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Antioxidant and Subchronic Toxicity Study of *Myrmecodia Platytyrae* (MyP) Water Extract

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ABSTRACT

In this study, *Myrmecodia platytyrae* (*MyP*) water extract was investigated to explain the antioxidant property and its safety. Water extract of *MyP* was prepared and tested for ORAC for each batch of preparations. The *MyP* water extract was administered daily onto three groups of experimental animals which were Low Dose (LD), Medium Dose (MD) and High Dose (HD) groups for a period of consecutive 28 days according to the OECD GLP guideline (OECD TG 407). The ORAC results showed that *MyP* water extract has an antioxidant activity for each batch. The subchronic toxicity test showed that *MyP* water extract product has no observable sub-chronic toxic effect on *Sprague Dawley* rats. The body weight of rats increased along with proportional food and water intake. In the same way, all hematological, biochemical parameters as well as histopathological observation do not show any abnormal finding. Gross observations, feed and water consumption, urine strip test and animals' weight during necropsy did not show any difference compared to the control group. In conclusion, *MyP* water extract is suggested to have a broad safety margin in experimental animals.

Key words: Myrmecodia platytyrae, antioxidant, safety and sub-chronic toxicity

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INTRODUCTION

Myrmecodia platytyrea was first described by Odoardo Beccari in 1884 (Huxley & Jebb, 1991) which was named as *M. antoinii,* Beccari 1884. However, it was moved to a subspecies of *M. platytyrea* by Huxley & Jebb in 1993. This plant can be found in South East Asian covering ASEAN countries, Papua New Guinea and Australia (Hamsar & Mizaton, 2012; Soeksmanto *et al*., 2010). Generally, *Myrmecodia*'s tuber contains the alkaloid, phenolics and terpenoids compounds (Prachayasittikul *et al*., 2008). Sanjaya *et al*., (2014) stated that *Myrmecodia* tuber has proven to be rich in bioactive constituents such as flavonoids, tocopherols, tannins and a variety of essential minerals. Moreover, Engida *et al*., (2013) reported that dry extract of *M. pendans* is a source of flavonoids such as kaempferol (13.767 mg/g), luteolin (0.005 mg/g), rutin (0.003 mg/g), quercetin (0.030 mg/g) and apigenin (4.700 mg/g). The previous study from Abdul Wahab *et al*., (2011) reported that stigmasterol is the major non-polar component from *M. platytyrea*.

Myrmecodia is traditionally used as herbal as a medicine plant for centuries to treat certain diseases (Subroto & Saputro, 2006). Treatments for malaria and leucorrhea are also use some of *Myrmecodia* family plants in Vietnam (Vo, 1996; Do, 2001). The people in Papua Island using this plant as decoction to treat many diseases such as ulcer, haemorrhoid,

nosebleed, backhead, allergy, uric acid disorder, stroke, coronary heart problem, tumor, cancer, hepatitis, rheumatism and, diarrhea (Prommee, 1988; Nguyen *et al*., 2004; Subroto & Saputro, 2006; Mizaton *et al*., 2010).

In this study, preparation of water extract of *M. platytyrea* was performed. Each batch of preparation was tested its antioxidant activity test using ORAC. Besides that, the extract also subjected to sub-chronic oral toxicity test. The OECD TG 407 test guideline was referred to conduct the study. The purpose of this study was to observe the potential sub-chronic toxicity effect of *MyP* water extract on *Sprague Dawley* rats, given through oral administration daily for 28 days.

METHOD

Preparation of 10 % *MyP* water Extract

Ten percent (10 %) of *MyP* water extract was prepared by adding 100 g *MyP* powder into a beaker containing 1000 ml distilled water and boiled at 100 ºC for 15 min. The solution was filtered and concentrated by using rotary evaporator at 50 ºC and freeze-dried. The 10 % extract powder of *MyP* water extract was stored at -80 ºC until used.

Preparation of Standardized *MyP* Water Extract

Oxygen radical absorbance capacity (ORAC) was performed on each batch of *MyP* water extract to determine its antioxidant properties. Each batch production had to maintain their properties. In the next *in vivo* study, the standardized *MyP* water extract was used for toxicity study. The following equation used to calculate extraction yield for standardized extract according to Pin *et al*., (2010):

Y = (Wd / Ve) x Rss x 100 Where:

Wd is the weight of dried extract (g),

Ve is the volume of aqueous filtered (mL) Rss is the ratio of solvent to solid (mL g^{-1}).

