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Research Article

STUDY OF AMELIORATIVE EFFECT OF VITAMIN C ON INDOXACARB INDUCED TOXICITY ON HEMATOBIOCHEMICAL PARAMETER IN MALE WISTAR RATS

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Abstract: This present study was undertaken to determine the ameliorative effect of vitamin C on indoxacarb toxicity. For this purpose, 20 male Wistar rats were randomly divided into four different groups as I to IV. Group I, II, III and IV received vehicle (control), IND (173.2 mg/kg b.wt.), VIT-C (200 mg/kg b.wt.) and IND+VIT-C (173.2 mg/kg b.wt. IND + 200 mg/kg b.wt. VIT-C) by oral gavages daily for 28 days. There was significantly ($P < 0.05$) decreased in TEC, Hb and HCT values of Groups II (IND) and IV (IND+VIT-C) rats when compared with control rats. The rats of Groups II and IV revealed significant decrease ($P < 0.05$) triglyceride level when compared with Group I (CMC) and Group III (VIT-C) rats. The rats of Groups II (IND) and IV (IND+VIT-C) showed significant ($P < 0.05$) increase in total antioxidant status in comparison to control Group rats. Ameliorative effect of vitamin C was observed on haematology and biochemical alterations in indoxacarb intoxicated rats.

Keywords: *Toxicity, Indoxacarb, Ameliorative, Vitamin C, Rats*

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Introduction

For decades, people firmly believed in the use of harmful chemical pesticides to rid gardens and crop field of pests. The usage of pesticides has resulted in soil pollution and air pollution. These pesticides are also hazardous to animal and plant life, as well as to human health [1]. Indoxacarb is a novel pyrazoline-derived oxadiazine insecticide having broad-spectrum efficacy against a variety of pests. Indoxacarb is a new insecticide for pest and vector control that belongs to the pyrrole class. The IUPAC (International Union of Pure and Applied Chemistry) name of indoxacarb is (S)-methyl 7-chloro-2, 5-dihydro-2-[(methoxycarbonyl) [4 (trifluoromethoxy) phenyl] amino]-carbonyl] indeno [1,2-e][1,3,4]oxadiazine-4a-(3H)-carboxylate [2]. It is frequently used as an insecticide in horticulture and agriculture that shows a strong action against lepidopteran pests of vegetables, fruits, cotton, peanuts, maize, trees, alfalfa, soybeans and other crops. However, indoxacarb's biological activity is not limited to insects, its extensive use poses considerable health hazards to plants, aquatic organisms, and humankind [3]. Furthermore, many people have claimed that pesticides might cause oxidative stress in many tissues. Indoxacarb has been shown to cause oxidative stress in a variety of tissues.

Vitamin C is most commonly used as antioxidant and is involved in a variety of biochemical processes in organisms. Vitamin C is a potent antioxidant that can donate a hydrogen atom to form the ascorbyl free radical, which is relatively stable. Vitamin C also improves iron absorption by converting Fe3+ from non-heme iron sources to Fe2+ [4]. Considering these facts, present study was conducted to evaluate sub-acute toxicity of indoxacarb and its amelioration with vitamin C in Wistar rats

Material and Methods

Animal procurement

A total of 20 male Wistar rats were procured from Pavo Research Solutions, Dashrath, Vododara, 391740, Gujarat, India. Before grouping and dosing the procured Wistar rats were maintained under acclimatization for 15 days.

Institutional animal ethics committee (IAEC) approval

The protocols were presented front of the Institutional Animal Ethics Committee (IAEC) on dated 13/01/2021 and approved vide letter No. VETCOLL/IAEC/2021/17/PROTOCOL-05 by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Science and Animal Husbandry, Sardarkrushinagar, 385 506, Gujarat, India.

Housing and environmental conditions

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

The toxicopathology of indoxacarb and its amelioration with vitamin C was studied on male Wistar rats. All the 20 rats were randomly divided into 4 different groups. Each group consisted of 5 males. The groups were numbered as Groups I to IV. Group I served as control and received only vehicle carboxymethyl cellulose, by oral gavage, while Group II received indoxacarb dissolved in carboxymethyl cellulose at 173.2 mg/kg b.wt. in male, by oral gavage. Group III received vitamin 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. in male and vitamin C 200 mg/kg b.wt. dissolved in distilled water, by oral gavage daily for 28 days

Numbering and identification

Animals were identified by marking their tails with a permanent colour marker.

