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Protective role of methanolic extract of medicinal plants in paracetamol induced toxicity in laying hens

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Abstract

The study was designed to assess the hepato-nephro protective role of indigenous medicinal plants in paracetamol induced toxicity in layers. A total of 165 laying birds were reared and alienated into 11 groups with 3 replicates each. The group G-1 was considered as negative control, while G-II was positive control and the other groups were fed with different doses of medicinal plants methonolic extracts@ 1, 100 and 1000 mg/Kg b.w. Toxicity was induced through paracetamol @ 750 mg/Kg b.w. Toxicity was measured by evaluating the hepatic specific biomarkers (ALT, AST, ALP and serum Protein). Gross histopathology of liver and kidney was performed to evaluate the curative potential of medicinal plants against paracetamol toxicity. Results of this study revealed that liver biomarkers in the birds treated with paracetamol showed elevated trend (AST=51.66, ALT=40.00, ALP=4385 and serum protein 4.89 IU/L) indicating severe hepatic damage. However, in G-4 fed with @ 100 mg/kg b.w methanolic extract of *Terminalia arjuna* showed significant reduction in liver biomarkers (AST=18.00, ALT=15.00, ALP=2350.33 and serum protein 9.03 IU/L). This low trend of liver biomarkers indicating that plant extract help in restoring the normal functional ability of the hepatocytes. Histological section of liver and kidney in G-1 showed significant pathological changes included hydopic degeneration, fatty infiltration, hemorrhages and leukocytic infiltration in liver section. The renal section revealedmild glomerulo-tubular degeneration changes with mild leukocytic infiltration. However, in the birds treated with *T. arjuna*@ 100 mg/kg b.w positive response with maximum regenerative potential. It was concluded that the methanolic extract of *Terminalia arjuna* @ 100 mg/kg have a significant hepato-nephroprotective response against paracetamol induce toxicity.

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Introduction

Herbal compound effect on prevention of acetaminophen (Paracetamol) toxicity in targeted and non-targeted tissue and organs has been extensively studied in mammalian models (Sharma *et al*., 2008; Makwana *et al*., 2012). Use of diclofenac sodium in veterinary practices is banned due to threat of extinction of vultures, hence paracetamol appeared safe alternative to diclofenac sodium (MoEF 2006; Swan *et al*., 2006). However the paracetamol has severe side effect when used persistently or in high dose. Limited studies have been conducted on antioxidant effect of medicinal plants on paracetamol toxicity in poultry production (Lindenthal *et al*., 1993; Kumar *et al*., 2009; Rangnathan *et al*., 2013).

Medicinal plants are used for curing and treatment of different diseases since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid and phenolic compounds (Shihabudeen *et al*., 2010). Their concentration may vary from plant to plant and from part to part.Medicinal plants have been reported for its antioxidant properties and inhibit peroxidation reactions (Criste *et al.,* 2013; Abdouli *et al*., 2014; Panaite *et al*., 2014). Medicinal plants contain active ingredients such as saponins, phenolic and flavonoids compounds that possess antibacterial and antioxidant properties (Panaite *et al*., 2014; Sharma *et al*., 2016: Malik *et al*., 2017: Vikas *et al*., 2018).

Modern medicines have little to offer for alleviation of hepatic diseases. Serum biomarkers of liver rise up in blood stream to indicate haptic damage. Different medicinal plants have been studied and reported to improve liver function. Medicinal plants normalize serum bio-markers by preventing intracellular enzymes leakage due to its membrane stabilizing activity, healing of hepatic parenchyma and regeneration of hepatocytes (Anusha, *et al*., 2011; Adewale, *et al*., 2014). Medicinal plants due to their antioxidant activates (Abdouli *et al*., 2014) prevent hepatotoxicity and keep the liver healthy (Chand *et al*., 2011: Ghosh *et al*., 2014: Ali *et al*., 2015: Ghosh and Gomes 2016).

The present study was conducted to determine the hepato-nephro protective properties of common indigenous medicinal plants e.g. *Trigonella foenum-graecum*, *Berberis lycium* and *Terminalia arjuna* in laying hens.

Materials and methods

Collection and identification of medicinal plants

The plants material (*fenugreek* seeds, *B. lyceum* bark and *T. arjuna* seed) were procured from local market. The plants were identified by the plant taxonomist Department of Weed Science, The University of Agriculture Peshawar, Pakistan. The Seeds of *Fenugreek*, *T. arjuna*, a and *B. lycium* bark were used in the trial.

Plant extracts preparation

The clean dried plant materials (seeds) were grinded to fine powder by electric grinder (Moulnix, 600 W LM-240, France). Approximately, 200g grinded powder from each plant was separately placed in 2000 mL of absolute methanol for two weeks with regular shaking. The extracts were then filtered through muslin cloth followed by Whatmann filter paper No.1. The methanol was removed in rotary evaporator (Heidolph, Laborota-4000 Germany). The dry extract kept in air tight container at 4 °C till further use. The plant extracts (5.0 g) were dissolved in 10% DMSO (5.0 mL) in falcon tube and mix properly with the help of vortex mixer (KMC-1300V, Korea). The tube was then placed in water bath for 30 minutes at 40 °C to properly dissolve all active compounds in stock solution. The final concentrations of working solution of each plant extract were made as 1, 100 and 1000 mg/kg b.w of the respective plants for *in-vivo* study (Dabur *et al.* 2004).

Experimental birds

Twenty six-weeks-old 165 disease free, layers were used for challenge experiment. The birds were placed in clean pens and were allowed to acclimatize for one week.

