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Safety profiling of pioglitazone and telmisartan combination by sub-chronic toxicity study in rat

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ABSTRACT

It has been reported that the major cause of mortality in diabetes is cardiovascular diseases and contribution of hypertension is significant in this context. Pioglitazone, a thiazolidinedione class of therapeutic agent is used to treat type 2 diabetes mellitus. Telmisartan, an angiotensin receptor blocker antihypertensive has been reported to have beneficial effect if co-administered with pioglitazone for the management of diabetes complications. The present research work aims to evaluate the safety/toxicity profile of this combination in rat model. The investigation was carried out after co-administering the drugs to the rats for 28 days at three dose levels of 50, 100 and 150 mg/kg covering low to high dose ranges. Various hematological and biochemical parameters were studied in addition to the histopathology of the major organs in order to evaluate the toxicity profile of the combination. Absence of mortality and histopathological changes as well as unaltered hematological and biochemical parameters was observed. This preliminary investigation concludes that the combination of pioglitazone and telmisartan can primarily be stated as safe in animals, even at the dose level which is several folds higher than the intended human dose. Thus, this combination can be explored in future to develop a rational therapy regimen to treat hypertensive diabetic patients.

1. Introduction

Presently, the worldwide prevalence of diabetes mellitus is becoming alarming and it is considered as one of the major causes of mortality affecting almost all age groups (Varalakshmi et al., 2014). Surprisingly, it is very rare nowadays that type-2 diabetes mellitus patient is free from mild to moderate level of hypertension. Literature review reveals that the major health problems associated with a pathophysiological mechanism for arterial damage in diabetes is hypertension (Parameshappa et al., 2010). The co-existence of hypertension and diabetes affects the same major organs and results in left ventricular hypertrophy, coronary artery disease, decreased renal function, development of diabetic retinopathy and several cerebral diseases (Parameshappa et al., 2010). The major factors for the development of hypertension in type-2 diabetic patients are nephropathy and insulin resistance. Raised insulin levels in diabetic patient increase sodium retention and promote re-absorption of sodium and glucose. Thus retention of sodium and fluid initiates hypervolemia which ultimately causes hypertension (Ranpise et al., 2014). In spite of having best medical therapies to control blood glucose level, diabetic patients suffer from poorer cardiovascular outcomes than nondiabetic individuals. In majority of complex diseases like diabetes, multiple medications are essential to achieve the best therapeutic control as several mediators are involved in their pathogenesis (Siddiq and Khan, 2013). Likewise, the control of blood glucose level and management of other cardiovascular risk factors should be considered as very vital for diabetes care. The American Heart Association recommends that patients with diabetes should be treated as high risk cardiovascular patient and requires more rigorous blood pressure targets for the prevention of cardiovascular events. Hence, concurrent administration of antihypertensive medication is almost essential for proper management of diabetes in an appropriate antidiabetic

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drug regimen. With respect to the management of blood pressures in diabetic patients, it is very difficult to maintain a systolic blood pressure in patients with diabetes. For achieving this target, the patients need to be treated with either angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) (Trikudanathan and Graham, 2008).

It is well established that ARB class of antihypertensives is better tolerated than ACE inhibitors (Schmieder et al., 2011). Amongst ARBs, telmisartan has been reported with fewer drug-related adverse events and provides superior blood pressure control than ACE inhibitors (Schmieder et al., 2011).

Telmisartan was originally developed for the treatment of hypertension only. Modern available research evidence suggests that the ability of telmisartan to partially activate peroxisome proliferator-activated receptor (PPAR)-γ is useful particularly in treating hypertensive patients with diabetes mellitus. By activation of PPAR-γ, it improves insulin sensitivity in hypertensive patients with insulin resistance and has beneficial effect to decrease the glucose levels as well (Suksomboon et al., 2012). It would be thus better if an ARB is used for controlling hypertension in diabetes which has both glucose-lowering and blood pressure controlling potentials. Furthermore, ARBs exert renoprotective effects in addition to their blood pressure lowering effect, as they have direct defensive action on the diabetic kidney (Balakumar et al., 2012).

Pioglitazone, an oral antidiabetic agent in the class of thiazolidinediones, is widely used in type 2 diabetes as insulin sensitizers (Kaga et al., 2015; Temboonkiat et al., 2012). Pioglitazone has several distinct advantages over other oral antidiabetic agents. Pioglitazone increases the level of high density lipoprotein (HDL), decreases the level of triglycerides and most importantly decreases the events of cardiovascular diseases (Inzucchi et al., 2015). Hence, combination therapy of pioglitazone and telmisartan can provide a better therapeutic regimen for controlling the blood glucose levels and other associated cardiovascular complications in diabetic hypertensive patients. The beneficial effects of modulation of PPAR activity by combined administration of pioglitazone with telmisartan in treating multi-component metabolic syndrome are well documented (Haque, 2012).