Table-1 *Effect of Indoxacarb and vitamin C on haematological parameters (Mean±SD, n=5) in male rats after daily oral administration for 28 days*

		Group I	Group II	Group III	Group IV
Parameter	Unit	(CMC)	(Indoxacarb)	(Vitamin C)	(IND + VIT-C)
TEC	10^6/ µL	8.23± 0.326[b]	6.30± 0.561[a]	8.51±1.240[b]	7.04±0.195[a]
Haemoglobin	g/dL	16.32± 0.572[b]	12.80± 1.227[a]	16.50± 1.100[b]	13.42± 0.733[a]
HCT	%	44.30± 3.585[b]	35.68± 2.564[a]	46.24± 6.048[b]	37.84± 2.535[a]
MCV	fL	58.44± 5.388	50.98± 9.294	56.14± 7.383	53.82± 5.040
MCH	Pg	19.84± 0.251	18.30± 1.044	19.92± 0.750	19.14± 2.958
MCHC	g/dL	38.50± 1.681	35.04± 3.411	38.98± 3.832	35.40± 2.723
PLT	10^3/µL	809.20± 158.996	781.00± 132.305	883.00± 62.909	851.40± 198.908
TLC	10^3/µL	8.62± 1.285	7.72± 1.392	8.24± 2.375	7.98± 0.823
Neutrophils	10^3/µL	1.34± 0.408	1.47± 0.596	1.38± 0.441	1.53± 0.342
Lymphocytes	10^3/µL	6.79± 1.059	5.83± 1.013	6.46± 2.130	5.91± 0.478
Monocytes	10^3/µL	0.39± 0.145	0.32± 0.148	0.32± 0.083	0.42± 0.133
Eosinophils	10^3/µL	0.11± 0.055	0.10± 0.028	0.08± 0.024	0.11± 0.054
Basophils	10^3/µL	0.00± 0.00	0.00± 0.00	0.0± 0.00	0.01± 0.031
RDW	%	21.60± 0.361	21.42±1.134	22.44± 0.397	22.18±0.402
RDW (a)	Absolute	36.48± 0.907	36.48± 6.288	33.94± 0.873	39.80± 5.826
MPV	fL	5.42± 0.249	5.64± 0.321	5.60± 0.212	5.68± 0.512

Mean bearing different superscripts in row differ significantly (p<0.05)

Table-2 *Effect of Indoxacarb and vitamin C on biochemical parameters (Mean± SD, n=5) in male rats after daily oral administration for 28 days*

Parameter	Unit	Group I	Group II	Group III	Group IV
		(CMC)	(Indoxacarb)	(Vitamin C)	(IND + VIT-C)
Alanine aminotransferase (ALT)	U/L	63.26± 12.300	60.20± 23.850	52.40± 4.321	68.38± 4.743
Aspartate aminotransferase (AST)	U/L	186.24± 42.595	177.16± 61.355	164.46± 39.326	196.48± 17.880
Alkaline Phosphatase (ALP)	U/L	240.60± 96.834	331.40± 81.638	270.60± 37.354	231.60± 52.577
Gamma Glutamyl Transferase (GGT)	U/L	14.00± 3.162	13.40± 3.050	14.80± 0.837	13.40± 1.817
Total Protein	g/dL	8.08± 0.427	8.03± 0.450	8.04± 0.518	8.70± 0.828
Albumin	g/dL	2.88± 0.130	2.94± 0.230	3.00± 0.235	2.88± 0.526
Urea	mg/dl	4.25± 4.760	37.77± 8.352	34.46± 6.592	44.23± 3.417
Uric acid	mg/dl	1.92± 0.581	1.92± 0.342	1.80± 0.604	2.60± 0.339
Creatinine	mg/dl	0.59± 0.129	0.70± 0.183	0.45± 0.168	0.73± 0.162
Triglyceride	mg/dl	109.22± 10.605[c]	62.69± 9.089[a]	90.10± 25.223[bc]	82.36± 16.072[ab]
Cholesterol	mg/dl	68.78± 11.686	84.73± 16.957	82.84± 9.265	75.02± 3.758
Glucose	mg/dl	72.60± 22.788	75.00± 21.909	69.60± 6.950	69.20± 3.701
Magnesium	mg/dl	2.98± 0.619	2.73± 0.731	1.73± 0.197	3.45± 2.662
Calcium	mg/dl	10.46± 0.488	10.54± 0.850	10.58± 0.335	10.74± 0.527
Phosphorus	mg/dl	8.08± 2.452	7.42± 1.057	6.48± 0.421	7.62± 0.507
Total Antioxidant Status (TAS)	(mmol/L)	1.21± 0.013[a]	1.40± 0.070[b]	1.24± 0.135[a]	1.38± 0.088[b]

Mean bearing different superscripts in row differ significantly (p<0.05)

The animals were identified by their cage labels and tail markings. Five rats were kept in one cage, with 1 ring, 2 rings, 3 rings, 4 rings, and no ring at the base of the tail, indicating the animal number of rats 1, 2, 3, 4 and 5.