Preparation of Standard dose

Keeping in view the preparation of silymarin as standard drug for evaluating the hepato-protective activity, the medicinal plants in the present study were powdered and weighted as per calculation of 1, 100 and 1000mg/kg b.w and then were made into suspension in 1% gum acacia (suspending agent) (Jagadish and Mahmood, 2008).

Paracetamol-induced toxicity in laying hens

Birds were randomized and divided into five groups consisting of five birds each in three replicates. Group I was served as negative control, while group II served as positive control with paracetamol induced toxicity. However, the remaing groups $MP_{(Fg)}E$, $MP_{(Bl)}E$ and $MP_{(Ta)}E$ were induced with paracetamol toxicity and fed with methanolic extract of medicinal plants (fenugreek, B. Lyceum and T. arjuna) at the rate of 1, 100 and 1000mg/kg body weight daily for two weeks (Table 1).

On day 8th, the Paracetamol suspension were given by oral route, at the dose rate of 750mg/kg body weight to all experimental birds except –ive control group for inducing toxicity.

Biochemical parameters

The biochemical parameters were estimated at the end of trail. The blood was collected from two birds per replicate and the samples were allowed to clot at room temperature followed by centrifugation at 4000 rpm for 15 minutes to separate the serum.

The serum was used for the estimation of serum AST, ALT, ALP and total protein as hepatic markers.

Histo-pathological study

At the end of research trial, three birds from each group were sacrificed and tissue of liver and kidney were collected and store in 10 % neutral buffered formalin. The tissues were processed for histo-pathological examination according to the standard procedure as described by Bancroft and Gamble (2007). The formalin-fixed tissue samples were processed for tissue sectioning, staining and detailed histopathology.

Statistical analysis of the data

The data were analysed through statistical package scientific analysis system (SAS) by using complete randomize design (CRD) as described by Steel and Torrie (1981).

Results

Effect of different methanolic plants extracts on paracetamol induced liver injury in birds with reference to biochemical changes in serum are given in Table 2.

Table 1. Layout of the experiment.

Group	Dose rate	Replicates		
		R1	R2	R3
G-I (Negative Control)	With no treatment	5	5	5
G-II (Positive control)	Induced toxicity with paracetamol suspension	5	5	5
$MP_{(Fg)}E$	1mg/kg	5	5	5
	100mg/kg	5	5	5
	1000mg/kg	5	5	5
$MP_{(Bl)}E$	1mg/kg	5	5	5
	100mg/kg	5	5	5
	1000mg/kg	5	5	5
$MP_{(Ta)}E$	1mg/kg	5	5	5
	100mg/kg	5	5	5
	1000mg/kg	5	5	5

$MP_{(Fg)}E$: Medicinal Plant (Fenugreek) Extract: $MP_{(Bl)}E$: Medicinal Plant (B. lycium) Extract: $MP_{(Ta)}E$: Medicinal Plant (T. arjuna) Extract: Positive Control: Induced Paracetamol.

Mean serum ALT was significantly effected in all treated groups. Positive control group showed highest level of ALT (51.66 IU/L). Treatment with *T. arjuna* at 100 mg/Kg body weight showed significant reduction in serum ALT (18.00 IU/L) as compared to the positive control. AST (15 IU/L) was significantly reduced in *T. arjuna* group at 100 mg/kg of body weight as compared to positive control. All the medicinal plants showed reduced value for AST as compared to positive control group. Varying levels of ALP values were recorded in the in experimental trials. Significant reduction in serum ALP (2350.33 IU/L) value was observed in the *T. arjuna* group treated with 100 mg/kg body weight as compared to positive control. Serum protein was significantly affected. Significantly highest (9.73 IU/L) value for serum protein was observed in negative control as compared to other treated groups.

Table 2. Liver enzymatic activities against the induced paracetamol toxicity in laying birds.

Groups	Dose level	Means±SE			
		ALT (IU/L)	AST (IU/L)	ALP (IU/L)	S. Protein (IU/L)
G-I Negative Control	Without supplementation	19.66^{h}±1.20	20.33^{f}±1.20	2413.33^{f}±27.43	9.73^{a}±0.13
Positive Control	Only Toxicity induced	51.66^{a}±0.33	40.00^{a}±0.57	4385.00^{a}±90.71	4.89^{h}±0.15
MPW$_{(Fg)}$	1 mg	29.00^{c}±0.57	16.33^{h}±0.88	2583.00^{e}±63.84	6.75^{ed}±0.13
	100 mg	30.00d^{e}±0.57	21.00^{d}±0.57	2679.67^{e}±14.07	7.60^{f}±0.20
	1000 mg	31.66d^{e}±0.66	23.00^{ef}±0.57	2664.67^{e}±8.74	8.45^{cb}±0.25
MPW$_{(Bl)}$	1 mg	20.33^{g}±0.33	22.33^{d}±0.88	3098.33^{c}±94.50	5.54^{g}±0.06
	100 mg	20.00^{g}±0.57	18.66^{h}±0.33	2415.33^{f}±23.38	7.25^{ef}±0.36
	1000 mg	45.00^{b}±0.57	33.33^{b}±0.88	3939.33^{b}±51.00	7.94^{cd}±0.24
MPW$_{(Ta)}$	1 mg	23.00^{f}±0.57	25.00^{c}±0.57	3138.00^{dc}±102.56	7.41^{ed}±0.32
	100 mg	18.00^{h}±0.57	15.00^{g}±0.57	2350.33^{f}±13.54	9.03^{ed}±0.33
	1000 mg	32.00^{e}±0.57	23.00^{ed}±0.57	3263.67^{d}±33.41	7.61^{b}±0.31
P-Value		0.0001	0.0001	0.0001	0.0001

Means in column with superscript are significantly different at α=0.05.