But, the safety profile of established safe drugs may even get altered when they are administered as a combination of two or more. Complex drug interactions may result in an alteration of the toxicity profile of each ingredient rendering some drugs potentially toxic (Rao and Goldfrank, 1998). These potential drug interactions in terms of toxicological perspective should be studied well and documented properly before proceeding for their co-administration. In respect of pioglitazone and telmisartan, a number of toxicity studies have been carried out individually or in combination with other drugs of their own category (Berthet et al., 2011; Chinnam et al., 2012; Duan et al., 2009; Fouad et al., 2015; Gawly et al., 2009; Khudhair and Numan, 2014; Nandi et al., 2013; Sengupta et al., 2012; Weinberg et al., 2011). However there is no toxicity data available in support of possibility for co-administering these two particular drugs.

Thus, the aim of this study was to investigate the toxicity profile of the pioglitazone and telmisartan combination after simultaneous oral administration in rat to find out whether this combination is safe and free of any additional toxicity when they are co-administered.

2. Materials and methods

2.1. Chemicals

Telmisartan and pioglitazone were obtained from Hangzhou Hyper Chemicals Limited (Zhejiang, China). Formalin, ethanol, Xylene, hematoxylin and eosin were purchased from Fisher Scientific (M) Sdn. Bhd. Malaysia.

2.2. Animal husbandry and maintenance

Wistar albino rats (6 weeks age) of each sex (male and female) were used for this study. Rats were purchased from the commercial animal supplier We Love Pets, Petaling Jaya, Malaysia. The animals were grouped and housed in the wire cages with six animals per cage under controlled laboratory conditions. Animals were kept under good laboratory conditions (temperature 22 ± 2 °C; 48± 8% relative humidity) and were subjected to dark and light cycle (12 h/12 h) for 10 days before commencing the experiment to adjust them with the new environment and to overcome stress possibly incurred during transit. During this maintenance period, they had free access to standard dry pellet diet (Hartz, USA) and water ad libitum (La Boost Health Beverages Mfg Sdn Bhd, Malaysia). Only healthy and non pregnant animals were assigned for the study. The study was approved by the Institutional Animal Ethics Committee of Lincoln University College, Malaysia.

2.3. Toxicity study

2.3.1. Experimental design

The healthy male and female Wistar albino rats having weight between 178 and 195 g were divided into three treatment groups corresponding to pioglitazone (PIO), telmisartan (TLM) and mixture fraction (50:50 on weight basis) of pioglitazone-telmisartan combination (PTC) treatment and one control group. Each treatment group was subdivided into high, intermediate and low dose groups. Each subgroup consists of 6 male and 6 female rats similar to the control group. The drug was administered orally at three dose levels of 50, 100 and 150 mg/kg body weight corresponds to low dose, intermediate dose and high dose, respectively. For the PTC group, the PIO and TLM were administered simultaneously combining the individual dose levels of each drug at 50, 100 and 150 mg/kg body weight. The control group was treated with normal saline. The test substances were administered daily orally in graduate doses to the experimental animals for a period of 28 days. The study was performed to evaluate some basic important toxicity parameters in reference to the Organization for Economic Cooperation and Development (OECD) guidelines (OECD/OCDE 407, 2008).

2.3.2. Clinical observation

All the animals were observed daily for clinical signs of toxicity after dosing. Additionally, the animals were observed thrice in a day (immediately prior to dosing, in the morning and in the afternoon) for morbidity and mortality.

2.3.3. Body weight trends

Body weights of each rat were measured at the initiation of the treatment and once a week throughout the treatment period.

2.3.4. Food and water consumption

Food and water consumption in a group of 12 rats in two cages (six rats in each cage) were measured at the starting of treatment and weekly throughout the treatment period. The amount of food and water were calculated before they were supplied to each cage and their remnants were measured the next day to calculate the differences, which were regarded as daily food and water consumption (g/rat/day).

2.3.5. Hematology

For hematological investigation, rat blood was collected through

retro-orbital route into the tubes containing EDTA after completion of 28 day drug treatment and was analyzed by an automated bio-analyzer (ADVIA 120 Hematology System, Siemens, Germany). The whole blood was used for the hematological investigation without any further processing. Hematological parameters including red blood corpuscles (RBC) count, hemoglobin (HB) concentration, total white blood corpuscles (WBC) count and platelet (PLT) count were examined.