In Vivo Subchronic Toxicity Test

Sub-chronic toxicity experiment consisted of four groups. Each of experimental group consisted of male and female representative. The rat species was *Sprague dawley*. The *MyP* water extract was administered daily to three groups of experimental animals which were Low Dose (LD), Medium Dose (MD) and High Dose (HD) groups for a period of consecutive 28 days. Every group consisted of five rats for the male group and a similar number for the female group. The animals were given *MyP* water extract depending on their individual body weight (BW) and their experimental group which consists of LD (100 mg/kg BW), MD (200 mg/kg BW) and HD (400 mg/kg BW) respectively while the normal control group was given reverse osmosis (RO) water. During the period of administration, the animals were observed closely for onset and progression of toxic effect. On week four, the rats were housed individually in metabolic cages for two days to collect urine samples. Urine collection also was done to trace any toxicity effect in the urine. Besides that, volume of urine secreted in 24 hours period was determined. At the end of the observation period, necropsy was performed and blood samples were withdrawn from each animal for hematology and clinical biochemistry analysis.

STATISTICAL ANALYSIS

Statistical analysis was performed appropriately. Calculation for each variable measured including the mean value and standard deviation (SD) were performed. The result with $p<0.05$ was considered as statistically significant ($\alpha= 0.05$).

Results and Discussion

Antioxidant study on *MyP* water extract

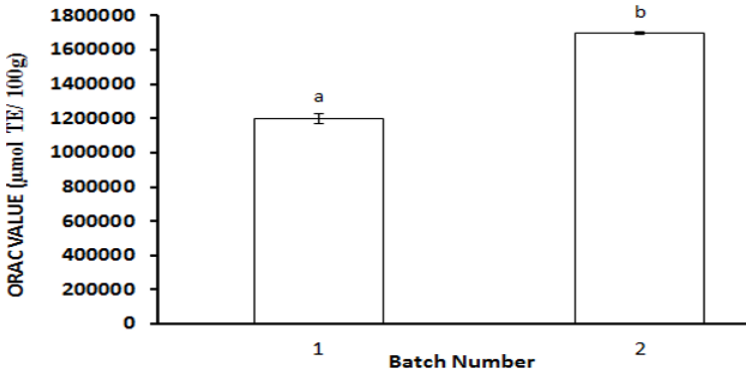

Figure 1: ORAC value for each batch of MyP water extract preparation.

Each batch of *MyP* water extract showed very high of antioxidant status. The first batch has 1, 200, 000 µmol TE/ 100g ORAC value. However, the second batch displayed higher reading of ORAC value which was 1, 696, 000 µmol TE/ 100g. This result is equivalent to Engida *et al*., (2013) which stated that *Myrmecodia* plant is a good source of antioxidant.

Sub-chronic toxicity study

Mortality

There was no treatment-related mortality of animals at any dose level tested and all animals survived until necropsy.

Body Weight and Body Weight Changes

Table 1: Bodyweight in control and rats treated with MyP water extract (gram/week)

Gender/Groups	**Week/ Bodyweight**			
Female	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	257.56 ± 21.23	278.92 ± 27.11	291.23 ± 16.28	311.67 ± 12.83
High Dose	261.44 ± 8.54	286.89 ± 11.01	301.76 ± 23.72	317.27 ± 28.83
Medium Dose	255.73 ± 17.84	277.50 ± 10.73	305.63 ± 21.83	327.64 ± 12.28
Low Dose	269.11 ± 28.11	271.96 ± 9.72	317.45 ± 21.38	330.56 ± 8.22
Male	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	258.92 ± 12.28	288.13 ± 10.27	315.52 ± 13.18	346.27 ± 21.89
High Dose	273.29 ± 17.63	300.62 ± 8.12	332.12 ± 21.28	352.17 ± 18.82
Medium Dose	251.23 ± 21.18	291. 21 ± 9.13	317.32 ± 11.78	338.11 ± 26.01
Low Dose	267.53 ± 18.11	289.12 ± 11.21	315. 22 ± 9.71	349.11 ± 17.37

Values are expressed as a mean ± standard deviation, n = 5.

No significant difference as compared to control group, ($p<0.05$).