The histological appearances of the liver damage occurring after a paracetamol overdose were described in liver biopsies from experimental birds. Positive control group showed varying level of hepatic damage. The histological changes were observed in the positive control group were fatty infiltration along with hemorrhages and leukocytic infiltration (Fig-2 and 3) as compared to negative control group (Fig-1).

Negative control showed well defined histological structures without any signs of hepatic and vascular inflammatory changes. Treatment with 100 mg medicinal plants extracts showed significant regeneration of the hepatocytes as compared to 1 and 1000 mg dose rates. Increase dose level up to 1000 mg exhibit toxic effect on liver histology. Treatment with *Terminalia arjuna* (Fig-4 and 5) and *Berberis lycium* (Fig-6 and 7) showed mild infiltration with regeneration of hepatocytes as compared to positive control group.

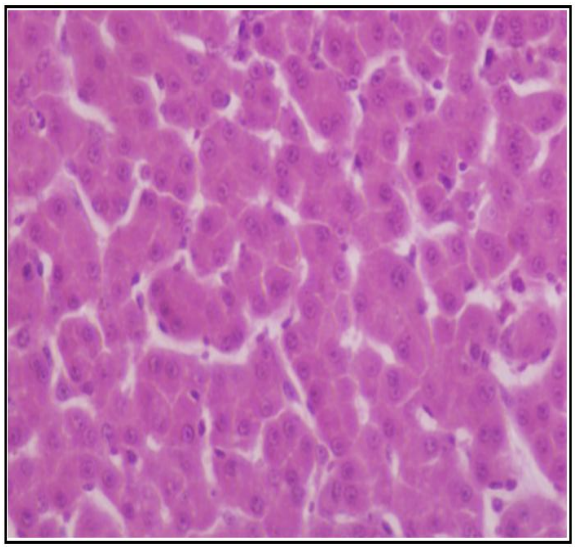

Fig. 1. The liver section show normal structure of hepatocytes and sinusoidal spaces at 40X in negative control.

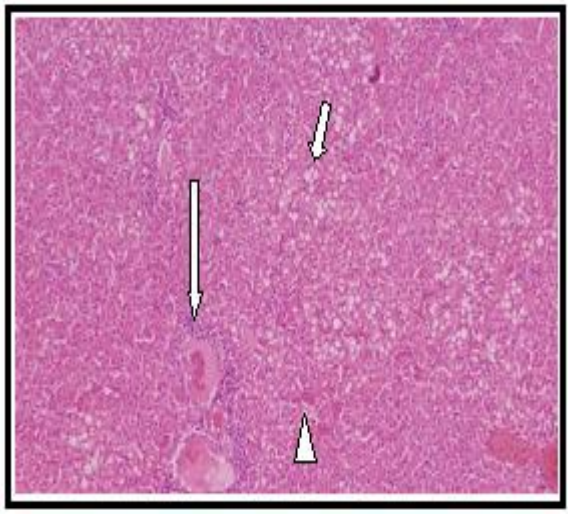

Fig. 2. Section of liver showing fatty infiltration (Short Arrow) along with haemorrhages (Arrow Head) and leukocytic infiltration (long Arrow) 10X. (Positive Control).

Moderate leukocytic infiltration were recorded in hepatocytes in fenugreek treated group (Fig-8 and 9). Normal histology of the glomerulus and renal tubules was found in kidney tissue of bird that received DMSO (Negative Control) vehicle only (Fig-10 and 11).

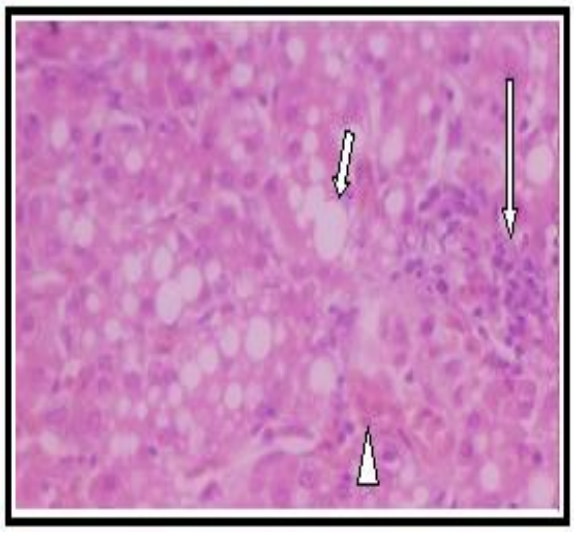

Fig. 3. Section of liver showing fat lobules (Short Arrow) along with haemorrhages (Arrow Head) and leucocytic infiltration (long Arrow) 40X. (Positive Control).

Paracetamol induced (positive control) showed inter tubular haemorrhages with severe leukocytic infiltration and glomerular atrophy (Fig-12 and 13). Birds treated with herbal extracts exhibited mild vascular changes in the kidneys comparable to those observed in administration of paracetamol group, however, no signs of inflammatory changes were observed, except at the lowest and highest dose of herbal extracts (1 and 1000 mg).

