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Hepatoprotective and nephroprotective efficacy of *Cichorium intybus* following imidacloprid induced subchronic toxicity in WLH cockerels

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ABSTRACT

The current research work was undertaken to evaluate the hepatoprotective and nephroprotective potential of *Cichorium intybus* following subchronic exposure of imidacloprid in white leghorn (WLH) chicks. Thirty, 6 to 8 weeks old chicks of 300-350 g weight were randomly and equally divided into five groups. Group I served as control and was fed normal grower ration and other were fed medicated ration containing *Cichorium intybus* leaf powder @ 5000 ppm in Group II, imidacloprid @ 100 ppm in Group III, imidacloprid @ 100 ppm + silymarin @ 100 ppm in Group IV and imidacloprid @ 100 ppm + *Cichorium intybus* leaf powder @ 5000 ppm in Group V, respectively, for 8 weeks. Biochemical parameters at 4th and 8th week revealed a significant increase in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, lactate dehydrogenase, total bilirubin, indirect bilirubin, creatinine, blood urea nitrogen (BUN) with an increase in absolute and relative organ weights of liver and kidney after 8 weeks and a significant decline in total protein, albumin and globulin in imidacloprid treated Group III as compared to control (Group I). However, the simultaneous administration of *Cichorium intybus* leaf powder (CILP) in Group V revealed amelioration in these parameters at par with Groups I and IV. Thus, amelioration of imidacloprid induced hepatotoxic and nephrotoxic effects following simultaneous CILP administration indicates hepatoprotective and nephroprotective potential of *Cichorium intybus* in imidacloprid intoxicated cockerels.

Keywords: Chicory, Hepatic, Insecticide, Renal, Silymarin

Imidacloprid, a chloronicotyl compound, is an insecticide of neonicotinoid class. This insecticide has high insecticidal activity, low mammalian toxicity as well as selective insecticidal action (Jeschke *et al.* 2010). Its accidental, occupational, careless and prolonged exposure have been reported to elicit adverse effects including gastrointestinal and metabolic disturbances, oxidative stress, hepatotoxicity, nephrotoxicity, neurotoxicity, carcinogenicity, teratogenicity and untoward effect on reproductive system and embryonic development in animals and birds (Abd-Elhakim *et al.* 2018, Maletha *et al*. 2021). In the recent years, imidacloprid has raised concern because of its ability to cause colony collapse disorder in bees, egg shell thinning and decline in egg production (Kapoor *et al*. 2011).

Cichorium intybus, a perennial herbaceous plant of the Asteraceae family, is commonly known as chicory and kasni. This plant has various active phytoconstituents of medicinal importance (Pushparaj *et al*. 2007, Street *et al*. 2013, Maletha *et al*. 2020). The traditional system of medicine reports chicory to be a medicinal herb due to its multifarious therapeutic properties like hepatoprotective, gastroprotective, anti-inflammatory, antioxidant, anticancer, immunological, sedative, analgesic, antimicrobial, anthelmintic, wound healing, antidiabetic and hypolipidemic activity (Al-Snafi 2016, Mousa *et al.* 2017). *Cichorium intybus* has been reported to have growth promoting activity (Sarwar 2013, Saeed *et al.* 2015).

This plant's products are premixed with feed by the feed industry which may improve the quality of chicken and minimise the load of chemical growth promoter in poultry meat. There is a scarcity of literature on the protective effect of *Cichorium intybus* against pesticide induced toxic effect. In view of this, the study was undertaken to evaluate the ameliorating potency of *Cichorium intybus* against imidacloprid intoxication in white leghorn (WLH) cockerels.

MATERIALS AND METHODS

Plant materials, chemicals and reagents: The seeds of *Cichorium intybus* were procured from Medicinal Research Development Centre of the University and sown in mid of October and the leaves were collected in January. The leaves were washed, cleaned, shade dried under fan followed by further drying in fan equipped incubator and then grinded in electric grinder to make fine homogenous powder. The plant was identified as *Cichorium intybus* L.

