RESEARCH ARTICLE

Pathomorphological Studies on Experimentally Induced Indoxacarb Toxicity and its Amelioration with Vitamin C in Male Wistar Rats

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Abstract

The present study was undertaken to determine the ameliorative effect of vitamin C on indoxacarb induced toxicity in rats. Twenty male Wistar rats were randomly divided into four different groups as I to IV. Rats of Group I, II, III and IV received vehicle carboxymethyl cellulose (control), indoxacarb (173.2 mg/kg b.wt.), vitamin C (200 mg/kg b.wt.) and indoxacarb + vitamin C, respectively, by oral gavages daily for 28 days. Clinical symptoms that were seen in indoxacarb group II rats were also observed in indoxacarb + vitamin C group IV rats, though to a lesser degree. Grossly, stomach was distended and filled with undigested feed materials in Groups II and IV rats. Histopathological changes (degeneration, necrosis and congestion) in the indoxacarb group II were increased when compared to the control group. These microscopic lesions were also visible in the Group IV animals to a lesser extent. In general, vitamin C has ameliorative effect on histopathological changes in indoxacarb intoxicated rats.

Key words: Amelioration, Indoxacarb, Rats, Toxicopathology, Vitamin C.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.3.03

Introduction

Pesticides remain the most efficient approach for protecting plants and animals against a wide range of pests. Nowadays, the use of pesticides became popular in developing countries and their use is indistinguishably associated with the enhancement of human welfare. The pesticides exert a positive effect on plant health in terms of insect pests, increased productivity, improved crop storage and disease control, but inadvertent uses negatively affect food safety (Zikankuba *et al*., 2019). Pesticides are a key group of environmental chemicals that have the potential to harm plants, animals and human. Indoxacarb is a promising new foliar broad-spectrum insecticide that belongs to the oxadiazine class (Harder *et al*., 1997) which is manufactured by DuPont, USA (Koli *et al.,* 2019). Indoxacarb is a synthetic, non-systemic insecticide that can replace the organophosphate insecticide (Doerr *et al*., 2004). Indoxacarb's active metabolite works uniquely by blocking sodium channels in nerve cells, inducing paralysis and death in the target pest species (Lapied *et al*., 2001).

Vitamin C is a water-soluble antioxidant that has been shown to neutralize reactive oxygen species (ROS) and reduce oxidative stress (Yu *et al*., 2008). Vitamin C is a potent, efficient and relatively inexpensive antioxidant. It is a potent reducing agent and an excellent source of electrons for free radicals that are looking for an electron to regain their stability (Duarte and Lunec, 2005). This study was therefore aimed to evaluate the pathomorphological changes in experimentally induced indoxacarb toxicity in rats and its amelioration with vitamin C.

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How to cite this article: Rathod, P.B., Patel, J.G., Modh, S.P., Raval, S.H., Parmar, R.S., Sindhi, P.I., & Joshi, D.V. (2023). Pathomorphological Studies on Experimentally Induced Indoxacarb Toxicity and its Amelioration with Vitamin C in Male Wistar Rats. Ind J Vet Sci and Biotech. 19(3), 12-16.

Source of support: Nil

Conflict of interest: None.

Submitted: 22/03/2023 **Accepted:** 30/04/2023 **Published:** 10/05/2023

Materials and Methods

Experimental Animals

A total of 20 male Wistar rats were procured from Pavo Research Solutions, Dashrath, Vododara-391 740, Gujarat,

India, following approval of the protocols by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Science and Animal Husbandry, Sardarkrushinagar-385 506, Gujarat vide letter No. VETCOLL/IAEC/2021/17/PROTOCOL-05. Before grouping and dosing, the procured rats were maintained for 15 days' acclimatization. Animal management and treatment procedures complied with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental Design

All the 20 rats were randomly divided into 4 different groups, numbered as Groups I to IV, each of 5 animals. Rats of Group I served as control and received only vehicle carboxymethyl cellulose, while those of Group II received indoxacarb (IND) dissolved in carboxymethyl cellulose at 173.2 mg/kg b.wt., by oral gavage, daily for 28 days. Group III received vitamin C (VIT-C) dissolved in distilled water at the dose of 200 mg/kg b.wt. and Group IV received indoxacarb dissolved in carboxymethyl cellulose at 173.2 mg/kg b.wt. and vitamin C 200 mg/kg b.wt. dissolved in distilled water, by oral gavage, daily for 28 days.