Table 2: Body weight changes in control and rats treated with MyP water extract (gram/week)

Gender/Groups	**Week/ Bodyweight changes**			
Female	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	21.36 ± 5.22	12.31± 8.25	20.44 ± 5.75	20.51 ± 7.25
High Dose	25.45 ± 6.29	14.87 ± 7.36	15.51 ± 4.25	11.72 ± 8.85
Medium Dose	21.77 ± 6.03	28.13 ± 2.67	22.01 ± 7.96	15.05 ± 5.20
Low Dose	12.85 ± 7.36	35.49 ± 7.44	13.11 ± 8.96	12.31 ± 7.95
Male	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	29.21 ±9.63	27.39 ± 8.34	30.75 ± 4.01	15.91± 9.62
High Dose	27.33 ± 5.64	31.5 ± 8.83	20.05 ± 6.83	26.82 ± 7.88
Medium Dose	39.98 ± 5.63	26.11 ± 7.93	20.79 ± 7.32	34.58 ± 8.03
Low Dose	21.59 ± 7.24	26.1 ± 6.78	33.89 ± 6.53	33.76 ± 6.36

Values are expressed as a mean ± standard deviation, *n = 5*.

No significant difference as compared to control group, ($p<0.05$).

The treatment of the extract did not affect the body weight of treated rats as shown in Table 1 and Table 2. Animals of both sexes in control as well as in *MyP* water extract treated groups showed an identical pattern of body weight changes. There were no significant differences in weight gain observed at week 4 of treatment in the extract treated group as compared to control group. Furthermore, body weight gain of treated rats with different concentration showed no significant changes. Generally, the excessive decrease in the body weight of animal can indicate an adverse effect caused by drugs and chemicals (Teo *et al*., 2002). Since the body weight of *MyP* extract treated groups did not tremendously drop, the extract probably safe and not cause acute toxic.

Food and Water Consumption

Table 3: Food consumption by control and rats treated with *MyP* water extract (gram/week)

Gender/Groups	**Week/ Food Consumption**			
Female	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	89.26 ± 5.82	90. 34 ± 7.27	91.48 ± 4.29	93.72 ± 9.25
High Dose	92.36 ± 8.28	93.76 ± 6.10	89.27 ± 7.30	96.29 ± 8.23
Medium Dose	99.73 ± 8.20	90.55 ± 9.29	88.21± 7.20	87.99 ± 8.92
Low Dose	79.3 ± 7.35	80.28 ± 7.82	87.22 ± 8.99	90.22 ± 9.52
Male	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	124.77 ± 10.28	118.82 ± 8.72	138.12 ± 9.54	139.93 ± 8.77
High Dose	137.82 ± 9.02	123.39 ± 8.40	127.53 ± 10.48	132.12 ± 5.53
Medium Dose	126.28 ± 9.31	126.19 ± 10.24	126.02 ±12.97	139.21 ± 7.63
Low Dose	110.39 ± 11.42	113.83 ± 10.07	132.18 ± 13.29	137.32 ± 9.04

Values are expressed as a mean ± standard deviation, $n = 5$.

No significant difference as compared to control group, ($p<0.05$).

Table 4: Water consumption by control and rats treated with MyP water extract (ml/week)

Gender/Groups	**Week/ Food Consumption**			
Female	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	115.65 ± 6.74	117.46 ± 7.85	137.48 ± 7.97	129.97 ± 7.56
High Dose	127.75 ± 8.56	125.96 ± 8.76	133.34 ± 6.78	143.68 ± 7.85
Medium Dose	117.97 ± 8.98	122.43 ± 8.89	133.82 ± 6.24	135.86 ± 8.85
Low Dose	105.65 ± 4.76	132.57 ± 9.49	143.89 ± 7.67	139.64 ± 9.67
Male	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	132.46 ±10.61	147.46 ±13.21	149.27 ±13.72	154.10 ±9.63
High Dose	138.58 ±24.53	159.35 ±14.28	157.12 ±12.83	169.46 ±8.33
Medium Dose	142.75 ±19.35	156.79 ±12.73	155.07 ±13.84	159.46 ±10.63
Low Dose	147.64 ±17.75	159.16 ±13.28	159.58 ±10.38	153.28 ±13.21

Values are expressed as a mean ± standard deviation, $n = 5$.

No significant difference as compared to control group, ($p<0.05$).

