Ann. For. Res. 67(1): 524-533, 2024
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

ALTERATIONS IN HISTOARCHITECTURE AND BIOCHEMICAL MARKERS OF LIVER AND KIDNEY FUNCTIONS ON SUB-ACUTE EXPOSURE OF IVERMECTIN IN ALBINO RATS

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Abstract
The present study was performed to evaluate alterations in histoarchitecture and biochemical markers of liver and kidney function on sub-acute exposure of Ivermectin in albino rats. Albino rats weighing 180-200 gm were treated with Ivermectin orally and divided into two groups, each group consistedof six rats. The rats of group I served as control. However, rats of group II were treated with ivermectin @ 5 mg/kg b.wt.Blood samples were collected on day 28 of experiment with the help of capillary tube for estimation of biochemical parameters andliver and kidney tissues samples were collected for histopathological examination. Ivermectin altered the functions of liver and kidney as indicated by the alterations in the biochemical markers and histopathological examinations of liver and kidney.Ivermectin significantly enhanced the concentration of biochemical markers of liver function viz. AST, ALT, ALP and Bilirubin as compared to control. The concentration of biochemical markers of kidney function viz. BUN and creatinine were significantly enhanced after ivermectin administration, as compared to control.Hepatomegaly with congestion and loss of demarcation between cortex and medulla in kidney were observed. Histopathologically, hydropic degeneration, vacuolation and congestion of hepatic tissues and marked congestion along with haemorrhages were found on histoarchitecture study of kidney tissue.Ivermectin have ability to alter the histoarchitecture and biochemical markers of liver and kidney in albino rats after the sub-acute exposure for 28 days.

Key words: Albino rats, Ivermectin, Biochemical parameters, histoarchitecture.

Introduction

Avermectins are a class of macrocyclic lactones with insecticidal and antiparasitic properties. They are the most effective and well-developed class of endectocides and are active against both endo and ectoparasites. Ivermectin, the best studied semi-synthetic derivate of avermectin, has been considered one of the most successful discoveries in the fight against infections caused by roundworm parasites (Azevedo *et al.,* 2019).

Although, Ivermectin is a potent antiparasitic drug, it poses a serious toxicity threat to animals by damaging the various organs of the body systems. These damaging effects are seen in dose-dependent and dose-time dependent manners. The reason is that, ivermectin is lipophilic hence, tend to accumulate in fatty tissues and in the liver, it induces oxidative stress leading to tissue damage through lipid peroxidation (Nasr *et al.,* 2016). Various studies on ivermectin have proven its ability to induce nephrotoxicity in many animals like mice, bats, rabbits and rats (DeMarco *et al.,* 2002; Ahmed *et al.,* 2019; Ahmed*et al*., 2020). The main molecular mechanism through which

ivermectin exerts nephrotoxic effect is the lipid peroxidation which results from the action of reactive oxygen species (Nasr *et al.,* 2016). This oxidative damage results in histopathological changes like interstitial nephritis, glomerular damage, interstitial infiltration areas of round cells and tubular necrosis as well as elevated levels of serum creatinine, urea and the uric acid in the blood (Abd-Elhady and Abou-Elghar, 2013). In toxicity analysis, a combined evaluation of various biochemical parameters provides better identification of the organ being damaged by the drug under investigation (Salman *et al.,* 2022).

Material and Methods

Experimental Animals

The study was conducted on healthy albino rats, weighing around 180-200 g. The experiment was approved by the Institutional AnBreimal Ethical Committee (IAEC) of College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur. Before the start of the experiment the rats were kept in laboratory condition for a period of 7 days for acclimatization. The rats were maintained with good hygienic conditions and kept in colony cages under standard managemental conditions and provided with standard feed and water *ad libitum*.

Experimental design –Twelve rats were randomly divided into two groups with six rats in each.

Group	Treatment
I	Control (Normal saline) once daily, orally for 28 days
II	Ivermectin (5 mg/kg) once daily, orally for 28 days

Collection of Samples

Blood Samples:

Blood was collected aseptically on day 0 (pre-treatment) and day 28 (post-treatment) from retro orbital plexus with the help of capillary tube, as described by Archar and Riley (1981). Blood was collected in heparinized vials and used for biochemical parameters study.