2.3.6. Serum biochemistry

The rat blood for clinical chemistry was collected in tubes devoid of any anticoagulant and allowed to clot at room temperature. The blood samples were then centrifuged at 3000 rpm for 10 min within 30 min after collection, and the serum was separated. The separated serum was used for the investigation of serum biochemistry. Serum levels of total protein (TP), blood urea, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were estimated by an automated bioanalyzer (COBAS INTEGRA 400 Plus, Roche Diagnostics, Switzerland).

2.3.7. Histopathology

After cervical decapitation under anesthesia, the major organs (liver, kidney, lung and heart) were collected for histopathological examinations. After collection, the organs were quickly transferred into the fixative agent 10% formalin.

The organs were processed by an automated processor (Leica Tissue Processor, Model TP1020, Singapore) for facilitating paraffin wax impregnation of the tissues. The organs were subjected to dehydration to remove the aqueous fixative fluids from the tissues using different concentration of alcohol solutions (50%, 70%, 80%, 95% and 100%). Organs were then cleaned to replace the dehydrating fluid with xylene. Finally, the organs were infiltrated with the embedding agent.

After processing, the organs were embedded into the paraffin blocks from which thin sections were cut out. Embedding centre (Leica, Model EG1160, Singapore) comprising wax dispenser, cold plate and heat storage area for molds was used. The tissues were removed from the cassettes, placed into the blocks and molten paraffin was poured over them to form a paraffin block. The embedded tissues in paraffin block were sectioned using a rotary microtome (Leica, Model RM2245, Singapore). The paraffin block was cut at 6 μm. Once the sections were cut, they were floated on a water bath (Leica, Model HI1210, Singapore) at 50 °C to remove wrinkles. Then they were picked up on a glass microscope slide. The glass slides were then placed on a hot plate (Leica, Model HI1220, Singapore) at 56 °C to dry the slides immediately and then placed in a warm oven (Memmert, Model 100–800, Germany) at 56 °C for about 15 min for adhering the sections to the slide.

Standard hematoxylin and eosin (H&E) stains were applied for staining the paraffin sections. The slides were de-paraffinized by applying xylene for 5 min. The slides were then hydrated with different concentration of alcohol solutions (90%, 80%, 70% and 50%) for 2 min each and then immersed in a running tap water for 5 min. The sectioned tissues were then stained with Hematoxylin and counter stained with Eosin. The tissues were washed with tap water, dehydrated by rinsing in increasing concentration of alcohol (70%, 80% and 90%), rinsed in xylene and placed in xylene II. Finally, a drop of distyrene, plasticizer and xylene (DPX) was placed on top of the sections before covering with the cover slip. The slides containing the tissues were examined by a digital microscope (Leica, Model CH-9435, Switzerland).

2.4. Statistical analysis

Statistical analyses for hematological and biochemical examinations were performed by comparing the treatment groups with the control group. An initial analysis to test for homogeneity of variance of the data was performed by parametric Levene's test with the help of SPSS software (version 21). The homogeneity of variance of the data was confirmed as the p-value in the Levene's test was found to be above 0.05 for all the groups (Martin and Bridgmon, 2012). Then the data were subjected to one-way analysis of variance (ANOVA). Data are presented as mean ± standard deviation (SD). Level of significance in all tests was taken as $p < 0.05$.

3. Results and discussion

3.1. Clinical observation

None of the experimental rats were found to show any untoward clinical signs like making noise immediately after treatment, struggling etc. There was no mortality observed in the entire study.

3.2. Body weight, food and water consumption

Change in body weight, food and water intake in the control group and treated groups were recorded and compared. A significant increase in food and water intake for the rats of PIO treated group was observed. Whereas, the food and water intake pattern of the rats of PTC treated group were almost similar to the control group. Additionally, PIO treated rats gained significant weight while TLM and PTC treated rat did not. The combined administration of PIO and TLM prevented the PIO-induced body weight gain. This is possibly due to improved carbohydrate and lipid metabolism by PIO, a reported weight gain effect of a full PPAR agonist (Schupp et al., 2004). On the other hand, partial agonists of PPAR like TLM may have the capacity to retard the weight gain by promoting fat distribution, adipocyte differentiation and energy expenditure (Sugimoto et al., 2006; Benson et al., 2004). Thereby, TLM can attenuate weight gain even in the absence of any effects on absolute

Table 1
Group mean Hematology, PIO [a].