Additionally, weekly collected data on body weight changes, food consumption and water consumption of the treated animals as depicted in Table 3 and 4 did not show any significant difference compared to control animals. All the three parameters measured displayed increase of food, water intake and body weight changes. Food intake is a complex process, partially regulated by gastrointestinal hormones, which mediates sensing and signalling related to food intake control in the central nervous system (Al Shukor et al., 2016).

Nature, Severity and Duration of Clinical Observations

Table 5: Clinical observation in female control and rats treated with *MyP* water extract

Observation	**Control group**	**High Dose**	**Medium Dose**	**Low Dose**
Digestion	No observation	No observation	No observation	No observation
Body weight	Normal	Normal	Normal	Normal
Temperature	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal
Rate of respiration	Normal	Normal	Normal	Normal
Change in skin	No observation	No observation	No observation	No observation

Drowsiness	No observation	No observation	No observation	No observation
Sedation	No observation	No observation	No observation	No observation
Eye color	No observation	No observation	No observation	No observation
Diarrhea	No observation	No observation	No observation	No observation
General physique	Normal	Normal	Normal	Normal
Coma	No observation	No observation	No observation	No observation
Death	Alive	Alive	Alive	Alive

Table 6: Clinical observation in male control and rats treated with *MyP* water extract

Observation	**Control group**	**High Dose**	**Medium Dose**	**Low Dose**
Digestion	No observation	No observation	No observation	No observation
Body weight	No observation	No observation	No observation	No observation
Temperature	No observation	No observation	No observation	No observation
Food intake	No observation	No observation	No observation	No observation
Urination	Normal	Normal	Normal	Normal
Rate of respiration	Normal	Normal	Normal	Normal
Change in skin	No observation	No observation	No observation	No observation
Drowsiness	No observation	No observation	No observation	No observation
Sedation	No observation	No observation	No observation	No observation
Eye color	No observation	No observation	No observation	No observation
Diarrhea	No observation	No observation	No observation	No observation
General physique	Normal	Normal	Normal	Normal
Coma	No observation	No observation	No observation	No observation
Death	Alive	Alive	Alive	Alive

Furthermore, the behavior observations revealed no pathological changes associated with the administration of *MyP* water extract. All animals both in the control group and *MyP* water extract treated groups appeared healthy as recorded in Table 5 and Table 6. There were no abnormalities concerning changes in temperature, urination, the rate of respiration, change in the skin including skin and fur, eyes, eye color, mucous membranes, and behavioral patterns (walking backward). No toxicity signs such as drowsiness, sedation, diarrhea, coma, hypoactivity, and alteration in the locomotor activity or deaths were recorded during the administration and recovery periods by oral route with *MyP* water extract in doses of 100, 200 and 400 mg/kg.

Table 7: Urine analysis result for female control and rats treated with *MyP* water extract

GROUP	RAT ID	Volume (ml)	Leukocyte	Nitrite	Urobilinogen	Protein	pH	Blood	Specific gravity	Ketone	Bilirubin	Glucose
Control Group (RO Water)	R1	17	-	-	N	NA	8.5	-	1	-	NA	-
	R2	17	-	-	N	100	8.5	-	1	-	NA	-
	R3	16	-	-	N	NA	8.5	-	1	-	NA	-
	R4	15	-	-	N	NA	8.5	-	1	5	NA	-
	R5	12	-	-	N	100	8.5	-	1	-	NA	-
High Dose (2g/kg BW)	R6	11	-	-	N	NA	8.5	-	1	-	NA	-
	R7	14	-	-	N	NA	8.5	-	1	5	NA	-
	R8	21	-	-	N	NA	8.5	-	1	-	NA	-
	R9	20	-	-	N	NA	8.5	-	1	-	NA	-
	R10	16	-	-	N	100	8.5	-	1	-	NA	-
Medium Dose (1g/kg BW)	R11	13	-	-	N	NA	8.5	-	1	-	NA	-
	R12	13	-	-	N	100	8.5	-	1	-	NA	-
	R13	16	-	-	N	NA	8.5	-	1	-	NA	-
	R14	17	-	-	N	NA	8.5	-	1	-	NA	-
	R15	15	-	-	N	300	8.5	-	1	-	NA	-
Low Dose (0.63g/kg BW)	R16	12	-	-	N	NA	8.5	-	1	-	NA	-
	R17	13	-	-	N	NA	8.5	-	1	-	NA	-
	R18	15	-	-	N	NA	8.5	-	1	-	NA	-
	R19	14	-	-	N	NA	8.5	-	1	-	NA	-
	R20	10	-	-	N	NA	8.5	-	1	5	NA	-