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Imidacloprid (17.8% SL of Maharaja) and silymarin (Silybon®-140) were purchased from the local market. Biochemical parameters were estimated using diagnostic kits of AUTOSPAN®, Arkray Healthcare Pvt. Ltd. (Gujarat) and Tulip Diagnostics (P) Ltd (Goa).

Experimental design: Thirty WLH cockerels of 6 to 8 weeks age, 300 to 350 g weight were procured from Instructional Poultry Farm, Nagla, GBPUAT, Pantnagar. The study was conducted after approval by the IAEC (Institutional Animal Ethics Committee) with approval number IAEC/CVASc/VPT/412.

The chicks were maintained under standard managemental condition in deep litter system and given grower ration prepared as per the Bureau of Indian Standards (BIS)-2007 specification and drinking water *ad lib.* during the study. After acclimatization for 15 days, chicks were randomly and equally divided into five groups, each group having six birds, respectively. Group I served as control and was given grower ration whereas other groups were given medicated ration containing *Cichorium intybus* leaf powder (CILP) @ 5000 ppm in Group II, imidacloprid (IM) @ 100 ppm in Group III, imidacloprid (IM) @ 100 ppm + silymarin (SM) @ 100 ppm in Group IV and imidacloprid (IM) @ 100 ppm + *Cichorium intybus* leaf powder (CILP) @ 5000 ppm in Group V, respectively for 8 weeks. The dose of imidacloprid and *Cichorium intybus* leaf powder was selected on the basis of available literature. After 8 weeks, all birds were sacrificed humanely after etherisation, and liver and kidney were collected for measuring the absolute and relative organ weights.

Biochemical analysis: At the end of 4th and 8th week, 2 ml of blood sample was collected from each bird to separate serum for the estimation of biochemical parameters. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatinine, blood urea nitrogen (BUN), total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, and globulin were estimated within 72 h of blood collection using diagnostic kits (AUTOSPAN® and Tulip) and the results were recorded on UV-VIS spectrophotometer.

Statistical analysis: The data obtained was analysed using SPSS version 17.0 for significant statistical differences among groups at 4 and 8 weeks interval by employing two way ANOVA at 5% level of significance (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

A significant ($p<0.05$) increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) levels was seen in imidacloprid group III cockerels as compared to other

Table 1. Effect of feeding *Cichorium intybus* leaf powder (CILP) on liver enzymes (IU/L) following simultaneous administration of imidacloprid @ 100 ppm in feed for 8 weeks in WLH cockerels

Group	ALT		AST		ALP		GGT		LDH	
	4th week	8th week	4th week	8th week	4th week	8th week	4th week	8th week	4th week	8th week
I	21.5±0.55^{b}	22.5±0.67^{c}	98.5±1.83^{bc}	103±5.84^{bc}	202±5.71^{b}	201±7.06^{b}	11.1±0.58^{b}	12.5±0.77^{b}	323±8.48^{b}	331 ±8.41^{b}
II	21.4±0.33^{b}	21.8±0.51^{c}	94.0±5.00^{c}	99.1±4.22^{c}	199±6.11^{b}	200±7.02^{b}	10.7±0.57^{b}	12.1±0.74^{b}	319±9.86^{b}	328±7.83^{b}
III	30.4±0.75^{aB}	42.1±4.76^{aA}	129±4.69^{aB}	147±7.29^{aA}	243±4.88^{aB}	268±5.45^{aA}	19.4±1.30^{aB}	22.8±1.10^{aA}	413±9.28^{aA}	439±11.6^{aA}
IV	25.1±0.97^{bB}	27.5±1.74^{bA}	103±1.50b^{cA}	106±1.94^{bcA}	206±3.19^{bA}	210±3.86^{bA}	13.5±0.54^{bA}	14.1±0.51^{bA}	331±12.4^{bA}	347±10.7^{bA}
V	25.6±0.87^{aB}	27.7±1.38^{bA}	105±1.59^{bcA}	108±2.29^{bcA}	209±2.21^{bA}	214±2.52^{bA}	14.0±0.53^{bA}	15.0±0.52^{bA}	336 ±11.6^{bA}	351±12.6^{bA}