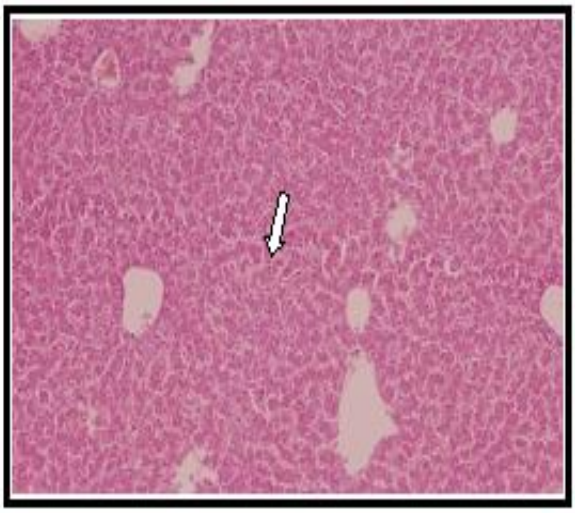

Fig. 4. Liver section showing mild leukocytic infiltration (Arrow) with almost normal architectural pattern at 10x. (BL-100).

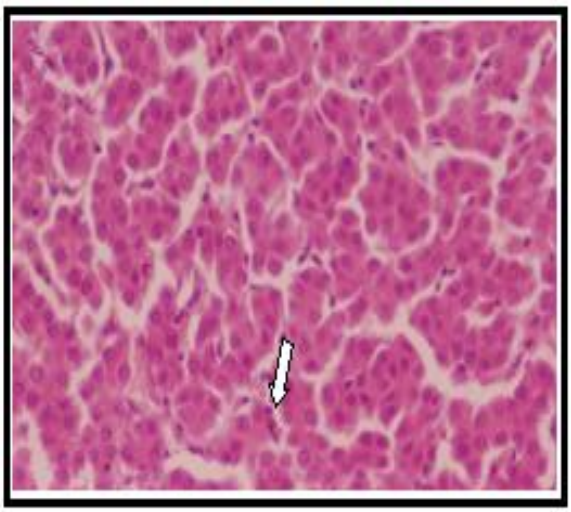

Fig. 5. Liver section show mild leukocytic infiltration (Arrow) at 40X. (BL-100).

Best results were found at 100 mg administration of all herbal extracts. Among the herbal extracts treatment *Terminalia arjuna* (Fig-14 and 15) and *Berberis lycium* (Fig-16 and 17) showed significant tubular regeneration changes with mild leukocytic infiltration. Administration of fenugreek at level of 100 mg showed mild leukocytic infiltration with tubular epithelial changes (Fig. 18 and 19).

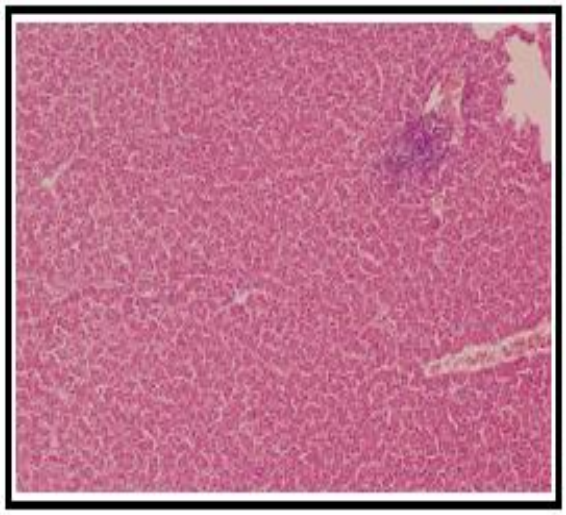

Fig. 6. Liver section showing almost normal structure with mild leukocytic infiltration at 10X in TA-100 mg group.

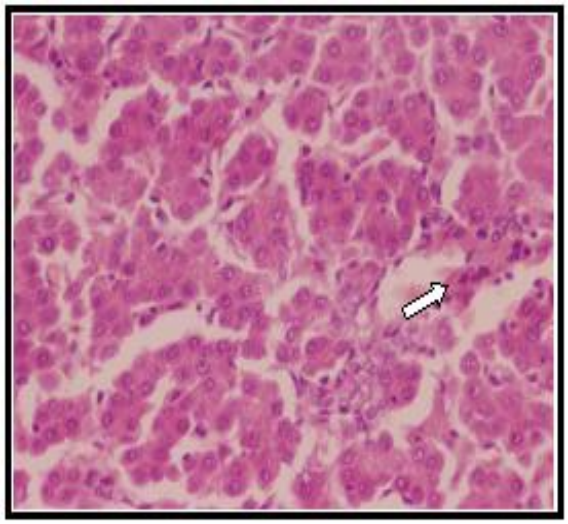

Fig. 7. Liver section showing almost normal structure with mild leukocytic infiltration (Arrow) at 40X in TA-100 mg group.

Discussion

In the present study, the effect of methanolic extract of three plants including fenugreek, *Berbeis lycium* and *Terminillia arjuna* were examined on paracetamol induced liver toxicity in birds. The result revealed significant decreased level of ALT, AST and ALP in the birds treated with *T. arjuna* as compared to positive control group induced with paracetamol toxicity.

The possible way to normalize the serum bio-markers of liver by medicinal plants might be due to the prevention of the leakage of intracellular enzymes by their membrane stabilizing activity and healing of hepatic parenchyma and the regeneration of hepatocytes (Anusha *et al.,* 2011; Adewale *et al.,* 2014).