Pathomorphological Studies

All the rats survived were euthanized on the 29th day of the study. The rats were fasted overnight before necropsy. All survived/sacrificed rats and those found dead during the study period were subjected to a detailed necropsy. Brain, stomach, intestines, liver, kidneys, adrenals, spleen, heart, thymus, trachea, lungs, sex organs, urinary bladder and lymph nodes were collected in 10% neutral buffered formalin. Testes and epididymides were initially collected in modified Davidson fluid for 48 h, then briefly washed and preserved in 10% neutral buffered formalin. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin. Formalin-fixed tissues were processed following routine method of dehydration in graded alcohol, clearing in xylene and embedding in paraffin. Sections of 5 µ thicknesses were obtained and stained by hematoxylene and eosin (H&E) and examined under microscope (Suvarna *et al*., 2012).

Results And Discussion

Organs, *viz.*, liver, kidneys, lungs, heart, brain, spleen, adrenals, thymus, trachea, pituitary glands, thyroid, parathyroids, salivary glands, seminal vesicles, testes, epididymides, prostate, urinary bladder, stomach and all parts of intestine were examined grossly and tissues were collected for histopathological examination from rats of all Groups during necropsy. In the present study, test related changes were noted in spleen, kidneys, stomach and liver.

Gross Pathology

In comparison to control, no appreciable gross lesions were found in any organs, except in stomach of Groups II (IND) and VI (IND + VIT-C) rats, where it was distended and filled with undigested feed materials (Fig. 1). This might have occurred due to alteration in gastrointestinal tract motility following intoxication.

Histopathology

In comparison to control, the liver of the indoxacarb group showed histological abnormalities, characterized by mononuclear cells infiltration with bile duct hyperplasia, portal vein congestion, minimal sinusoidal congestion, micro-vesicular vacuolation and minimal infiltration of inflammatory cells (Fig. 2). Group IV rats treated with indoxacarb + vitamin C also showed multifocal vacuolation with minimal sinusoidal congestion and multifocal bile duct hyperplasia with minimal infiltration of inflammatory cells (Fig. 3) as compared to normal liver of control rats.

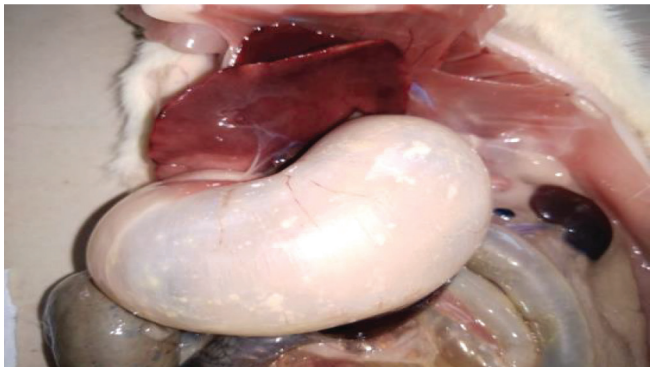

Fig. 1: Stomach of Group II rat distended and filled with undigested feed materials

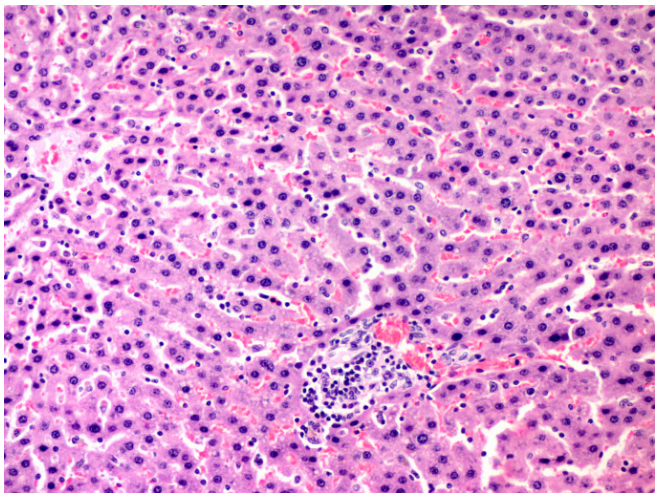

Fig. 2: Liver section of Group II rat showing moderate sinusoidal congestion, micro vesicular vacuolation and focal microgranuloma. H & E 200X