N: Normal, NA: Not Available, - : Negative

Table 8: Urine analysis result for male control and rats treated with *MyP* water extract

GROUP	RAT ID	Volume (ml)	Leukocyte	Nitrite	Urobilinogen	Protein	pH	Blood	Specific gravity	Ketone	Bilirubin	Glucose
Control Group (RO Water)	R21	15	-	-	N	NA	8.5	-	1	-	NA	-
	R22	14	-	-	N	NA	8.5	-	1	-	NA	-
	R23	13	-	-	N	NA	8.5	-	1	-	NA	-
	R24	15	-	-	N	NA	8.5	-	1	-	NA	-
	R25	19	-	-	N	NA	8.5	-	1	-	NA	-
High Dose (2g/kg BW)	R26	11	-	-	N	NA	8.5	-	1	-	NA	-
	R27	14	-	-	N	NA	8.5	-	1	-	NA	-
	R28	15	-	-	N	NA	8.5	-	1	-	NA	-
	R29	21	-	-	N	NA	8.5	-	1	-	NA	-
	R30	11	-	-	N	NA	8.5	-	1	-	NA	-
Medium Dose (1g/kg BW)	R31	13	-	-	N	NA	8.5	-	1	-	NA	-
	R32	13	-	-	N	NA	8.5	-	1	-	NA	-
	R33	15	-	-	N	NA	8.5	-	1	-	NA	-
	R34	16	-	-	N	NA	8.5	-	1	-	NA	-
	R35	15	-	-	N	NA	8.5	-	1	-	NA	-
Low Dose (0.63g/kg BW)	R36	12	-	-	N	-NA	8.5	-	1	-	NA	-
	R37	13	-	-	N	NA	8.5	-	1	-	NA	-
	R38	15	-	-	N	NA	8.5	-	1	-	NA	-
	R39	14	-	-	N	NA	8.5	-	1	-	NA	-
	R40	10	-	-	N	NA	8.5	-	1	-	NA	-

N: Normal, NA: Not Available, T: Trace, - : Negative

As shown in table 7, there were no abnormalities found in the female rat. However, ketone bodies were detected in rat 7 and rat 20 which from 400 mg/kg and 100 mg/kg group respectively. Rat 4 from control group also showed detection of ketone bodies in its urine. The presence of ketone bodies without any other pathological signs such as diabetes in the rat's urine have related with stress and might be the rat still not acclimated with the metabolic cages. Table 8 did not show any abnormalities in the male rat.

Table 9: Haematological values of female control and rats treated with *MyP* water extract

	Control (RO Water)	High Dose (400 mg/kg BW)	Medium Dose (200 mg/kg BW)	Low Dose (100 mg/kg BW)
Female				
WBC	8.55 ± 2.72	8.35 ± 3.54	8.92 ± 3.11	8.75± 3.96
Lymph#	6.73 ± 1.37	6.81 ± 2.73	6.39 ± 1.96	6.72 ± 1.67
Mon#	0.19 ± 0.016	0.20 ± 0.035	0.18 ± 0.038	0.21 ± 0.042
Gran#	0.39 ± 0.021	0.43 ± 0.036	0.37 ± 0.041	0.42 ± 0.038
Lymph%	71.80±15.78	69.88 ± 23.73	71.49 ± 20.45	71.53 ± 19.53
Mon%	1.45 ± 1.85	1.79 ± 1.83	1.81 ± 1.42	1.77 ± 1.29
Gran%	24.86 ± 4.87	25.82 ± 4.69	23.48 ± 5.21	25.67 ± 6.32
RBC (106/µL)	7.78 ± 2.17	7.57 ± 2.63	7.95 ± 1.38	7.76 ± 2.79
HGB (g/dL)	13.67± 1.27	13.88 ± 3.78	13.02 ± 5.63	13.33 ± 3.28
HCT (%)	43.79±2.35	44.99 ± 3.29	40.56 ± 4.11	45.78 ± 5.33
MCV (fL)	54.45 ± 13.73	55.63 ± 18.31	52.44 ± 19.83	53.97 ± 21.79
MHC (pg)	20.25 ± 7.28	19.76 ± 9.85	23.88 ± 7.35	27.67 ± 11.46
MCHC (g/dL)	35.28 ± 12.83	33.57 ± 18.16	32.87 ± 13.97	34.83 ± 20.24
RDW (%)	11.89 ± 8.17	12.58 ± 7.39	11.58 ± 9.93	13.68 ± 6.92