Gr. No.	Dose		Hb (g/dL)	Platelets (x 10^3/mL)	RBC (x 10^6/cmm)	Total WBC (x 10^3/cmm)
I	Control (0 mg/kg)	Mean	13.34	7.33	7.90	9.59
		± SD	0.68	0.55	0.76	1.65
II	Low (50 mg/kg)	Mean	13.25	7.28	7.68	8.83
		± SD	0.45	0.62	0.45	1.49
III	Middle (100 mg/kg)	Mean	13.13	7.25	7.53	9.24
		± SD	0.60	0.58	0.64	1.92
IV	High (150 mg/kg)	Mean	13.05	7.08	7.44	10.01
		± SD	0.59	0.49	0.44	1.81

[a] N = 12 animals in each group; Difference of values in treatment groups were not statistically significant compared to control in one-way ANOVA with post-hoc Tukey HSD Test.

Table 2
Group mean Hematology, TLM [a].

Gr. No.	Dose		Hb (g/dL)	Platelets (x 10^3/mL)	RBC (x 10^6/cmm)	Total WBC (x 10^3/cmm)
I	Control (0 mg/kg)	Mean	13.34	7.33	7.90	9.59
		± SD	0.68	0.55	0.76	1.65
II	Low (50 mg/kg)	Mean	13.21	7.50	7.71	9.18
		± SD	0.66	0.49	0.47	1.79
III	Middle (100 mg/kg)	Mean	13.04	7.23	7.60	10.10
		± SD	0.58	0.64	0.45	1.40
IV	High (150 mg/kg)	Mean	12.89	7.44	7.40	9.29
		± SD	0.61	0.54	0.51	1.51

[a] N = 12 animals in each group; Difference of values in treatment groups were not statistically significant compared to control in one-way ANOVA with post-hoc Tukey HSD Test.

Table 3
Group mean Hematology, PTC [a].

Gr. No.	Dose		Hb (g/dL)	Platelets (x 10^3/mL)	RBC (x 10^6/cmm)	Total WBC (x 10^3/cmm)
I	Control (0 mg/kg)	Mean	13.34	7.33	7.90	9.59
		± SD	0.68	0.55	0.76	1.65
II	Low (50 mg/kg)	Mean	13.14	7.62	7.62	9.17
		± SD	0.39	0.53	0.46	1.71
III	Middle (100 mg/kg)	Mean	13.02	7.34	7.49	10.03
		± SD	0.61	0.54	0.51	1.62
IV	High (150 mg/kg)	Mean	12.79	7.53	7.36	9.64
		± SD	0.56	0.57	0.39	1.51

[a] N = 12 animals in each group; Difference of values in treatment groups were not statistically significant compared to control in one-way ANOVA with post-hoc Tukey HSD Test.

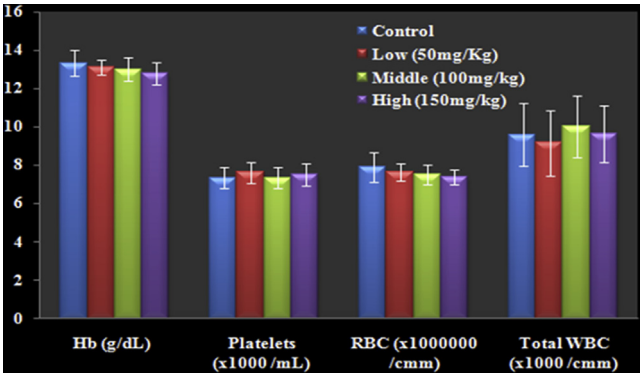

Fig. 1. Group mean hematology, PTC.

food intake. In this study, TLM induced attenuation of weight gain may be related to an intrinsic increase in caloric expenditure as well as due to retarding the unwanted increase in appetite and food absorption caused by PIO.

3.3. Hematology

Both PIO and TLM inhibited the Hb and RBC count in the animals which showed to behave in a dose dependent manner. Their combination (PTC) also was found to reduce the Hb and RBC count in the same way. But no potentiation in toxicity in terms of reducing Hb and RBC level was noted in co-administered group. No alteration in WBC count was observed in any of the drug treated rats (Tables 1–3) (Fig. 1).

The statistical difference of the mean hematological parameters among control and drug treated groups was evaluated by using one way ANOVA and determining critical F value (F_{crit}). The F_{crit} value for 3 degree of freedom for between treatments and 44 degree of freedom for within treatments is 2.82 at 0.05 significance level (48 animals: 3 groups containing 12 rats per dose level and a control group) (NIST/SEMATECH e-Handbook of Statistical Methods, 2013). Since the F ratio obtained in the present experiment is less than the F_{crit} value (Table 4), it can be concluded that the difference was not significant ($p > 0.05$). This result indicates that there was no significant effect on the hematology of the rats when treated with PIO and TLM either alone or in combination. The little inhibition in Hb and RBC count in the drug treated groups may be due to the well known dose-related bone marrow suppression effect of PPAR agonists and hemodilution effect of thiazolidinediones (Aleo et al., 2003).