Histopathologically, kidneys of 1/5 (20%) control rats showed minimal congestion and accumulation of proteinaceous material in tubular lumen. No any other significant findings were noted in kidneys of control rats. Group II (indoxacarb) rats' kidneys showed mild to moderate accumulation of proteinaceous material in tubular lumen (Fig. 4), regenerative tubules, congestion and tubular necrosis when compared with control rats. Regenerative tubules were characterized by flattened epithelium, tubule epithelial cell basophilia and nuclear crowding (Fig. 5). In case of Group IV (IND + VIT-C) rats, kidneys showing lesser pathological lesions as compared to Group II (IND) due to vitamin C amelioration.

The microscopic lesions in spleen composed pigmentation, megakaryocytosis, and atrophy of white pulp in Group II (IND) and Group IV (IND + VIT-C) rats as compared to control rats. In Group II (IND) rats, spleen showed severe depletion of white pulp (Fig. 6). In comparison to Group II, protective effects were seen in vitamin C supplemented Group IV rats with minimal to moderate depletion of white pulp, presence of golden yellow colored hemosiderin pigments and presence of numerous basophilic islands in red pulp of spleen (Fig. 7).

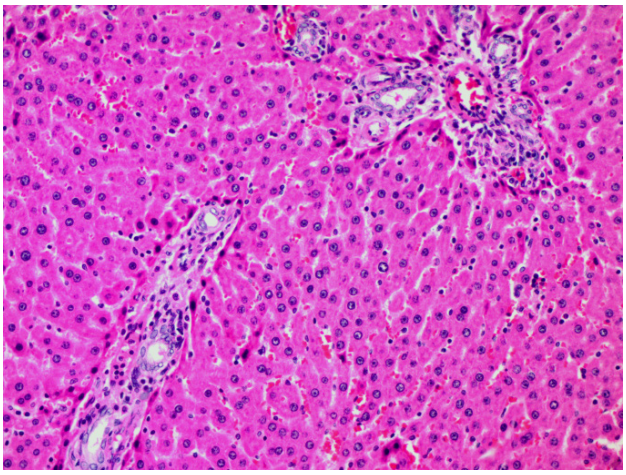

Fig 3: Liver section of Group II rat showing multifocal bile duct hyperplasia with minimal infiltration of inflammatory cells. H & E 200X

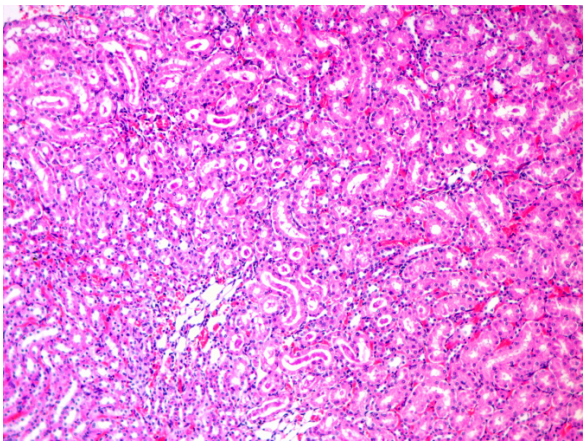

Fig. 4: Kidney section of Group II rat showing accumulation of eosinophilic proteinaceous material in tubular lumen. H & E 100X

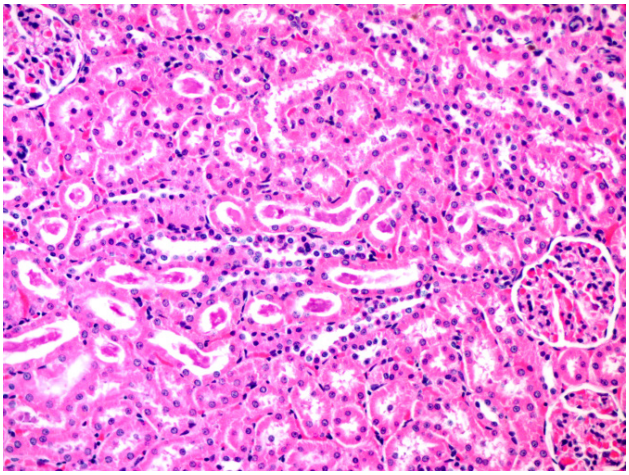

Fig. 5: Kidney section of Group II rat showing proteinaceous material in tubular lumen and regenerative tubules characterized by flattened epithelium, tubule epithelial cell basophilia and nuclear crowding. H & E 200X