Table 10: Haematological values of male control and rats treated with *MyP* water extract

	Control (RO Water)	High Dose (400 mg/kg BW)	Medium Dose (200 mg/kg BW)	Low Dose (100 mg/kg BW)
Male				
WBC	9.12 ± 1.26	9.04 ± 5.88	9.20 ± 4.22	9.11 ± 5.34
Lymph#	7.21 ± 1.41	7.03 ± 2.13	7.93 ± 3.86	7.13 ± 3.37
Mon#	0.65 ± 0.31	0.52 ± 0.73	0.67 ± 0.28	0.84 ± 0.32
Gran#	0.99 ± 0.63	1.04 ± 0.74	0.96 ± 0.73	0.71 ± 0.39
Lymph%	65.30 ± 17.77	63.27± 23.49	67.28± 27.32	68.28± 23.82
Mon%	1.61 ±1.48	2.74± 1.19	1.92± 0.92	1.72± 0.93
Gran%	27.35 ±14.60	29.18± 15.23	28.78± 13.23	26.71± 10.98
RBC (106/µL)	7.76 ± 0.25	8.13± 0.93	8.24± 0.82	7.77± 0.95
HGB (g/dL)	14.19 ± 1.78	15.29 ± 2.49	13.93 ± 2.47	16.94 ± 2.94
HCT (%)	45.29 ±3.74	48.18 ± 4.19	49.20 ± 4.39	41.49 ± 8.28
MCV (fL)	51.24 ± 5.31	55.62 ± 7.83	50.71 ± 8.97	52.38 ± 8.47
MHC (pg)	20.36 ± 0.24	21.39 ± 5.09	22.45 ± 6.21	23.49 ± 7.73
MCHC (g/dL)	37.21 ± 4.53	33.92 ± 6.29	38.59± 7.39	37.53 ± 6.63
RDW (%)	10.36 ± 1.26	14.07 ± 3.67	17.69 ± 7.36	16.17 ± 5.78

WBC: White Blood Cells, RBC: Red Blood Cells, HGB: Haemoglobin, HCT: Hematocrit, PLT: Platelet.

Values are expressed as a mean ± standard deviation, $n = 5$. No significant difference observed compared to control group.

The hematopoietic system is one of the most susceptible targets to toxic substances and is an important parameter for assessing the physiological and pathological status in human and animal (Santos *et al*., 2013). The results for female and male are presented in Table 9 and Table 10, respectively. No obvious change of haematological parameters was observed in the *MyP* extract-treated female rats. Since the haematological parameters values are within the normal range for the species, the observed variations are not biologically meaningful to be considered as a sign of any toxicity (Giknis and Clifford, 2006). In this study, all values of measured parameters and its minor fluctuations haematology analysis were within normal limits. This indicated that the *MyP* water extract had provided no adverse effects on circulating blood cells.

Table 11: Biochemical parameters of female control and rats treated with MyP water extract

	Control (RO water)	**High Dose** (400 mg/kg BW)	**Medium Dose** (200 mg/kg BW)	**Low Dose** (100 mg/kg BW)
Total protein (g/L)	6.8 ± 2.67	7.1 ± 3.57	6.5 ± 3.02	7.2 ± 4.04
Albumin (g/L)	3.9 ± 2.85	3.57± 2.76	3.75± 2.48	3.69± 2.68
Liver enzyme				
ALP (U/L)	32.41±14.18	35.57±16.76	38.47±17.34	36.86±18.56
ALT (U/L)	27.56 ± 9.11	28.47 ± 9.27	26.85 ± 6.25	26.85 ± 7.74
AST (U/L)	67.67±23.65	68.40±27.51	64.86±38.57	67.38 ±6.64
Renal enzyme				
Urea (µmol/L)	7.76± 2.26	7.59± 3.29	7.47± 4.48	8.29± 3.76
Creatinine (µmol/L)	91.53± 35.32	96.90± 30.38	94.69± 39.28	89.94± 36.74
Glucose (mmol/L)	6.81± 1.30	6.64± 1.34	6.77± 1.62	6.69± 1.68

Values are expressed as a mean ± standard deviation, $n = 5$.
No significant difference observed as compared to control group.