Values in the table are Mean±SE (n=6). Group I, Control; Group II, CILP @ 5000 ppm; Group III, IM @ 100 ppm; Group IV, IM @ 100 ppm + SM @ 100 ppm + CILP @ 5000 ppm; CILP, *Cichorium intybus* leaf powder; SM, Silymarin; IM, Imidacloprid. Values having different superscripts (a, b and c) within a column differ significantly ($p<0.05$). Values having different superscripts (A and B) within a row differ significantly.

groups (Group I, II, IV and V) at 4 and 8 weeks interval (Table 1). The concurrent administration of *Cichorium intybus* leaf powder (CILP) in Group V resulted in significant ($p<0.05$) reduction in all serum enzymes and the results were comparable to that of Group IV and control.

A significant ($p<0.05$) increase in ALT, AST, ALP and GGT activities in Group III exposed to imidacloprid suggests its hepatotoxic effect in cockerels. ALT, primarily found in liver, plays an important role in amino acid metabolism, gluconeogenesis and serves as an important biomarker of liver damage (Thapa and Walia 2007). Its elevation in blood results from degeneration of hepatocytes resulting in release of enzymes into blood circulation. AST is an important enzyme which is involved in amino acid metabolism. AST elevation in blood reflects hepatocellular damage, however, being present in other organs, it is a non-specific hepatic biomarker (Dufour *et al.* 2001). An increase in AST activity in imidacloprid intoxicated cockerels might have occurred due to degenerative changes in liver resulting in increased cellular permeability leading to spillage of AST into the blood stream. Findings of our study are in agreement with the previous studies which reported an increase in serum AST and ALT activities following imidacloprid exposure in pigeons (Abu Zeid *et al.* 2019), Japanese quails (Emam *et al.* 2018) and white leghorn cockerels (Gupta *et al.* 2014).

The elevated ALP activity in blood results due to the cholestasic liver disease causing obstruction of the bile ducts resulting in inhibition of ALP secretion and subsequently its release into the blood stream (Burtis and Ashwood 1999). The increased ALP activity as seen in imidacloprid treated Group III in the present study might have been due to its hepatotoxic effect, causing hepatic malfunctioning as a result of damage to the hepatocytes, and interfering the transport mechanism leading to enhanced release of ALP into the blood. GGT is a membrane bound glycoprotein used diagnostically for hepatic diseases, however, is not considered a specific biomarker like ALT because apart from the liver, it is also found in kidney, heart, brain, spleen, seminal vesicles, pancreas, bile duct and gall bladder (Goldberg 1980). Its level increases in cases of cholestatic liver diseases. The increase in GGT level due to imidacloprid in present study might have been due to the inhibition of bile secretion after hepatic damage which might have augmented the GGT activity. An increase in ALP and GGT activity following exposure to imidacloprid suggest its hepatotoxic effect and the results are in agreement with the previous findings (El-Halwagy *et al.* 2018, Badawy *et al.* 2018). The reduction in the levels of ALT, AST, ALP and GGT by *Cichorium intybus* leaf powder (CILP) suggests its ameliorative effect, reflecting its hepatoprotective potency which might be due to the presence of antioxidants like flavonoids and phenols (Mathur *et al.* 2014, Maletha *et al*. 2020). These findings are in corroboration with the previous findings of Helal *et al.* (2011) and Li *et al.* (2014) in rats, where reduction in levels of ALT, AST, ALP and GGT was seen following *Cichorium intybus* administration. The hepatoprotective efficacy of *Cichorium intybus* has also been reported against carbon tetrachloride and thioacetamide induced hepatotoxicity in rats (Elgengaihi *et al.* 2016, Keshk *et al.* 2019).