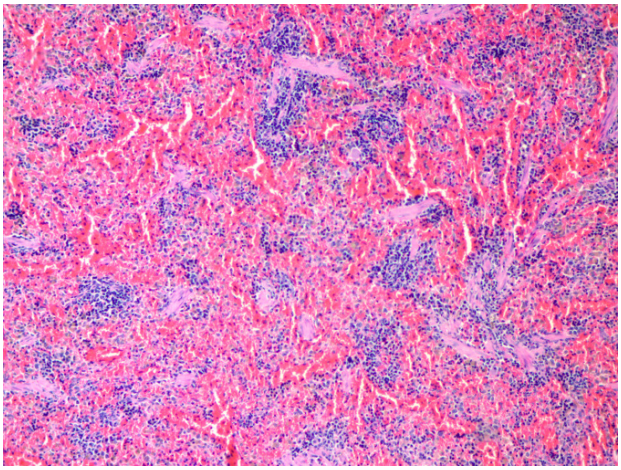

Fig. 6: Spleen section of Group II rat showing depletion of white pulp, presence of golden yellow colored hemosiderin pigments and presence of numerous basophilic islands of erythropoiesis in red pulp. H & E 100X

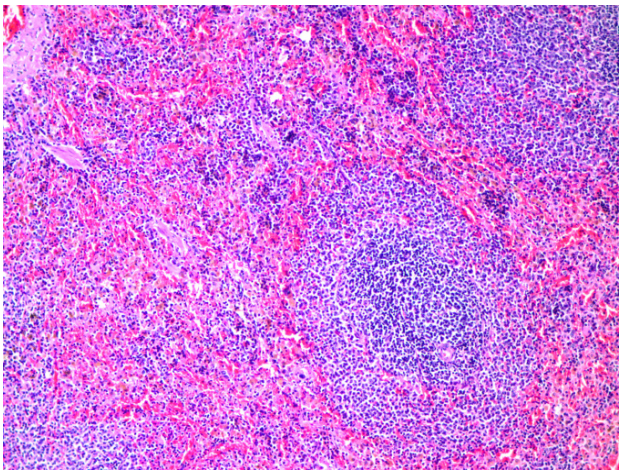

Fig. 7: Spleen section of Group II rat showing minimal to moderate depletion of white pulp and presence of numerous basophilic islands in red pulp. H & E 100X

In non-glandular stomach, indoxacarb dosing induced mild to moderate orthokeratotic hyperkeratosis and prominent keratohyalin granules in Group II (indoxacarb) as well as in Group IV (indoxacarb + vitamin C) rats with less severity as compared to Groups I (control) and III (vitamin C). Minimal infiltration of inflammatory cells was seen in Groups II and IV rats. In comparison to control Group I (Fig. 8), rats of indoxacarb Group II showed marked orthokeratotic hyperkeratosis characterized by diffuse thickening of stratum corneum without retention of nuclei (Fig. 9). Hyperkeratosis was noted in 4 of 5 (80%) rats. Non-glandular stomach of Group III (VIT-C) revealed normal stratified squamous epithelium with thin keratin layer. In indoxacarb with vitamin C supplemented rats, hyperkeratosis was noted in 2 of 5 (40%) rats.

Microscopic lesions in lungs of rats treated with indoxacarb were composed of severe oedema, moderate alveolar congestion and infiltration of inflammatory cells (Fig. 10) as compared to Group I (control) and Group III (VIT-C) (Fig 11). Lungs showed moderate congestion and inflammatory cells infiltration in Group IV rats.

