.96
Cu (microgm/L)	447.01 ± 21.01	669.3 ± 20.06
Fe (microgm/L)	1214.43 ± 39.26	1087.21 ± 34.26

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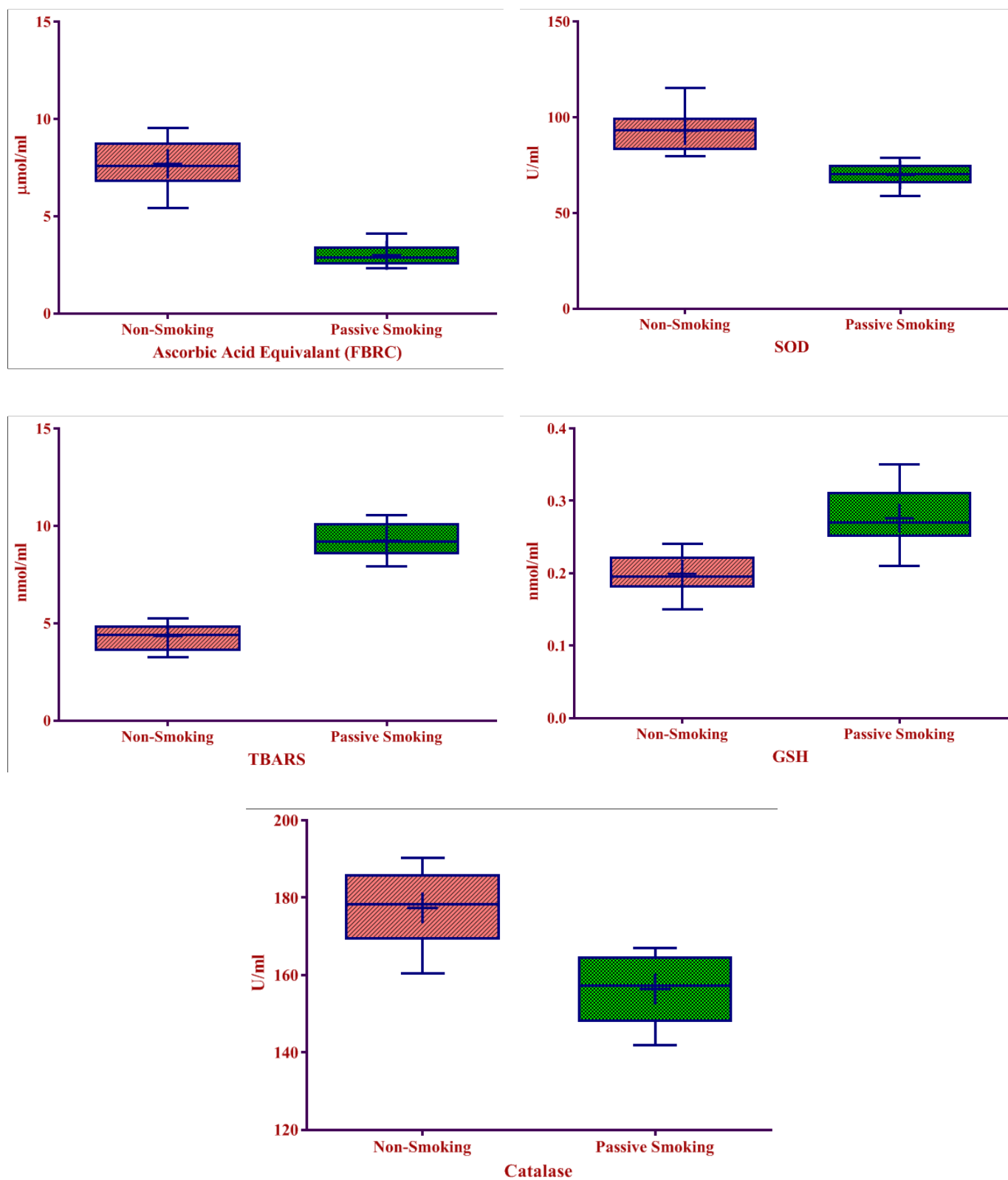
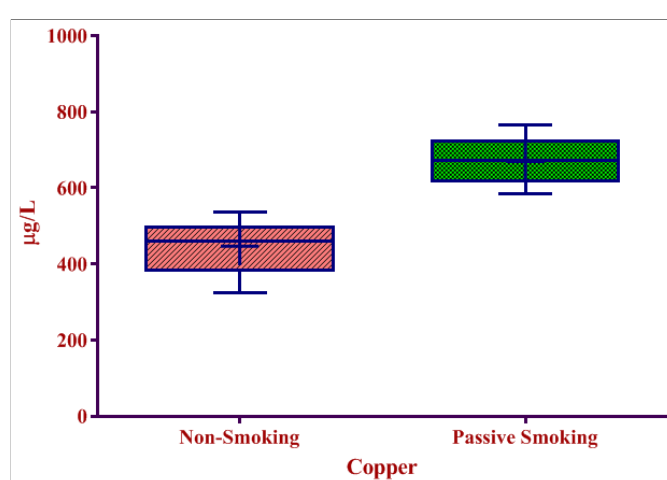