Table 12: Biochemical parameters of male control and rats treated with MyP water extract

	Control (RO water)	High Dose (400 mg/kg BW)	Medium Dose (200 mg/kg BW)	Low Dose (100 mg/kg BW)
Total protein (g/dL)	6.7 ± 2.46	7.03 ± 3.64	6.79 ± 3.78	6.87 ± 3.40
Albumin (g/dL)	4.5 ± 1.47	5.04 ± 2.74	4.44 ± 3.75	4.97 ± 2.86
Liver enzyme				
ALP (U/L)	55.37 ± 21.46	60.35 ± 33.79	58.57 ± 29.56	55.89 ± 23.85
ALT (U/L)	33.56 ±17.23	45.87 ± 16.45	40.47 ± 17.45	37.89 ± 20.71
AST (U/L)	67.45 ± 21.6	66.57 ±24.75	65.89 ± 21.97	64.68 ± 27.45
Renal enzyme				
Urea (mmol/L)	7.31 ± 2.15	7.45 ± 2.96	7.58 ± 2.86	7.56 ± 2.84
Creatinine (mmol/L)	88.30 ± 25.1	87.68 ± 30.64	79.99 ± 29.75	90.87 ± 35.93
Glucose (mmol/L)	6.60 ± 1.20	6.53 ± 1.04	6.55 ± 1.38	6.58± 1.70

Values are expressed as a mean ± standard deviation, $n = 5$.

No significant difference observed as compared to control group.

The increase of some enzymes and proteins such as ALT, AST, gamma-glutamyltransferase and bilirubin are an indicative of hepatocellular effects (OECD, 2008b and Brandt *et al*., 2009). Inclinations of biomarkers such as creatinine and blood urea nitrogen are a sign of nephron functional injury (Lameire *et al*., 2005). Furthermore, no significant changes in biochemical parameters of rats treated orally with *MyP* water extract as shown in Table 11 and Table 12. There were no significant increases of UREA observed in the all *MyP* water extract groups for both female and male. In addition, ALT in the *MyP* water extract 400 mg/kg group for male showed slightly increase compared to control group. However, no significant increase of ALT and AST in the *MyP* water extract group for female groups. Besides that, female rats show no significant increase of ALP in 400 mg/kg. There were no significant increases in ALB and TP in the *MyP* water extract. These biochemical parameters indicated of non-toxic aspects of *MyP* water extract towards kidney and liver which was further confirmed by findings of histopathological examinations and organ weight observation.

Table 13: Relative organ weight in (g/100g BW) of female rats treated with *MyP* water extract.

Organ	Groups/ Relative organ weight in gram per 100 g BW			
	Control (RO Water)	High Dose (400 mg/kg BW)	Medium Dose (200 mg/kg BW)	Low Dose (100 mg/kg BW)
Heart	0.172 ± 0.023	0.174 ± 0.032	0.170 ± 0.023	0.169 ± 0.044
Lung	0.257 ± 0.037	0.266 ± 0.047	0.269 ± 0.051	0.277 ± 0.082
Liver	1.789 ± 0.131	1.876 ± 0.252	1.725 ± 0.145	1.693 ± 0.217
Spleen	0.085 ± 0.025	0.091 ± 0.032	0.093 ± 0.042	0.089 ± 0.043
Kidney (Left)	0.213 ± 0.052	0.215 ± 0.063	0.220 ± 0.072	0.214 ± 0.035
Kidney (Right)	0.209 ± 0.014	0.201 ± 0.016	0.204 ± 0.015	0.203 ± 0.018
Adrenal (Left)	0.0059 ± 0.005	0.006 ± 0.002	0.007 ± 0.003	0.0059 ± 0.001
Adrenal (Right)	0.0057 ± 0.002	0.0058 ± 0.004	0.006 ± 0.005	0.006 ± 0.004
Ovary (Left)	0.014 ± 0.005	0.012 ± 0.005	0.014 ± 0.004	0.013 ± 0.005
Ovary (Right)	0.013 ± 0.004	0.012 ± 0.005	0.013 ± 0.003	0.012 ± 0.004
Bladder	0.016 ± 0.005	0.014 ± 0.005	0.015 ± 0.004	0.017 ± 0.006
GIT	0.264 ± 0.061	0.260 ± 0.037	0.259 ± 0.042	0.266 ± 0.053
Stomach	0.399 ± 0.056	0.397 ± 0.061	0.372 ± 0.055	0.391 ± 0.067

Values are expressed as mean ± standard deviation, $n = 5$.