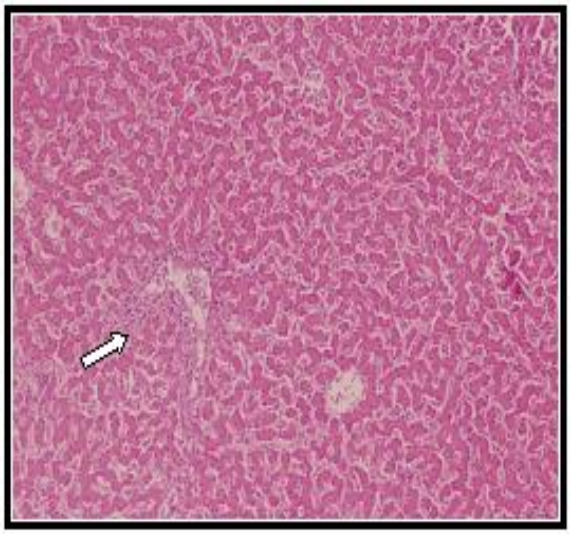

Fig. 8. Liver section show moderate leukocytic infiltration (Arrow) at 10X. (Fg-100).

These results were justified by the findings of a study who reported that *T. arjuna* significantly normalized the serum liver biomarkers (Chaudhari and Mahajan, 2016).

The *T. arjuna* act as antioxidant by preserving the integrative of glutathione and superoxide dismutase to scavenge the free radicals produce during liver injury (Henderson, 2016).

However, in the positive control group the paracetamol oxidative product conjugate with sulphydryal groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity. Normally, ALT (formerly called serum glutamate-pyruvate transaminase SGPT) is found inside liver cells. Whereas, AST is present in the cytosol of hepatocyte and inside the mitochondria (Raj, 2012).

However, when the liver is get inflamed or injured by injurious agents, these biomarkers are released into the bloodstream.

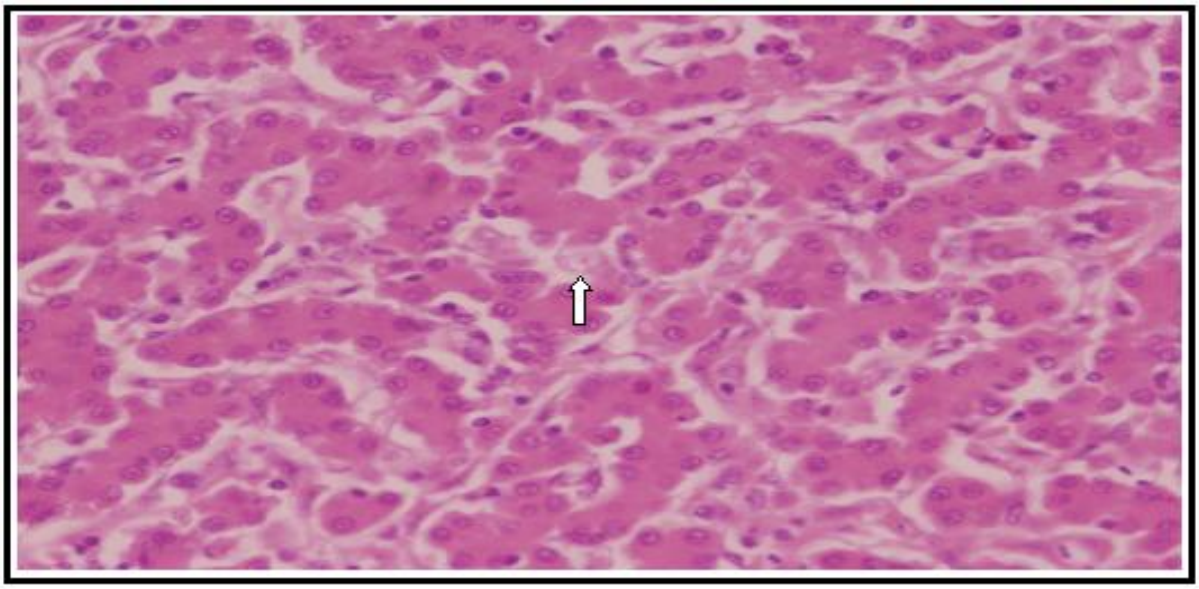

Fig. 9. Liver section show mild hepatocyte swelling (Arrow) and moderate leukocytic infiltration 40X (Fg-100).

Measuring blood levels of biomarkers can give important information about the status of the liver. The present findings revealed that increase level of serum ALT, AST, ALP in positive group compared to the other treated medicinal plants groups in birds indicated that paracetamol cause their release of these enzymes from the hepatocytes.

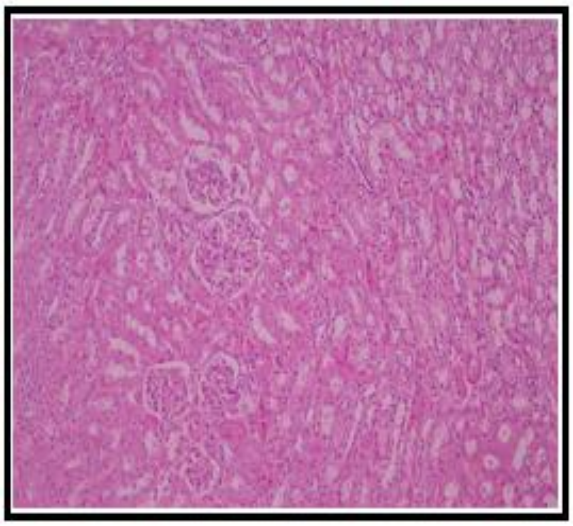

Fig. 10. Kidney section at 10X show normal structural pattern. (Negative control).