Test compound

For the 28 days study, indoxacarb was purchased from local agro center and vitamin C from Sigma-Aldrich.

Clinical Pathology

Haematological profile

The rats fasted overnight before blood collection and necropsy. On 29th day of study, from all survived rats, blood was collected from the retro-orbital plexus with the help of a heparinized capillary tube in clot activator for clinical chemistry and sterilized vials containing 4.0 % potassium ethylene diamine tetra acetic acid (K3 EDTA) as an anticoagulant for estimation of hematology. Following haematological parameters were analysed by using Automated Blood Analyzer (Exigo haematology analyzer, Boule Medical AB, Sweden) through impedance method. Haemoglobin (Hb) (g/dL), Total Erythrocyte Count (TEC) (106/µL), Packed Cell Volume (PCV) or Haematocrit HCT (%), Mean Corpuscular Volume (MCV) (fL), Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dL), Mean Corpuscular Haemoglobin (MCH) (pg), Total Leukocytes Count (TLC) (103/µL), Differential Leukocyte Count (DLC), Red cell Distribution Width (RDW) (%), Red cell Distribution Width (RDW) (a) and Mean Platelet Volume

Biochemical profile

Following biochemical parameters were analysed by commercially available reagent kits through Fully Automated Biochemistry Analyzer (RANDOX-RX Monaco, United Kingdom). Alanine Aminotransferase (ALT) (U/L), Aspartate Aminotransferase (AST) (U/L), Alkaline Phosphatase (ALP) (U/L), Urea (mg/dL), Uric acid(mg/dL), Creatinine (mg/dL), Total Protein (TP) (g/dL), Albumin (g/dL), Cholesterol (mg/dL), Triglycerides (mg/dL), Glucose (mg/dL), Magnesium (mg/dL), Calcium (mg/dL), Phosphorous (mg/dL), Gamma-Glutamyltransferase (GGT) (U/L), Total Antioxidant status (mmol/l).

Statistical analysis

The data generated on various parameters like, haematological and Biochemical were analyzed by using Analysis of Variance (AOV) procedure of R program (2020) [5] software (version 3.6.3) to know the effect of different treatment groups.

Results and Discussion

Haematological profile

The mean of the haematology data of all the male rats of Groups I, II, III and IV is presented in [Table-1]. In the present study, there was no any statistically significant difference in the haematology data of group III male rats as compared to Group I (control) male. Group II (IND) and IV (IND + VIT-C) male showed significant ($P < 0.05$) decrease in TEC, Hb and HCT value, when compared with Group I (control) and Group III (VIT-C) male rats, respectively [Table-1]. But, mean values of TEC, Hb and HCT were increased in male rats received co-treatment of vitamin C with indoxacarb when compared to only indoxacarb administered male rats. There was no any statistically significant difference in TLC, DLC, Platelets, MCV, MCH, MCHC, RDW and MPV data of Groups II, III and IV rats as compared to Group I (control).

Hematological parameters like decrease in TEC, haemoglobin content and haematocrit as observed in the present study agreed with the findings of

Abdelrasoul [6], Koli *et al*. [7] and Modh [8]. However, shit *et al*. [9] found a decrease in hemoglobin content, percentage of lymphocytes and monocytes, erythrocyte and leukocyte counts, as well as an increase in the percentage of eosinophils and neutrophils, implying that indoxacarb has some adverse effects on mice blood cells. Malek [10] observed significant reduction in mean values of RBC and Hb with increase in MCV and reticulocyte counts, suggestive of mild haemolytic anaemia. These effects could be due to adverse effects of insecticide on bone marrow or direct destruction of blood cells.

The pre-treatment with vitamin C noticeably prevented the effect of indoxacarb on TEC, haemoglobin content and Haematocrit. In the present study, co-treatment with vitamin C improved the haematological parameters depressed by indoxacarb, which is in agreement with earlier studies of Nisar *et al*. [11].