Biochemical Parameters

Plasma was separated from heparinised blood samples and refrigerated at 4˚C for biochemical studies. The following biochemical markers of liver and kidney function were estimated by using Semi-auto analyzer with respective commercially available kits of ERBA, manufactured by Transasia Bio-Medicals Ltd., Daman

1. Aspartate aminotransferase (AST) (IU/L)

2. Alanine transaminase (ALT) (IU/L)

3. Alkaline phosphatase (ALP) (IU/L)

4. Bilirubin (mg/dl)

5. Albumin (g/dl)

6. Creatinine (mg/dl)

7. Blood urea nitrogen (BUN) (mg/dl)

Pathological Studies

All the rats of each group were humanely euthanized by inhalation anaesthesiaon day 28 of study. Liver and kidney were dissected out and examined for changes in gross appearance and histopathological architecture (Gridley, 1960).

Gross Pathology -

Liver and kidney were subjected to detailed post mortem examination. Gross lesions on liver and kidney were recorded. Rats showing gross lesions like hepatomegaly, congestion, haemorrhagic areas and other changes were suspected for histopathological changes.

Histopathological Study

Tissue pieces from liver and kidney were collected in 10 per cent formalin and processed for histopathological examination (Gridley, 1960).

Processing of Tissues

The tissue samples were then processed by dehydration, clearing and embedding in paraffin.

Section Cutting

Approximately 5 μm thin sections were cut by rotary microtome and ribbon section formed was placed in water bath. Floating sections were taken on clean glass slides smeared with egg albumin as adhesive for histopathology.

Staining of Tissues

Routine Staining: Hematoxylin and Eosin (H&E)

Hematoxylin and Eosin staining was performed as per the method described by Gridley (1960) with slight modification. The sectioned slides were stained with hematoxylin and eosin, mounted with DPX (distyrene plasticizer xylene) and covered with coverslips for further histopathological examination.

Statistical Analysis

Means and standard error were obtained as per standard procedure. Parameters were analyzed by using the method of complete randomized design with two groups of six animals each. The difference between groups was tested statistically for their significance (Snedecor and Cochran, 1994).

Results and Discussion

Biochemical Staudies

In the present research work,biochemical study was carried out to study the alterations in biochemical markers of liver and kidney functions on sub-acute exposure of ivermectin in albino rats.

Biochemical Markers of Liver Function

AST and ALT

The mean values of AST in control were 41.10±0.79 IU/Land 40.55±0.82 IU/L on day 0 and day 28 of the study, respectively. In Ivermectin treated group, the mean values of AST were 40.55±0.85 IU/Land 86.91±0.84 IU/L on day 0 and day 28 of the study, respectively. Ivermectin significantly enhance the value of AST on day 28 of the study and the increase was 114.33 per cent.

The mean values of ALT in control were 83.95±1.63 IU/Land 83.80±1.71IU/L on day 0 and day 28 of the study, respectively. In Ivermectin treated group, the mean values of ALT were 84.40±0.95 IU/Land 174.60±1.99 IU/L on day 0 and day 28 of the study, respectively. Ivermectin significantly enhance the value of AST on day 28 of the study and the increase was 106.87 per cent.

ALP (Alkaline Phosphates)

The mean values of ALP in control were 185.75±0.84 IU/L and 195.53±0.76 IU/L on day 0 and day 28 of the study, respectively. In Ivermectin treated group, the mean values of ALP were 184.51±1.14IU/Land 340.70±0.99 IU/L on day 0 and day 28 of the study, respectively. Ivermectin significantly enhance the value of ALP on day 28 of the study and this increase was 84.65 per cent.

Albumin And Bilirubin

The mean values of albumin in control were 3.95±0.27 g/dL and 3.83±0.24 g/dL on day 0 and day 28 of the study, respectively. In Ivermectin treated group, the mean values of albumin were 4.16±0.09 g/dL and 1.50±0.15 g/dL on day 0 and day 28 of the study, respectively. Ivermectin significantly decrease the value of albumin on day 28 of the study and this decrease was 63.94 per cent.