3.4. Serum biochemistry

Liver and kidney are the most important organs participate in the metabolism and excretion of drugs. Therefore, these two organs were selected to study the effect of the combination on histopathology of these organs. Liver transaminases (SGOT and SGPT) are

Table 4
ANOVA of hematological parameters.

Parameter	No. of subjects	Calculated F value			F_{crit}	P value	Statistically significant?
		PIO	TLM	PTC			
Hemoglobin	48	0.58	1.14	1.96	2.82	>0.05	No
Platelets	48	0.45	0.58	0.83	2.82	>0.05	No
RBC	48	1.40	1.67	2.13	2.82	>0.05	No
Total WBC	48	1.01	0.80	0.56	2.82	>0.05	No

Table 5
Group mean serum biochemistry, PIO [a].

Gr. No.	Dose		Total protein (g/dL)	Urea (mg/dL)	SGOT (IU/L)	SGPT (IU/L)
I	Control (0 mg/kg)	Mean	70.08	6.15	93.08	51.75
		± SD	3.99	0.50	7.04	6.03
II	Low (50 mg/kg)	Mean	69.50	6.08	92.08	51.00
		± SD	2.94	0.43	5.84	5.49
III	Middle (100 mg/kg)	Mean	68.92	5.95	90.33	50.58
		± SD	3.15	0.44	7.61	5.96
IV	High (150 mg/kg)	Mean	68.67	5.83	88.75	47.75
		± SD	3.23	0.37	6.58	4.69

[a] N = 12 animals in each group; Difference of values in treatment groups were not statistically significant compared to control in one-way ANOVA with post-hoc Tukey HSD Test.

Table 6
Group mean serum biochemistry, TLM [a].

Gr. No.	Dose		Total protein (g/dL)	Urea (mg/dL)	SGOT (IU/L)	SGPT (IU/L)
I	Control (0 mg/kg)	Mean	70.08	6.15	93.08	51.75
		± SD	3.99	0.50	7.04	6.03
II	Low (50 mg/kg)	Mean	70.42	6.23	93.83	52.08
		± SD	3.40	0.53	7.58	6.19
III	Middle (100 mg/kg)	Mean	70.75	6.32	95.00	53.08
		± SD	3.11	0.46	7.05	7.30
IV	High (150 mg/kg)	Mean	69.92	6.43	98.33	56.00
		± SD	2.68	0.57	6.08	5.61

[a] N = 12 animals in each group; Difference of values in treatment groups were not statistically significant compared to control in one-way ANOVA with post-hoc Tukey HSD Test.

Table 7
Group mean serum biochemistry, PTC [a].

Gr. No.	Dose		Total protein (g/dL)	Urea (mg/dL)	SGOT (IU/L)	SGPT (IU/L)
I	Control (0 mg/kg)	Mean	70.08	6.15	93.08	51.75
		± SD	3.99	0.50	7.04	6.03
II	Low (50 mg/kg)	Mean	69.50	6.09	92.42	50.92
		± SD	3.18	0.43	6.56	6.32
III	Middle (100 mg/kg)	Mean	69.08	6.24	94.25	51.67
		± SD	2.94	0.47	6.09	6.57
IV	High (150 mg/kg)	Mean	68.83	6.13	94.17	50.83
		± SD	2.66	0.52	6.15	6.35

[a] N = 12 animals in each group; Difference of values in treatment groups were not statistically significant compared to control in one-way ANOVA with post-hoc Tukey HSD Test.

useful biomarkers of liver injury with some degree of intact liver function (Johnston, 1999; McClatchey and Kenneth, 2002; Mengel and Schwiebert, 2005). Treatment with PIO was found to decrease the Total protein (TP), urea, SGPT and SGOT level, whereas TLM treated rats exhibited increased serum concentration of urea, SGPT, and SGOT. Interestingly, when they were co-administered, the inhibitory effect of PIO on serum level of urea, SGPT, and SGOT were nullified and the serum concentration of these parameters were shown to be almost similar to the control group (Tables 5–7) (Fig. 2). This alteration in biochemical parameters confirms the beneficial effect of this combined treatment on the liver and kidney functions.