Lactate dehydrogenase (LDH) is an important cytosolic enzyme involved in carbohydrate metabolism. It is ubiquitous in nature, present in almost all tissues and serves as an important biomarker of tissue damage (Graham *et al.* 2013). The elevation in the LDH levels in imidacloprid treated chicks indicates the cellular damage which might have occurred due to the generation of free radicals under oxidative stress. The previous studies conducted by Khalil *et al.* (2017) in rats and Abu Zeid *et al.* (2019) in pigeon also reported an increase in LDH activity following exposure to imidacloprid. Similarly, reduction in LDH levels following *Cichorium intybus* administration in rats has also been reported by Helal *et al.* (2011) and Sharma *et al.* (2019**)** suggesting protective effect of *Cichorium intybus*.

A significant ($p<0.05$) increase in levels of total and indirect bilirubin and reduction in serum total proteins, albumin and globulin was observed in imidacloprid treated Group III as compared to control at both 4 and 8 week intervals (Table 2). The treatment with CILP in Group V reversed these parameters and the results were at par with that of Group IV and control. Bilirubin is an endogenous anion derived from the regular degradation of haemoglobin. Elevation in bilirubin results either due to biliary obstruction, hemolysis and renal failure. The rise in bilirubin occurs due to the obstruction either within the liver or outside the liver. The elevated total and indirect bilirubin levels indicate hepatocellular dysfunction (Dufour *et al.* 2001). The increased total and indirect bilirubin levels in imidacloprid treated group might be due to the adverse effects of imidacloprid on liver causing hepatic damage as is evident from elevated ALT, AST, ALP and GGT levels. Liver plays a vital role in the synthesis and secretion of the wide variety of proteins. Proteins are structurally altered by free radicals resulting in functional inactivation (Aly *et al.* 2009). The significant decrease in serum proteins, albumin and globulin level due to imidacloprid in Group III might be attributed to hepatotoxicity by imidacloprid as is also evident from elevated ALT, AST ALP and GGT reported in this study or because of the damage in the filtration system of kidney resulting in leakage and passage of proteins to urine. The findings of this investigation are in accordance with the reports of Gupta *et al.* (2014) and El-Halwagy *et al.* (2018) in white leghorn (WLH) cockerels and rats, respectively. Ameliorative effect shown by CILP may be attributed to the potent antioxidant action of *C. intybus* which might have reversed the imidacloprid induced hepatic damage. This fact is also supported by the study conducted by Saeed *et al.* (2015) who reported hepatoprotective and growth promoting activity of *Cichorium intybus.*

Imidacloprid significantly ($p<0.05$) increased the levels of BUN and creatinine in Group III as compared to other

Table 2. Effect of feeding *Cichorium intybus* leaf powder (CILP) on liver function parameters (mg/dl) following simultaneous administration of imidacloprid @ 100 ppm in feed for 8 weeks in WLH cockerels