The mean values of bilirubin in control were .47±0.05 g/dL and 0.49±0.05 g/dL on day 0 and day 28 of the study, respectively. In Ivermectin treated group, the mean values of bilirubin were 0.53±0.06 g/dLand 3.03±0.38 g/dL on day 0 and day 28 of the study, respectively. Ivermectin significantly enhance the value of bilirubin on day 28 of the study and this increase was 471.69 per cent.

Biochemical markers of kidney function

Serum Creatinine and BUN

The mean values of serum creatinine in control were 0.35±0.03 g/dL and 0.33±0.03 g/dL on day 0 and day 28 of the study, respectively. In Ivermectin treated group, the mean values of serum creatinine were 0.41±0.02 g/dLand 1.46±0.15 g/dL on day 0 and day 28 of the study, respectively. Ivermectin significantly enhance the value of serum creatinine on day 28 of the study and this increase was 256.09 per cent.

The mean values of blood urea nitrogen in control were 15.83±0.46 mg/dL and 16.00±1.20 mg/dL on day 0 and day 28 of the study, respectively. In Ivermectin treated group, the mean values of BUN were 15.61±0.46 mg/dL and 52.25±1.15 mg/dL on day 0 and day 28 of the study, respectively. Ivermectin significantly enhance the value of BUN on day 28 of the study and this increase was 234.72 per cent.

Table 01: Effect of subacute exposure ofivermectin on Biochemical markers of liver functionin albino rats

Biochemical markers	**Control**		**Ivermectin (5 mg/kg) once daily, orally for 28 days**
AST (IU/L)	**Day 0**	41.10^{a}±0.79	40.55^{a}±0.85
	Day 28	40.55^{c}±0.82	86.91^{a}±0.84
ALT (IU/L)	**Day 0**	83.95^{a}±1.63	84.40^{a}±0.95
	Day 28	83.80^{c}±1.71	174.60^{a}±1.99
ALP (IU/L)	**Day 0**	185.75^{a}±0.84	184.51^{a}±1.14
	Day 28	195.53^{c}±0.76	340.70^{a}±0.99
Albumin (g/dl)	**Day 0**	3.95^{a}±0.27	4.16^{a}±0.09
	Day 28	3.83^{a}±0.24	1.50^{c}±0.15
Bilirubin (mg/dl)	**Day 0**	0.47^{a}±0.05	0.53^{a}±0.06
	Day 28	0.49^{c}±0.05	3.03^{a}±0.38

- Values are mean of six observations
- Means with different superscripts in same rows differs significantly *(P≤0.05)*

Table 02: Effect of subacute exposure of ivermectin on Biochemical markers of kidney function in albino rats

Biochemical markers	Control		Ivermectin (5 mg/kg) once daily, orally for 28 days
Serum Creatinine (mg/dl)	Day 0	$0.35^{a}\pm0.03$	$0.41^{a}\pm0.02$
	Day 28	$0.33^{c}\pm0.03$	$1.46^{a}\pm0.15$
BUN (mg/dl)	Day 0	$15.83^{a}\pm0.46$	$15.61^{a}\pm0.46$
	Day 28	$16.00^{c}\pm1.20$	$52.25^{a}\pm1.15$

- Values are mean of six observations
- Means with different superscripts in same rows differs significantly *($P\leq0.05$)*

Pathological studies

The gross and microscopic changes in liver and kidney were recorded in rats during the experimental study. The gross and histopathological alterations observed in liver and kidney have been described below:

Gross pathalogy

Group-I (Control)

No appreciable gross and microscopic changes were observed in liver and kidney in control group (Plate 01,02 and 03,04).

Group-II (Ivermectin)

Gross lesions

Grossly, hepatomegaly, congestion and haemorrhagic areas were observed (Plate 05). Cut section of kidney showed congestion with no clear-cut demarcation between cortex and medulla (Plate 06).