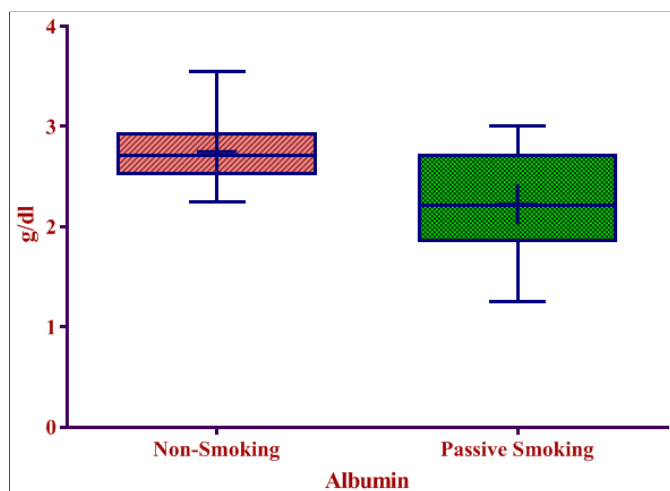
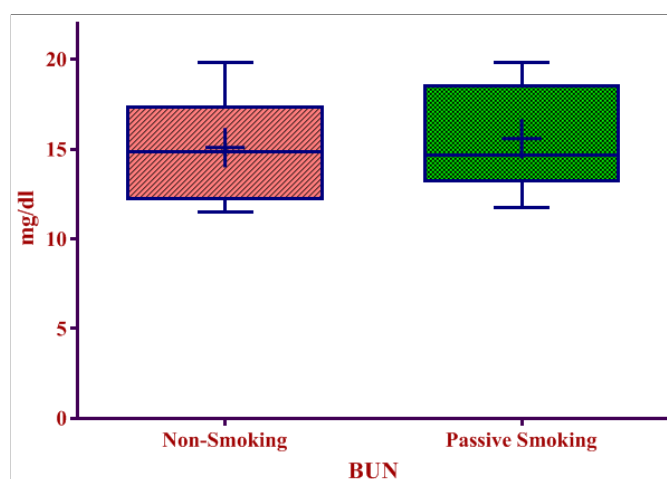
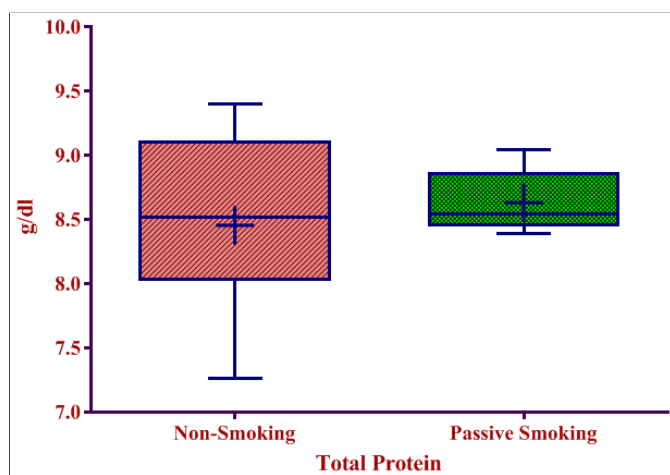
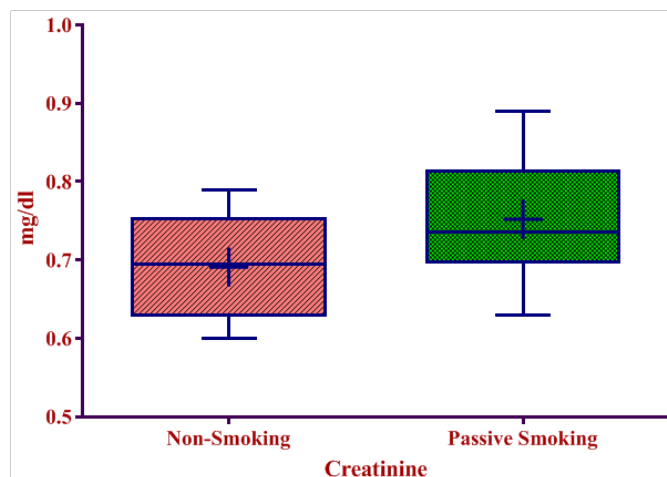
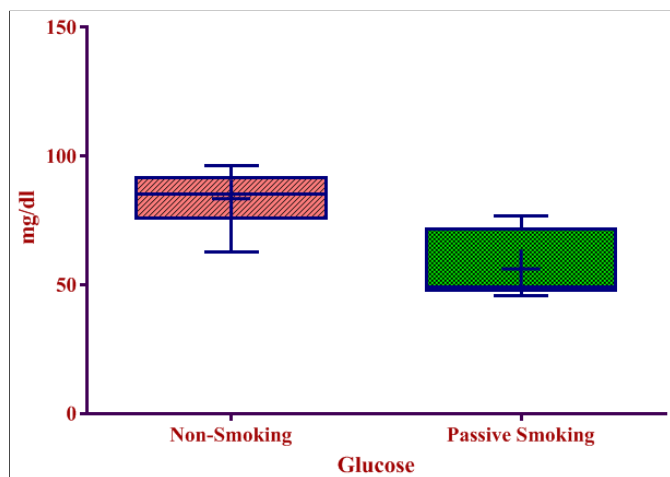