To check the statistical differences of biochemical parameters between the groups, one way ANOVA was performed and the results are presented in Table 8. The calculated F ratio was less than the F_{crit} value (2.82) for all the parameters (NIST/SEMATECH e-Handbook of Statistical Methods, 2013). Therefore, the alterations in biochemical parameters observed in the study were not statistically significant and thus not clinically relevant. Moreover, the changes observed in different biochemical parameters were beneficial when PIO and TLM were given in combination.

3.5. Histopathology

There were no significant changes in weight of liver, kidney, lung and heart observed as a result of the treatments of PIO and TLM singly and when co-administered. Histopathological examinations

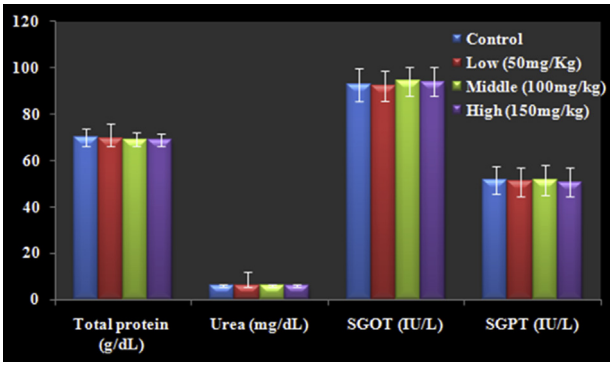

Fig. 2. Group mean blood chemistry, PTC.

Table 8
ANOVA of biochemical parameters.

Parameter	No. of subjects	Calculated F value			F_{crit}	P value	Statistically significant?
		PIO	TLM	PTC			
Total protein	48	0.43	0.15	0.34	2.82	>0.05	No
Urea	48	1.23	0.64	0.22	2.82	>0.05	No
SGOT	48	0.95	1.33	0.23	2.82	>0.05	No
SGPT	48	1.18	1.12	0.07	2.82	>0.05	No

of liver, kidney, lung and heart at the highest dose level did not show any significant changes. Representative picture of histopathological examinations of liver and kidney in control as well as treated groups for highest dose level is shown in Fig. 3. Both the control and drug treated rats showed the normal histological picture of liver i.e; normal hepatocytes with the presence of nuclei, cytoplasm and well distinct hepatic laminae. The section of kidney of control and drug treated rats also exhibited a normal histological picture i.e; normal glomerulus, nuclei and cytoplasm. The results of the histopathological examination suggesting no detrimental changes or morphological disturbances resulted from the administration of the drugs for 28 days.

4. Conclusion

This study was performed to assess the toxicity profile of the combination treatment of pioglitazone and telmisartan after administering them orally in Wistar albino rats. It has been observed that, unwanted increase of body weight due to pioglitazone treatment can be nullified by telmisartan. The combination treatment resulted in a small decrease in hemoglobin and RBC level though within the normal range. Co-administration of telmisartan with pioglitazone showed to have beneficial effect in terms of alteration of the biochemical parameters in rats. There were no harmful changes found in the level of SGPT and SGOT in serum of PTC treated group when compared with the control rats which confirms lacking in hepatotoxic potential of the combination. In spite of the little changes, the blood chemistry results for the combination treated rats were mostly similar to the individually treated (PIO and TLM) and control rats. PIO-TLM combination treatment showed no detrimental effects on kidney as no significant differences were observed on urea and total protein concentrations with respect to the control.

Histopathological analysis also supported the safety data inferred from the physiological, biochemical and hematological studies after PIO-TLM treatment. Histopathological examination of liver, kidney, lung and heart of the animals with high dose group revealed no abnormality attributable to the treatment. Both the control and drug treated rats showed the normal histological picture.

Until now, there have been no published data to support the safety of the combination of pioglitazone and telmisartan in terms of their toxicity profile in any animal models. This study established that combination of pioglitazone and telmisartan is non-toxic in rats following oral administration until 28 days. Based on this short term toxicity study, the combination can primarily be stated as well tolerated, with a favorable safety profile as no evidence of adverse hematological, biochemical and histopathological effects in rats were observed. However, this study might be further explored in different experimental animal models covering a wider range of parameters for the evaluation of long term toxicity profile before concluding that combination of pioglitazone and telmisartan is safe for the treatment of hypertensive diabetic patients.