Microscopically, heart of control rats showed minimal congestion. Heart of Group II rats dosed with indoxacarb showed minimal to moderate congestion. Pre-treatment with vitamin C and indoxacarb administered rats also revealed same microscopic lesions as found in control and indoxacarb fed rats. These changes were considered as test article/ substance independent lesions.

Minimal decreased sperm concentration in lumen of epididymides was found in Groups I, II and IV rats. Some other incidental or test substance independent lesions were also noted in different organs. Other organs, *viz.,* small and large intestine, adrenals, thymus, trachea, salivary glands, seminal vesicles, prostate and urinary bladder did not reveal any appreciable microscopic lesions as compared to control.

In the present study, microscopic lesions were found in spleen, stomach, kidneys, lungs and liver of rats fed with

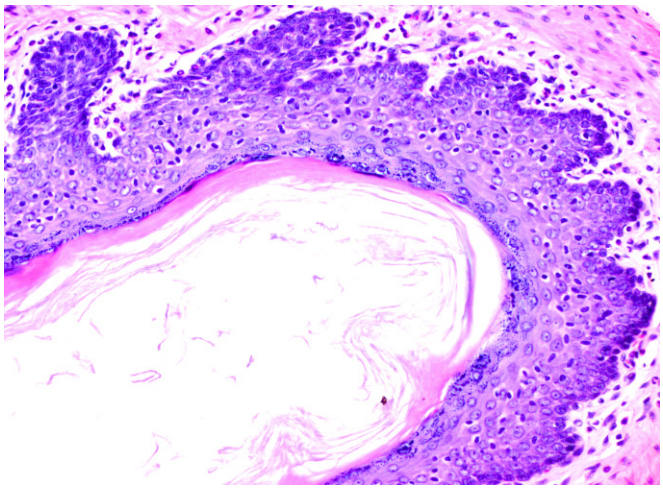

Fig. 8: Section of stomach of Group I rat showing normal stratified squamous epithelium with thin keratin layer. H & E 200X

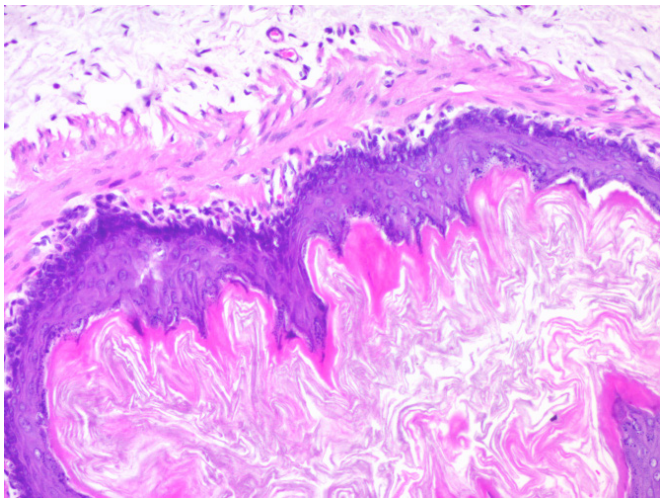

Fig. 9: Section of stomach of Group II rat showing marked orthokeratotic hyperkeratosis and prominent keratohyalin granules. H & E 200X

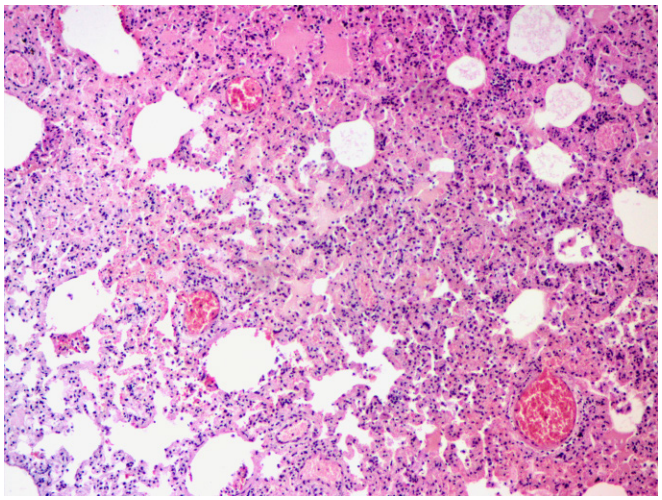

Fig. 10: Lung section of Group II rat showing severe oedema and haemorrhage with infiltration of mononuclear cells. H & E 100X