Where lowered total protein (TP) of positive control as compare to that of medicinal plants treated groups was might be due to stoppage of protein synthesis and increased execration of proteins with paracetamol. *T. arjuna* significantly protect the liver from paracetamol injury.

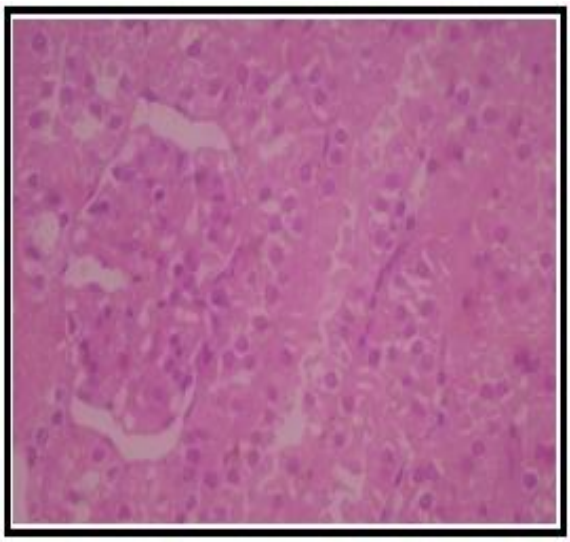

Fig. 11. Kidney section at 40X show normal structural pattern. (Negative control).

Similar findings were also reported that Terminalia spp. had the potential to restore the liver serum biomarkers enzymes in liver injury by toxic vehicle (Abdul-Vahab and Harindran, 2016).

The findings are supported by Haidry and Malik (2014) that *T. arjuna* significantly reversed the effects of cadmium (Toxicity Vehicle) and proved that it has hepatoprotective, and antioxidative potential. In another enzyme kinetic study, it was revealed that extracts of *T. arjuna* showed rapidly reversible non-competitive inhibition of all enzymes in human liver microsomes (Varghese *et al.,* 2015).

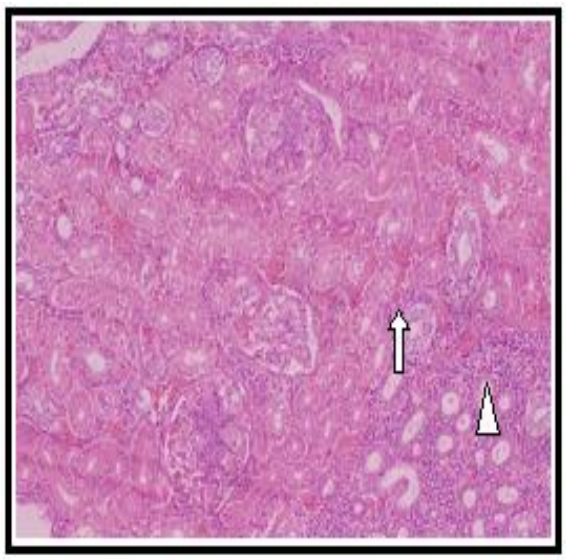

Fig. 12. Kidney section revealed inter tubular haemorrhages (Arrow) with severe leukocytic infiltration (Arrow head) at 10X. (Positive control).

The results of the present findings are also supported by the findings that *Terminalia arjuna* is good potent agent in liver protection against the paracetamol induced toxicity (Moulisha *et al.,* 2011).

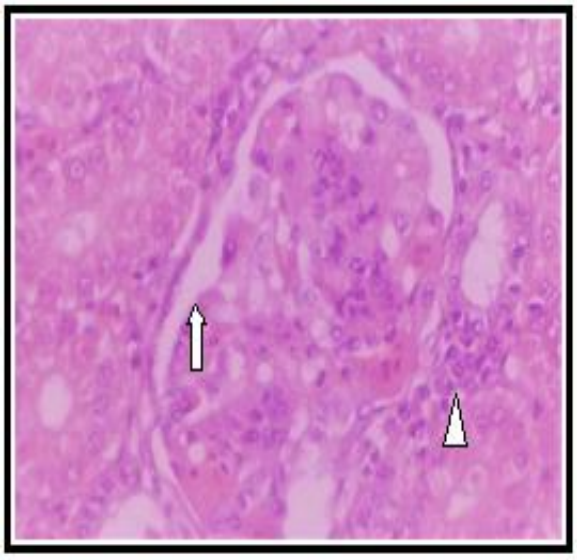

Fig. 13. Kidney section at 40X show glumerulonephritis (Arrow) with leukocytic infiltration (Arrow Head). (Positive control).

Pharmacological study of Sivalokanathan *et al.* (2006) supported the present findings by evaluating the *T. arjuna* hepatoprotective effect against the N-nitrosodiethylamine (DEN) induced liver cancer in male Wistar albino rats. They found that the plant show an antioxidant activity gainst DEN-induced liver cancer. Several other researchers reported the hepato-protective effect of *T. arjuna* against the commonly hepato-toxic compound of CCl_4 in lab animal (Prasenjit *et al.,* 2006).