Group	Total bilirubin		Direct bilirubin		Indirect bilirubin		Total protein		Albumin		Globulin	
	4th week	8th week	4th week	8th week	4th week	8th week	4th week	8th week	4th week	8th week	4th week	8th week
I	1.10±0.04^{bc}	1.14±0.04^{b}	0.24±0.009^{a}	0.24±0.001^{a}	0.86±0.03^{b}	0.90±0.03^{b}	3.83±0.15^{a}	3.99±0.20^{a}	1.98±0.06^{a}	2.11±0.05^{a}	1.86±0.09^{a}	1.88±0.15^{a}
II	1.04±0.03^{c}	1.09±0.04^{b}	0.23±0.005^{b}	0.23±0.002^{b}	0.82±0.03^{b}	0.86±0.04^{b}	3.96±0.15^{a}	4.18±0.18^{a}	2.06±0.03^{a}	2.12±0.03^{a}	1.90±0.18^{a}	2.06±0.17^{a}
III	1.32±0.04^{aB}	1.52±0.08^{aA}	0.21±0.004^{cA}	0.20±0.0009^{cA}	1.11±0.04^{aB}	1.32±0.08^{aA}	3.18±0.22^{bA}	3.05±0.30^{bA}	1.61±0.14^{bA}	1.67±0.13^{bA}	1.57±0.15^{aA}	1.37±0.18^{bA}
IV	1.12±0.03^{b}	1.15±0.04^{b}	0.24±0.001^{a}	0.24±0.003^{a}	0.88±0.03^{b}	0.91±0.04^{b}	3.63±0.19^{ab}	3.89±0.28^{a}	1.84±0.08^{ab}	1.91±0.07^{a}	1.79±0.13^{a}	1.99±0.21^{a}
V	1.16±0.04^{b}	1.18±0.04^{b}	0.24±0.002^{a}	0.24±0.001^{a}	0.92±0.04^{b}	0.95±0.04^{b}	3.67±0.16^{ab}	3.75±0.17^{a}	1.90±0.09^{a}	1.98±0.08^{a}	1.78±0.10^{a}	1.78±0.10^{ab}

Values in the table are Mean±SE (n=6). Group I, Control; Group II, CILP @ 5000 ppm; Group III, IM @ 100 ppm; Group IV, IM @ 100 ppm + SM @ 100 ppm; Group V, IM @ 100 ppm + CILP @ 5000 ppm; CILP, *Cichorium intybus* leaf powder; SM, Silymarin; IM, Imidacloprid. Values having different superscripts (a, b and c) within a column differ significantly ($p<0.05$). Values having different superscripts (A and B) within a row differ significantly.

groups at 4 and 8 week intervals, whereas the administration of CILP in Group V reduced these values and the results were at par with that of Group IV and control (Table 3). Moreover, the administration of CILP alone in Group II significantly ($p<0.05$) reduced the BUN levels as compared to control. Urea, the major nitrogenous end product of protein and amino acid metabolism constitutes the largest fraction of NPN component of blood, produced primarily by the liver and excreted through kidney. An elevation in BUN indicates impaired renal function. Both BUN and creatinine are considered as biomarker to assess kidney damage (Edelstein 2008). Creatinine is a breakdown product formed in muscles from creatine phosphate and is produced regularly at fairly constant rate by the animal body depending on muscle mass and removed from plasma by glomerular filtration (Zuo *et al.* 2008). The significant ($p<0.05$) increase in BUN and creatinine levels in Group III might be due to the damage to the kidney and impairment in GFR due to imidacloprid as also reported by Arfat *et al.* (2014) and Gupta *et al.* (2014) in mice and white leghorn cockerels, respectively. A significant ($p<0.05$) reduction in elevated BUN and creatinine levels in Group V following simultaneous administration of CILP suggests its nephroprotective action which might be due to the presence of antioxidants (Maletha *et al.* 2020). The

Table 3. Effect of feeding *Cichorium intybus* leaf powder (CILP) on BUN and creatinine (mg/dl) following simultaneous administration of imidacloprid @ 100 ppm in feed for 8 weeks in WLH cockerels

Group	BUN		Creatinine	
	4th week	8th week	4th week	8th week
I	4.80±0.40^{b}	5.26±0.31^{b}	0.55±0.02^{ab}	0.60±0.10^{b}
II	3.82±0.20^{c}	3.95±0.26^{c}	0.35±0.03^{b}	0.46±0.10^{b}
III	7.56±0.28^{aB}	9.82±0.32^{aA}	0.73±0.13^{aB}	1.03±0.19^{aA}
IV	5.64±0.24^{b}	5.75±0.31^{b}	0.59±0.05^{ab}	0.66±0.12^{b}
V	5.33±0.29^{b}	5.42±0.32^{b}	0.62±0.06^{ab}	0.68±0.10^{b}