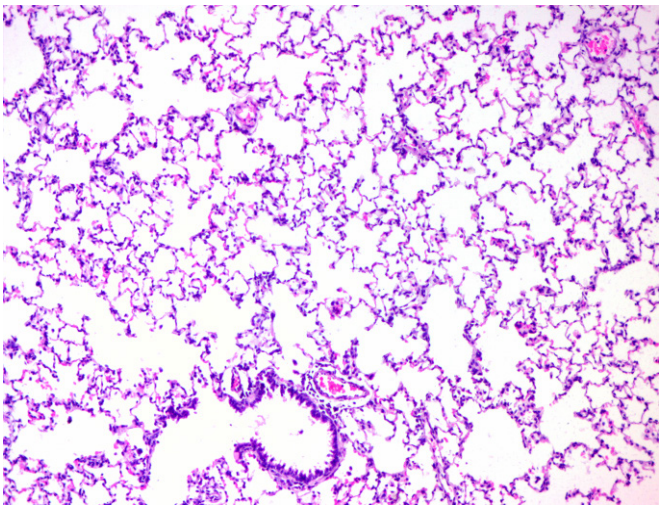

Fig. 11: Lung section of Group III rat showing normal architecture. H & E 100X

indoxacarb, which may be attributed to toxic effect of indoxacarb. Similar findings were noted previously in liver, kidney by Mabrouk *et al*. (2016), Abdelrasoul (2018) and Koli *et al.* (2019) in mice and rats due to indoxacarb toxicity. Abdelrasoul (2018) observed necrosis, pyknosis of neurons, congestion of cerebral blood vessel and perivascular cuffing with mononuclear cells in male rats following indoxacarb and abamectin plus indoxacarb toxicity. The lung lesions found in this investigation have never been reported earlier. Rats pre-treated with vitamin C and indoxacarb restored the microscopic changes in the present study. The administration of vitamin C before indoxacarb decreased the toxic effect of indoxacarb due to its power to eliminate the reactive oxygen species resulting from it. These ameliorative effects of vitamin C suggest its valuable impact on preventing toxicity resulted from the generation of reactive oxygen species, as it has radical scavenging property.

The stomach and spleen lesions found in this investigation have been reported earlier by Modh (2020). Hyperkeratosis in the non-glandular stomach is caused by a lack of mechanical abrasion caused by starvation or local irritation from test substances (Nolte *et al.,* 2016). Probably anorexia caused by indoxacarb may be responsible for hyperkeratosis of stomach in treated rats. The excess keratin in anorectic rats is most likely due to decreased mechanical clearance through meal passage. The relative decreased hyperkeratosis in stomach reported in the indoxacarb plus vitamin C group showed the protective nature of vitamin C on indoxacarb-evoked stomach hyperkeratosis.

Spleen lesions noted in the present study were in agreement with the findings of Malek (1997), who observed increased haemosiderin pigment and erythrocytic hyperplasia in the spleen of male and female rats treated at 60 ppm and above indoxacarb. Aging, cachexia, poor nutrition, toxicity, or chemotherapy can cause white pulp atrophy or decreased lymphocytes and macrophages cellularity in the spleen (Willard-Mack *et al.,* 2019). White pulp was depleted in all indoxacarb-treated rats, implying that indoxacarb dosage is responsible for such changes. Metabolite of indoxacarb is responsible for the hemolytic effects observed in the present study by lowering RBC count, Hb and HCT support to the extra-medullary hematopoiesis noted in spleen.

Conclusion

The result of our study showing less severe histopathological lesions in spleen, stomach, kidneys, lungs and liver following pre-treatment with vitamin C is a clear demonstration of the antioxidant effect of this vitamin. Moreover, vitamin C showed protective effects against cellular damage and harmful effects induced by indoxacarb.

Acknowledgement

The authors wish to express their gratefulness to Principal, College of Veterinary Science and Animal Husbandry, Sardarkrushinagr (Dantiwada) and Head, Professors and Staff member of Department of Veterinary Pathology, for technical support. Thanks are also extended for animal care and help.

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