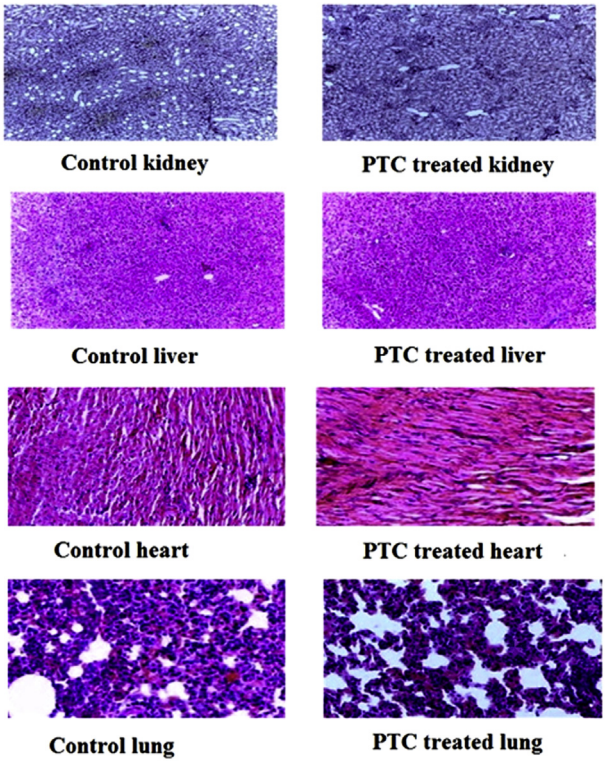

Fig. 3. Histopathology of control and PTC treated organs.

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Transparency document

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References

Aleo, M.D., Lundeen, G.R., Blackwell, Smith WM., Coleman, G.I., Stadnicki, S.W., Kluwe, W.M., 2003. Mechanism and implications of brown adipose tissue proliferation in rats and monkeys treated with the thiazolidinedione darglitazone, a potent peroxisome proliferator-activated receptor- γ agonist. J. Pharmacol. Exp. Ther. 305, 1173–1182.

Balakumar, P., Bishnoi, H.K., Mahadevan, N., 2012. Telmisartan in the management of diabetic nephropathy: a contemporary view. Curr. Diabetes Rev. 8 (3), 183–190.

Benson, S.C., Pershadsingh, H.A., Ho, C.I., Chittiboyina, A., Desai, P., Pravenec, M., Qi, N., Wang, J., Avery, M.A., Kurtz, T.W., 2004. Identification of Telmisartan as a