Biochemical profile

The mean of the haematology data of all the male rats of Groups I, II, III and IV is presented in [Table-2]. No statistically significant difference has been detected between the negative control Group I (CMC) and the positive control Group III (VIT-C) regarding biochemical and oxidative stress parameters. Biochemical parameters viz., Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), Total protein (TP), albumin, urea, uric acid, cholesterol, glucose, creatinine and along with minerals such as calcium, phosphorus and magnesium did not reveal any significant changes in any animals of treatment Group as compared to Group I (control) and Group III (VIT-C).

The male rats of Groups II and IV revealed significantly low ($P < 0.05$) triglyceride when compared with Group I and Group III (VIT-C) rats. Co-supplementation of vitamin C to the male rats intoxicated with indoxacarb significantly increased ($P < 0.05$) the triglyceride level, but still was less than that of the control.

The male rats of Group II (IND) and IV (IND + VIT-C) revealed significant ($P < 0.05$) increase in total antioxidant status when compare with Groups I and III (VIT-C) and did not reveal any significant changes in Group III (VIT-C). However, decrease of total antioxidant status was observed in pre-treatment with vitamin C to indoxacarb-intoxicated rats.

In the present study, ALT, AST, GGT, TP, albumin, urea, uric acid, cholesterol, glucose, creatinine and along with minerals such as calcium, phosphorus and magnesium did not alter significantly. In the same line, Modh [8] reported that sub-acute exposure to the indoxacarb has no effect on aforesaid biochemical parameters. However, in past studies due to indoxacarb toxicity, increased ALP, ALT, AST, BUN and creatinine levels [7,12,13] and decreased in total protein were reported [6,12]. Furthermore, according to Mabrouk *et al*. [12], significant depletion in values of TP, Albumin and significant elevation in ALT, ALP and GGT values were found in indoxacarb intoxicated rats. Similarly, indoxacarb did not produce any significant alterations in levels of creatinine and blood urea nitrogen in present work was reported by Goyal and Sandhu [14] in buffalo calves.

The total antioxidant status (TAS) is used to measure the overall antioxidant status of the body. Only one study reported an effect of indoxacarb on total antioxidant status, while a few studies did find an effect of indoxacarb on other oxidative stress parameters. In the current study, TAS was significantly ($P < 0.05$) elevated in male rats treated with indoxacarb. Similarly, this finding was coincided with finding of Modh [8]. Mudaraddi *et al*. [15] and Thoker [13] found a significant increase in the values of superoxide dismutase, glutathione transferase and catalase enzymes in mice. The increase in the mean TAS of exposed rats could be attributed to the antioxidant system's involvement in combating the increased free radical load and, most likely, the oxidative stress induced by indoxacarb toxicity in male rats. Interestingly, rats given indoxacarb in combination with vitamin C exhibited a gradual improvement in TAS. Our findings support the concept that supplementing with vitamin C as an antioxidant reduces indoxacarb-induced toxicity in male rats. Co-treatment of indoxacarb with vitamin C helped protect the rats against indoxacarb's harmful effects.

Conclusion

Effect of indoxacarb, a new generation insecticide of the oxadiazine class of chemistry, along with ameliorative effect of vitamin C on indoxacarb induced toxicity in 20 Wister rats. Indoxacarb significantly ($P < 0.05$) reduced Total Erythrocyte Count, Haemoglobin and Haematocrit in both male rats of Groups II and IV. Indoxacarb induced statistically significant reduction in triglyceride level in male rats of Groups II and IV. Total antioxidant status was significantly increased in male rats of Groups II and IV. Co-treatment with vitamin C improved haematobiochemical parameter alteration in the indoxacarb intoxicated group.

Application of research: With the research finding of the study, vitamin C having good protective effect on indoxacarb toxicity

Research Category: Toxicopathology

Abbreviations:

103 / µl: Thousands per microliter
106 / µL: Billions per microliter
ALP: Alkline Phosphatase
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
BUN: Blood Urea Nitrogen
b.wt.: Body weight
fL: Femolitre
HCT: Haematocrit
IND: Indoxacarb
MCH: Mean corpuscular haemoglobin
MCHC: Mean corpuscular haemoglobin concentration
MCV: Mean corpuscular volume
PLT: Platelet
RDW: Red cell distribution width
TEC: Total erythrocytes count
TLC: Total leucocytes count
TP: Total protein
TAS: Total antioxidant status

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Conflict of Interest: None declared

Ethical Committee Approval Number: VETCOLL/IAEC/2021/17/PROTOCOL-05

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