Values in the table are Mean±SE (n=6). Group I, Control; Group II, CILP @ 5000 ppm; Group III, IM @ 100 ppm; Group IV, IM @ 100 ppm + SM @ 100 ppm; Group V, IM @ 100 ppm + CILP @ 5000 ppm; CILP, *Cichorium intybus* leaf powder; SM, Silymarin; IM, Imidacloprid. Values having different superscripts (a, b and c) within a column differ significantly ($p<0.05$). Values having different superscripts (A and B) within a row differ significantly.

study conducted by Zaman *et al.* (2017) also reported nephroprotective activity of *Cichorium intybus* in rats.

An increase in absolute and relative organ weight of liver and kidney was observed in imidacloprid Group III as compared to control group which was further ameliorated following CILP administration in Group V as is evident from the decrease in absolute and relative organ weight (Table 4). The increase in absolute and relative organ weight in Group III can be correlated to the increase in serum enzymatic level of hepatic and renal biomarkers in Group III which was further ameliorated following *Cichorium intybus* leaf powder administration in Group V,

Table 4. Effect of feeding *Cichorium intybus* leaf powder (CILP) on absolute and relative organ weights (g) following simultaneous administration of imidacloprid @ 100 ppm in feed for 8 weeks in WLH cockerels

Group	Absolute organ weight		Relative organ weight	
	Liver	Kidney	Liver	Kidney
I	31.98±1.95[a]	2.48±0.31[a]	2.80±0.16[a]	0.22±0.03[a]
II	28.79±0.89[a]	2.40±0.39[a]	2.40±0.09[a]	0.20±0.03[a]
III	34.37±4.83[a]	3.30±0.43[a]	3.22±0.48[a]	0.31±0.04[a]
IV	28.13±2.08[a]	3.02±0.19[a]	2.49±0.20[a]	0.27±0.02[a]
V	26.84±2.56[a]	3.13±0.23[a]	2.39±0.23[a]	0.28±0.02[a]

Values in the table are Mean±SE (n=6). Group I, Control; Group II, CILP @ 5000 ppm; Group III, IM @ 100 ppm; Group IV, IM @ 100 ppm + SM @ 100 ppm; Group V, IM @ 100 ppm + CILP @ 5000 ppm; CILP, *Cichorium intybus* leaf powder; SM, Silymarin; IM, Imidacloprid. Values having different superscripts (a and b) within a column differ significantly ($p<0.05$).

as is also evident from reduction in serum hepatic and renal enzymes in Group V in the present study, suggesting it's hepatoprotective and nephroprotective activity.

Thus, amelioration of imidacloprid (@100 ppm) induced hepatotoxic and nephrotoxic effects following concurrent administration of *Cichorium intybus* leaf powder (CILP) @ 5000 ppm for 8 weeks in white leghorn cockerels suggests the hepatoprotective and nephroprotective activity of *Cichorium intybus*. The ameliorating properties of this plant leaves is due to the presence of medicinal phytoconstiuents which also act as growth promoters. Further detailed studies on the exact mechanism of action responsible for hepatoprotective and nephroprotective action of *Cichorium intybus* at the molecular level are required.

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REFERNCES

Abd-Elhakim Y, Mohammed H H and Mohamed W A. 2018. Imidacloprid impacts on neurobehavioral performance, oxidative stress, and apoptotic events in the brain of adolescent and adult rats. *Journal of Agricultural and Food Chemistry* **66**(51): 13513–24.

Abu Zeid E H, Alam R T H, Ali S A and Hendawi M Y. 2019. Dose-related impacts of imidacloprid oral intoxication on brain and liver of rock pigeon (*Columba livia domestica*), residues analysis in diferent organs. *Ecotoxicology and Environmental Safety* **167**: 60–68.