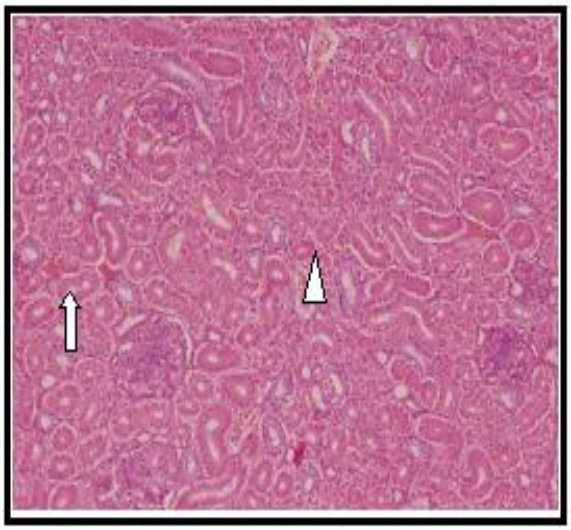

Fig. 14. Kidney section at 10X show tubular hemorrhages (Arrow) with leukocyitic infiltration (Arrow Head). (BL-100).

Histopathology of vital organs

Section of liver in induced paracetamol toxicity showed variable pattern/marked structural alteration. Dose dependent study on medicinal plants showed significant regenerative and protective changes in liver as compare to positive control.

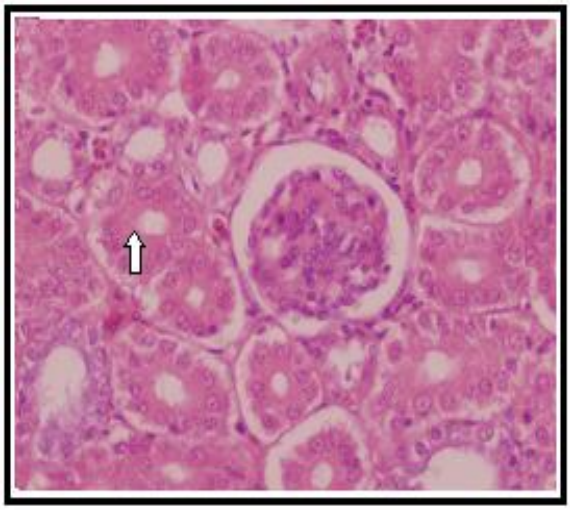

Fig. 15. Kidney section at 40X show tubular regeneration changes (Arrow). (BL-100).

Almost normal architecture, few regenerative hepatocytes, sinusoidal congestion was notice in *T. arjuna* treated group at the rate of 100 mg/kg b.w.

These findings are supported by the study of Abdul-Vahab and Harindran (2016). They reported that ethanolic extract of *T. arjuna* had good effect to protect hepatocytes from degradation. Also found regeneration of the cells and normal architecture of hepatic cell with less fatty changes.

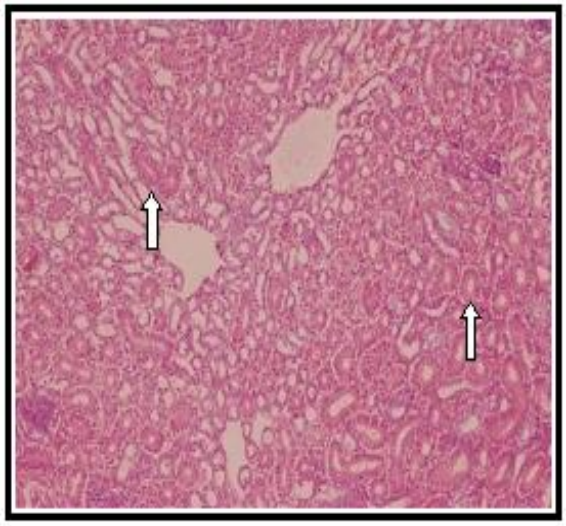

Fig. 16. Kidney section showing normal structure with some regenerative tubular changes (Arrow) at 10X. (TA-100).

The present findings are further supported by the work of Shankreppa*et al.* (2015). They concluded that mice treated with bark extract of *T. arjuna* brought significant level of hepato protection against high fat diet. Liver histopathology showed normal hepatic architecture.

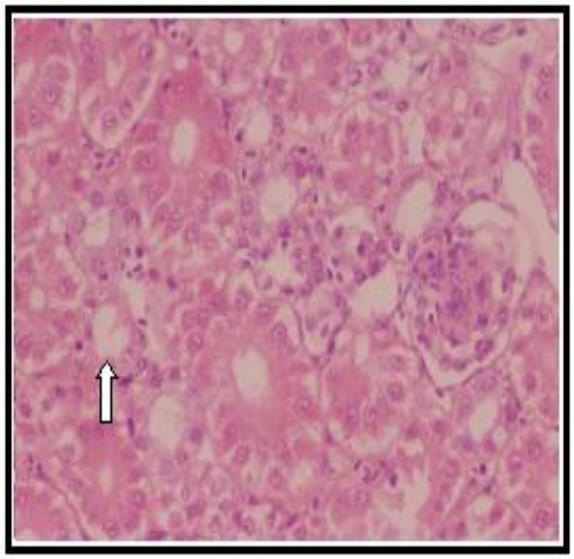

Fig. 17. Kidney section showing normal structure with some regenerative tubular changes (Arrow) at 40X. (TA-100).

Present study results are supported by the work of Hebbani *et al.* (2015) that bioactive compounds in aqueous extract of Terminaliaarjuna showed marked protection of hepatocytes from alcoholic induced toxicity in rats.