unique angiotensin II receptor antagonist with selective PPAR- γ modulating activity. Hypertension 43, 993–1002.
Berthet, S., Olivier, P., Montastruc, J., Maryse, L., 2011. Drug safety of rosiglitazone and pioglitazone in France: a study using the French PharmacoVigilance database. BMC Clin. Pharmacol. 11 (5), 1–6.
Chinnam, P., Mohsin, M., Shafee, L.M., 2012. Evaluation of acute toxicity of pioglitazone in mice. Toxicol. Int. 19 (3), 250–254.
Duan, S.B., Wang, Y.H., Liu, F.Y., Xu, X.Q., Wang, P., Zou, Q., Peng, Y.M., 2009. The protective role of telmisartan against nephrotoxicity induced by x-ray contrast media in rat model. Acta Radiol. 50 (7), 754–759.
Fouad, A., Albuali, W., Al-Mulhim, A., Jresat, L., 2015. Protective effect of Telmisartan treatment against arsenic-induced testicular toxicity in rats. Z. für Naturforsch. C 70, 7–8.
Gawly, H.W., Tawfik, M.K., Rashwan, M.E., Baruzaig, A.S., 2009. The effect of pioglitazone on the liver of streptozotocin-induced diabetic albino Wistar rats. Eur. Rev. Med. Pharmacol. Sci. 13 (6), 443–451.
Haque, S.F., 2012. Peroxisome proliferators Activated Receptor -γ Agonists: pioglitazone and telmisartan; potential therapeutic approaches to the metabolic syndrome. J. Diabetology 1 (2), 1–9.
Inzucchi, S.E., Bergenstal, R.M., Buse, J.B., Diamant, M., Ferrannini, E., Nauck, M., Peters, A.L., Tsapas, A., Wender, R., Matthews, D.R., 2015. Management of hyperglycemia in type 2 diabetes: a patient centered approach. Diabetes Care 38140–38149.
Johnston, D.E., 1999. Special considerations in interpreting liver function tests. Am. Fam. Physician 59 (8), 2223–2230.
Kaga, K., Araki, K., Kanesaka, M., Sugiura, M., Ho, K., Masuda, H., Kojima, S., Kaga, Y.N., 2015. Does pioglitazone increase the Risk of bladder Cancer in japanese diabetic patients? Med. Surg. Urology 4 (1), 1–3.
Khudhair, A., Numan, I., 2014. Possible cardio-protective effects of Telmisartan againist 5-fluorouracilinduced cadiotoxicity in Wister rats. Int. J. Pharm. Pharm. Sci. 6 (6), 397–400.
Martin, W.E., Bridgmon, K.D., 2012. Quantitative and Statistical Research Methods: from Hypothesis to Results. ISBN: 978-0-470-63182-9.
McClatchey, Kenneth, D., 2002. Clinical Laboratory Medicine. Lippincott Williams & Wilkins, p. 288. ISBN 978-0-683-30751-1.
Mengel, M.B., Schwiebert, L.P., 2005. Family Medicine: Ambulatory Care & Prevention. McGraw-Hill Professional, p. 268. ISBN 978-0-07-142322-9.
Nandi, U., Karmakar, S., Das, A.K., Ghosh, B., Padman, A., Chatterjee, N., Pal, T.K., 2013. Pharmacokinetics, pharmacodynamics and toxicity of a combination of metoprolol succinate and telmisartan in Wistar albino rats: safety profiling. Regul. Toxicol. Pharmacol. 65 (1), 68–78.
NIST/SEMATECH, 2013. e-Handbook of Statistical Methods, 2013. http://www.itl.nist.gov/div898/handbook/.
OECD, 2008. OECD Guidelines for the Testing of Chemicals. Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD/OCDE, p. 407.
Parameshappa, B., Venkat Rao, N., Shivaraj Gouda, T., Sen, S., Chakraborty, R., Md Basha, A., Ashok Reddy, A., Shanta Kumar, S.M., 2010. A study on drug-drug interaction between anti-hypertensive drug (propranolol) and anti-diabetic drug (glipizide). Ann. Biol. Res. 1 (3), 35–40.
Ranpise, N., Jamkar, P., Langote, H., 2014. Formulation and development of fixed dose combination of antihypertensive and antidiabetic agent for treatment of co-existent type two diabetes mellitus and hypertension. Indian J. Pharm. Educ. Res. 48, 109–117.
Rao, R.B., Goldfrank, L.R., 1998. Fixed-dose combination therapy: panacea or poison? Intensive Care Med. 24 (4), 283–285.
Schmieder, R.E., Bakris, G., Matthew, R., 2011. Telmisartan in incipient and overt diabetic renal disease. J. Nephrol. 24 (3), 263–273.
Schupp, M., Janke, J., Clasen, R., Unger, T., Kintscher, U., 2004. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor- γ. Circulation 109, 2054–2057.
Sengupta, P., Nandi, U., Pal, T.K., 2012. Development of safety profile evaluating pharmacokinetics, pharmacodynamics and toxicity of a combination of pioglitazone and olmesartan medoxomil in Wistar albino rats. Regul. Toxicol. Pharmacol. 62 (1), 7–15.
Siddiq, A., Khan, R.A., 2013. Effect on Hepatic Parameters and Hepatocytes following administration of antihypertensive, hypolipidemic and hypoglycemic drugs in combination (Part II). J. Appl. Pharm. Sci. 3 (2), 105–108.
Sugimoto, K., Qi, N.R., Kazdova, L., Pravenec, M., Ogihara, T., Kurtz, T.W., 2006. Telmisartan but not valsartan increases caloric expenditure and protects against weight gainand hepatic steatosis. Hypertension 47, 1003–1009.
Suksomboon, N., Poolsup, N., Prasit, T., 2012. Systematic review of the effect of telmisartan on insulin sensitivity in hypertensive patients with insulin resistance or diabetes. Clin. Pharmacol. Ther. 37 (3), 319–327.
Temboonkiat, S., Satyapan, N., Benjasuratwong, Y., Patarakitvanit, S., Tunkanitlert, J., Vudhironarit, T., Klangkalya, B., 2012. Clinical efficacy of pioglitazone: generic vs. original product. J. Med. Assoc. Thail. 95 (5), 58–62.
Trikudanathan, S., Graham, T., 2008. Cardioprotective strategies for patients with type 2 diabetes case study and commentary. J. Clin. Outcomes Manag. 15 (3), 133–146.
Varalakshmi, K., Subramanian, G., Sunitha Reddy, M., 2014. Fixed dose combination products of anti-hypertensive's and anti-diabetic's - indian regulatory perspective against USA and EU. Int. J. Pharm. Pharm. Sci. 6 (3), 13–16.
Weinberg, G.L., Ripper, R., Bern, S., Lin, B., Edelman, L., Digregorio, G., Piano, M., Feinstein, D.L., 2011. Pioglitazone attenuates acute cocaine toxicity in rat isolated heart: potential protection by metabolic modulation. Anesthesiology 114 (6), 1389–1395.