Al-Snafi A E. 2016. Medical importance of *Cichorium intybus*: A review. *IOSR Journal of Pharmacy* **6**: 41–56.

Aly H A, Lightfoot D A and El-Shemy H A. 2009. Modulatory role of lipoic acid on lipopolysaccharide induced oxidative stress in adult rat sertoli cells *in vitro*. *Chemico-Biological Interactions* **182**(2): 112–18.

Arfat Y, Mahmood N, Tahir M U, Rashid M, Anjum S, Zhao F, Li D J, Sun Y L, Hu L, Zhihao C and Yin C. 2014. Efect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice. *Toxicology Reports* **1**: 554–61.

Badawy M H, Ahmed N S and Attia A M. 2018. Sub-acute oral toxicity of imidacloprid and fipronil pesticide mixture in male albino rats; biochemical and reproductive toxicity evaluation. *Journal of Materials and Environmental Science* **9**(8): 2431–37.

Burtis C A and Ashwood E R. 1999. *Tietz Textbook of Clinical Chemistry.* 3rd edition. Saunders, Philadelphia.

Dufour D R, Lott J A, Nolte F S, Gretch D R and Koff R S. 2001. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis and monitoring. *Clinical Chemistry* **47**: 1133–35.

Edelstein C L. 2008. Biomarkers of acute kidney injury. *Advances in Chronic Kidney Disease* **15**(3): 222–34.

Elgengaihi S, Mossa A T, Refaie A A and Aboubaker D. 2016. Hepatoprotective efficacy of *Cichorium intybus* L. extract against carbon tetrachloride induced liver damage in rats. *Journal of Dietary Supplements* **13**(5): 570–84.

El-Halwagy M E A, Hussein R H, Hamza A H and Al-Bishri W M. 2018. Hepatoprotective effect of alpha lipoic acid versus intoxication with imidacloprid widely used in KSA in albino rats. *International Journal of Pharmaceutical Research and Allied Sciences* **7**(3): 224–32.

Emam H, Emam A and Abdel-Daim M. 2018. Antioxidant capacity of omega-3-fatty acids and vitamin E against imidacloprid-induced hepatotoxicity in Japanese quails. *Environmental Science and Pollution Research* **25**(12): 11694–702.

Goldberg D M. 1980. Structural, functional and clinical aspects of γ-Glumatyltransferase. *CRC Critical Reviews in Clinical Laboratory Sciences* **12**(1): 1–58.

Graham S S, Gail L W and Robin M W. 2013. Clinical pathology in non-clinical toxicology testing. *Haschek and Rousseaux's Handbook of Toxicologic Pathology.* 3rd edition. pp 565–94.

Gupta M, Singh S P and Pankaj N K. 2014. Evaluation of protective effect of *Erythrina variegata* and *Spirulina platensis* in cockerels intoxicated with imidacloprid. *Journal of Veterinary Pharmacology and Toxicology* **13**(2): 146–51.

Helal E, Samia M, Atef M and Ghada A. 2011. Effect of *Cichorium intybus* L. on fatty liver induced by oxytetracycline in albino rats. *Egyptian Journal of Hospital Medicine* **45**: 522–35.

Jeschke P, Nauen R, Schindler M and Elbert A. 2010. Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry* **59**: 2897–2908.

Kapoor U, Srivastava M K and Srivastava L P. 2011. Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats. *Food and Chemical Toxicology* **49**: 3086–89.

Keshk W A, Soliman N A, Ali D A and Elseady W S. 2019. Mechanistic evaluation of AMPK/SIRT1/FXR signalling axis, inflammation and redox status in thioacetamide induced liver cirrhosis: The role of *Cichorium intybus* L. (chicory) supplemented diet. *Journal of Food and Biochemistry* **43**(8): e12938.