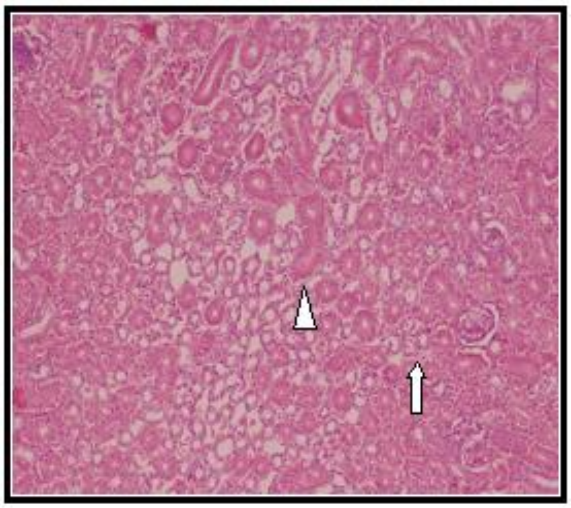

Fig. 18. Kidney section showing mild leukocytic infiltration (Arrow) with tubular epithelial changes (Arrow Head) at 10X. (Fg-100).

The bark extract of *Terminalia arjuna* significantly prevent the architectural changes in liver cell against heavy metal Lead (LA) toxicity in mice (Ghazwan *et al.,* 2013). The present findings are further supported by the work of Shirish (2011) that the aqueous slurry of the bark powder of *Terminalia arjuna* prevent the histopathogical changes in hepatocytes against the CCl_4 induced toxicity. Present results are in agreement to the Hyun-Sun *et al.* (2008) that *Terminalia* spp. extract significantly reduced the hepatic lesions induced by t-BHP in lab animal. Ragavan and Krishnakumari (2006) also noticed that the bark extract of *T. arjuna* showed partially reverse hepatic damage against high fat contact diet in mice. In agreement to Manna *et al.* (2006) TA-extract had significantly prevent the hepatocytes from CCl_4 induced toxicity.

Kidney section showed inters tubular haemorrhages with severe leukocytic infiltration and glomerular atrophy in positive control group. Medicinal plants extracts therapy showed almost normal architecture with mild vascular changes and regenerative processes as compared to positive control group. Li *et*

al. (2017) used Potassium bromate ($KBrO_3$) as renal toxicity vehicle in rats. They found that B. lycium extract had significantly reduced the renal tissue damage/injury against $KBrO_3$ toxicity. Hebbani *et al.* (2015) is in favour of the present findings by evaluating the TA extract as protective agent against alcohol induced toxicity on nephron. In agreement to Ghazwan *et al.* (2013) that TA extract had significantly reduced the renal toxicity. They found that TA had significant protect the Bowmane's space and reduced inflammatory infiltration. These findings were supported by the findings of El-Nekeety *et al.* (2009). However, TA significantly reduced renal injury and obviously, acted as a protective agent against LA-induced toxicity. The results are similar to the present findings. Previous study of Ragavan and Krishnakumari (2006) indicated same findings on kidney histopathology.

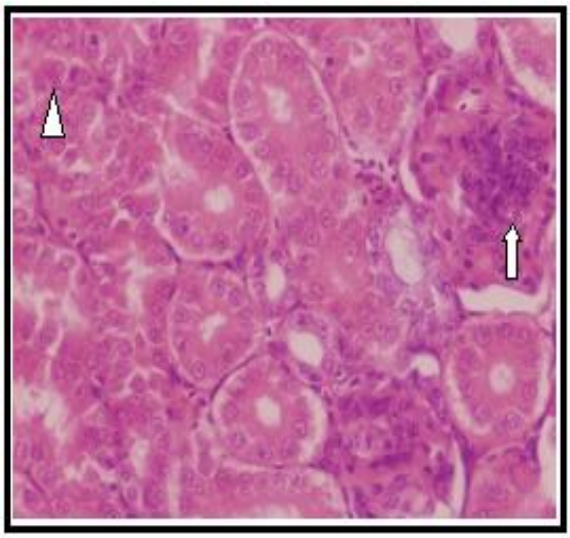

Fig. 19. Kidney section showing mild leukocytic infiltration (Arrow) with tubular epithelial changes (Arrow Head) at 40X. (Fg-100).

At safe level of paracetamol administration in human being and animal model (rat) has no significant effect on renal function, glomerular filtration rate, sodium excretion and prostagland in E2 (Prescott *et al.*, 1989, Trumper*et al.*, 1998). Its fact that paracetamol can cause acute and chronic nephrotoxicity at overdose which is characterized by necrosis and damage to proximal tubule. Henrich (1998) reported that habitual ingestion of paracetamol as analgesic can lead to renal papillary necrosis and chronic nephritis with progressive renal failure. Continues ingestion of low doses (500-1000 mg) can affect the renal system and may produce nephrotoxicity (Blantz, 1996).

Conclusion

Treatment with *T. arjuna*at dose rate of 100 mg/Kg body weight in experimental birds showed significant reduction in serum ALT level (18.00 IU/L) as compared to the positive control. *T. arjuna* group at the rate of 100 mg/kg of body weight significantly decrease the AST (15 IU/L) level as compared to paracetamol induced toxicity group. Significant reduction in serum ALP (2350.33 IU/L) value was observed in the *T. arjuna* group treated with 100 mg/kg body weight as compared to positive control. Serum protein level was comparatively normal in *T. arjuna* treated group as compared to positive control group showed that the treated extract significantly preserved the liver integrity. The histopathological findings revealed that significant regenerative potential was observed in *T. arjuna* and B. lycium groups.

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