Microscopic lesions

Histopathology of liver section shown congested portal vein, severe vacuolation and hepatocyte degeneration (Plate 07,08).

Microscopic lesion in kidney includes fibrosis, hematoma formation and severe glomerular congestion (Plate 09,10).

Conclusion

Ivermectin at the dose of 5 mg/kg body wt., orally, daily for 28 days in albino rats induced the liver and kidney impairment as indicated by altered biochemical markers and altered histological architecture. A significant increase in concentration of biochemical markers of liver function viz. ALT, AST, ALP and bilirubin was reported after subacute exposure of ivermectin however, the concentration of albumin was decreased. Biochemical markers of kidney functions were also increased significantly after the subacute exposure of ivermectin.

Pathological studies indicated hepatomegaly with congestion and loss of demarcation between cortex and medulla in kidneys in ivermectin treated group. Histopathologically, hydropic

degeneration, vacuolation and congestion of hepatic tissues and marked congestion along with haemorrhages were found on histoarchitecture study of kidney tissue.

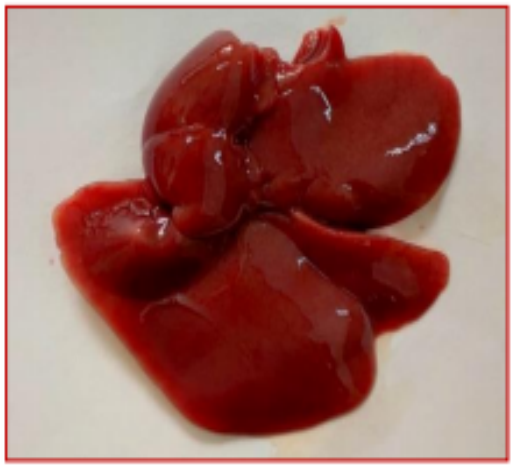

Plate 01: liver of rat in control group showing normal shape, size and consistency

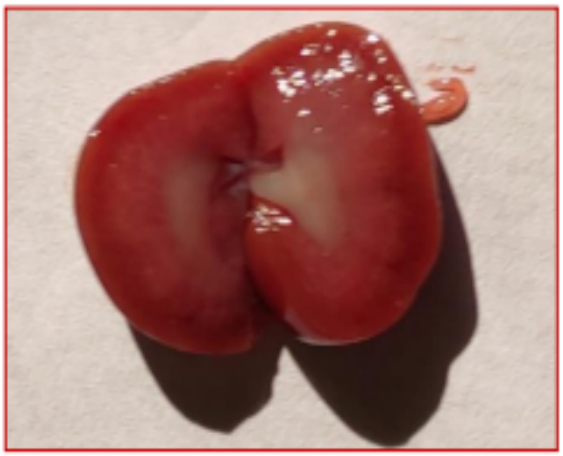

Plate 02: kidney of rat in control group showing normal architecture with clear demarcation between cortex and

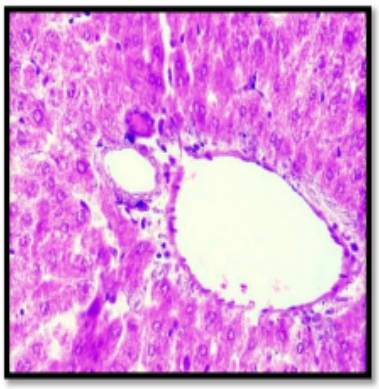

Plate 03: Microscopic section of liver of rat (control group) showing normal portal vein. H&E, X200

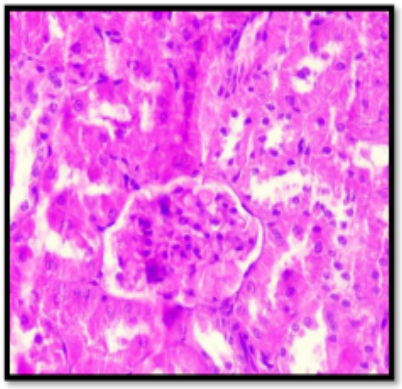

Plate 04: Microscopic section of kidney of rat (control group) showing normal glomerular. H&E, X400