Khalil S R, Awad A, Mohammed H H and Nassan M A. 2017. Imidacloprid insecticide exposure induces stress and disrupts glucose homeostasis in male rats. *Environmental Toxicology and Pharmacology* **55**: 165–74.

Li G Y, Gao H Y, Huang J, Lu J, Gu J K and Wang J H. 2014. Hepatoprotective effect of *Cichorium intybus* L., a traditional Uyghur medicine, against carbon tetrachloride-induced hepatic fibrosis in rats. *World Journal of Gastroenterology* **20**: 4753–60.

Maletha D, Singh S P, Ramanarayanan S, Pandey D and Taj G. 2020. *In vitro* phytochemical analysis and evaluation of antioxidant activity of *Cichorium intybus*. *Journal of Veterinary Pharmacology and Toxicology* **19**(2): 10–13.

Maletha D, Singh S P, Ramanarayanan S, Pandey D and Taj G. 2021. The ameliorating potential of *Cichorium intybus* against imidacloprid induced intoxication in WLH cockerels. *Journal of Veterinary Pharmacology and Toxicology* **20**(1): 52–56.

Mathur N, Pande K D, Aeri V, Kishore A, Joshi V, Madaan A and Verma R. 2014. Determination of antioxidant and hepatoprotective ability of flavonoids of *Cichorium intybus*. *International Journal of Toxicological and Pharmacological Research* **6**(4): 107–112.

Mousa M A, Osman A S and Hady H A. 2017. Performance, immunology and biochemical parameters of *Moringa oleifera* and/or *Cichorium intybus* addition to broiler chicken ration. *Journal of Veterinary Medicine and Animal Health* **9**(10): 255–63.

Pushparaj P N, Low H K, Manikandan J, Tan B H K and Tan C H. 2007. Antidiabetic effects of *Cichorium intybus* in streptozotocin induced diabetic rats. *Journal of Ethnopharmocology* **111**(2): 430–34.

Saeed M, Baloch A R, Wang M, Soomro R N, Baloch A M, Bux B A, Arian M A, Faraz S S and Zakriya H M. 2015. Use of *Cichorium intybus* leaf extract as growth promoter, hepatoprotectant and immune modulant in broilers. *Journal of Animal Production Advances* **5**(1): 585–91.

Sarwar M S. 2013. 'Effect of chicory leaves extract, extracted in water at different pH levels (3,7 and 12) on growth, nutrient digestibility, hematology, immune response and economics of broilers.' M.Sc. Hons. Thesis, University of Agriculture, Faisalabad, Pakistan.

Sharma M, Afaque A, Dwivedi S, Jairajpuri Z S, Shamsi Y, Khan M F, Khan M I and Ahmed D. 2019. *Cichorium intybus* attenuates streptozotocin induced diabetic cardiomyopathy via inhibition of oxidative stress and inflammatory response in rats. *Interdisciplinary Toxicology* **12**(3): 111–19.

Snedecor G W and Cochran W G. 1967. *Statistical Methods*. 6th Edition. Iowa State University Press, Ames, lowa.

Street R A, Sidana J and Prinsloo G. 2013. *Cichorium intybus*: Traditional uses, phytochemistry, pharmacology and toxicology. *Evidence Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2013/579319.

Thapa B R and Walia A. 2007. Liver function tests and their interpretation. *Indian Journal of Pediatrics* **74**: 663–71.

Zaman R, Alam A, Jafri M, Sofi G and Ahmed G. 2017. Nephroprotective effect of Beekh Kasni (Roots of *Cichorium intybus*) in the form of methanolic and aqueous extract in gentamycin induced rat models. *Journal of Pharmacognosy and Phytochemistry* **6**: 337–41.

Zuo Y, Wang C, Zhou J, Sachdeva A and Ruelos V C. 2008. Simultaneous determination of creatinine and uric acid in human urine by high performance liquid chromatography. *Analytical Sciences* **24**(12): 1589–92.