Figure 1. -- Antioxidants Status in dogs which were exposed to tobacco smoke and not exposed to tobacco smoke



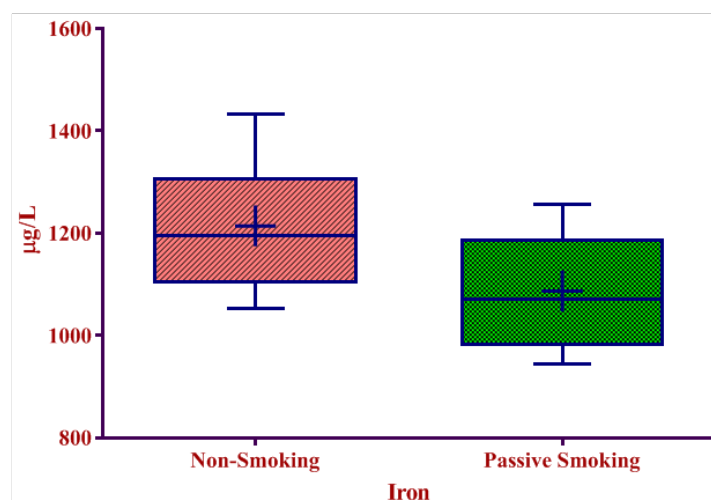


Figure 2. -- Biochemical parameters in dogs which were exposed to tobacco smoke and not exposed to tobacco smoke

Conclusion

This study reveals that tobacco smoke is highly harmful to dogs due to the toxins and free radicals. Dogs exposed to tobacco smoke experience severe oxidative stress which leads to direct cellular damage. Ultimately the resulting imbalance between free radicals and antioxidants accelerates aging, damages vital tissues and increases the risk of chronic health issues such as cognitive decline, heart disease and cancer this passive tobacco smoke exposure also induces oxidative stress which is associated with alterations in glucose metabolism, protein metabolism, renal biochemical indices and trace-element homeostasis in dogs.

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Authors

Amit Kumar

Assistant Professor, Department of Veterinary Physiology & Biochemistry, Mahala Veterinary College, Reengus, Sikar, Rajasthan, India

Anil Gattani

Professor, Department of Veterinary Physiology & Biochemistry, C.O.V.Sc. & A.H., Jabalpur, Madhya Pradesh, India

Sanju Mandal

Assistant Professor, Department of Veterinary Physiology & Biochemistry, C.O.V.Sc. & A.H., Jabalpur, Madhya Pradesh, India

Aafreen Khan

PhD Scholar, Division of Veterinary Biochemistry, ICAR-IVRI Izatnagar, Bareilly, Uttar Pradesh, India

Ravinder Malhan

PhD scholar Division of Physiology and Climatology ICAR IVRI Izatnagar, Bareilly Uttar Pradesh, India

Shashank Vishvakarma

Assistant Professor, Department of Veterinary Gynaecology & Obstetrics, Bhumika College of Veterinary Science and Research Centre, Mahendragarh, Haryana, India

Eshani Dixit

M.V. Sc, Department of Veterinary Microbiology, C.O.V.Sc. & A.H., Jabalpur, Madhya Pradesh, India

Sankalp Sahu

M.V. Sc, Department of Veterinary Surgery & Radiology, C.O.V.Sc. & A.H., Jabalpur, Madhya Pradesh, India

Shivani Dhurve

M.V. Sc, Department of Livestock Products Technology, C.O.V.Sc. & A.H., Jabalpur, Madhya Pradesh, India