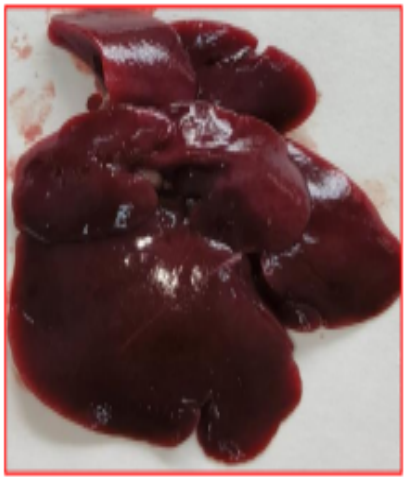

Plate 05: liver of rat in group treated with ivermectin showing hepatomegely with congestion

Plate 06: kidney of rat in group treated with ivermectin showing congetion and loss of demarcation between cortex and medulla

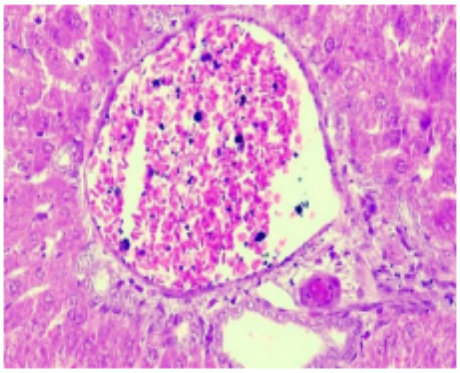

Plate 07: Microscopic section of liver of rat (Ivermectin group) showing congested portal vein. H&E, X400

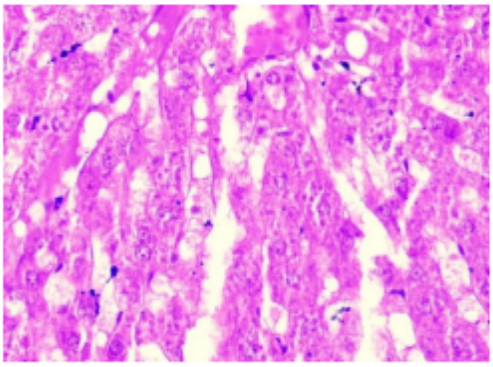

Plate 08: Microscopic section of liver of rat (Ivermectin group) showing severe vacuolation and hepatocyte degeneration. H&E, X400

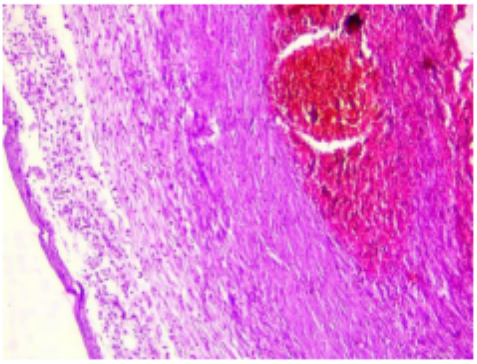

Plate 09: Microscopic section of kidney of rat (Ivermectin group) showing fibrosis and hematoma formation. H&E, X100

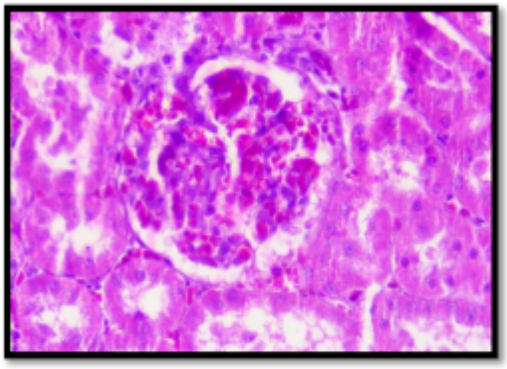

Plate 10: Microscopic section of kidney of rat (Ivermectin group) showing severe glomerular congestion. H&E, X400

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