No significant difference observed as compared to control group.

Table 14: Relative organ weight in (g/100g BW) of male rats treated with *MyP* water extract

Organ	Groups/ Relative organ weight in gram per 100 g BW			
	Control	High Dose	Medium Dose	Low Dose
	(RO Water)	(400 mg/kg BW)	(200 mg/kg BW)	(100 mg/kg BW)
Heart	0.361 ± 0.048	0.365 ± 0.031	0.359 ± 0.040	0.361 ± 0.019
Lung	0.497 ± 0.071	0.488 ± 0.034	0.487 ± 0.068	0.490 ± 0.061
Liver	3.162 ± 0.064	3.099 ± 0.093	3.132 ± 0.162	3.137 ± 0.173
Spleen	0.169 ± 0.058	0.172 ± 0.062	0.171 ± 0.053	168 ± 0.025
Kidney (Left)	0.421 ± 0.055	0.437 ± 0.041	0.441 ± 0.035	0.438 ± 0.061
Kidney (Right)	0.411 ± 0.075	0.423 ± 0.024	0.402 ± 0.098	0.429 ± 0.031
Adrenal (Left)	0.0069 ± 0.002	0.0069 ± 0.001	0.0067 ± 0.003	0.0069 ± 0.004
Adrenal (Right)	0.007 ± 0.001	0.007 ± 0.001	0.0069 ± 0.001	0.007 ± 0.003
Testis (Left)	0.5900 ± 0.1	0.610 ± 0.071	0.597 ± 0.032	0.599 ± 0.057
Testis (Right)	0.57 ± 0.12	0.610 ± 0.074	0.588 ±0.054	0.600± 0.064
Bladder	0.036 ± 0.021	0.032 ± 0.024	0.031± 0.021	0.032 ± 0.024
GIT	0.320 ± 0.047	0.317 ± 0.046	0.292 ± 0.038	0.329 ± 0.031
Stomach	0.475 ±0.025	0.497 ± 0.037	0.443 ± 0.056	0.489 ± 0.086

Values are expressed as mean ± standard deviation, $n = 5$.

No significant difference observed as compared to control group.

Relative organ weight (ROW) values have been shown to reflect the pathological changes in impaired organs (Piao *et al*., 2013). Table 13 and Table 14 showed the ROW of animal used in this study. In female group, animals treated with *MyP* water extract 100 mg/kg BW showed no significant difference in ROW as compared to control. The female rats from *MyP* water extract 400 mg/kg BW group also showed no significant difference in ROW of liver and kidney. In male group, animals from *MyP* 100 mg/kg BW treated group did not show an increase in ROW of the liver. Furthermore, male rats treated with *MyP* water extract 400 mg/kg BW showed no significant difference in ROW of heart, left kidney and right kidney. Also, there were no statistically significant differences were found in ROW of the organs including spleen, adrenals, stomach, bladder, testes and, ovaries in both male and female groups at any dose level. All the minor fluctuations in ROW values that fell within the normal range are not considered as a sign of organ damage (Aleman *et al*., 1998). However, necropsy of these main organs or tissues showed that no treatment-related alterations in ROW and no obvious pathological changes.

Necropsy Findings

All the animals were within limits during necropsy. Post-mortem evaluations noticed no observable toxic effects. The assessment of both macro and microscopic pathological changes in the organs of treated animals are the basis of a safety assessment. In this study, the macroscopic analysis, the *MyP* water extract, at all doses tested and produced no changes in the treated animals' vital and reproductive organs in the qualitative analysis.

Detailed Description of All Histopathological Findings

Histopathology results from *MyP* treated group rats showed no significant changes from the control group. Examination of a section of the kidney in *MyP* water extracts treated rats show normal renal architecture. Renal glomeruli, collecting tubules, interstitial tissue and, blood vascular channels observed as in normal condition. Interstitium of the kidney did not show any sign of necrosis or degeneration. In photomicrographs of *MyP* water extract treated rats liver, no focal or diffuse foci of necrosis of hepatocytes, and infiltration of chronic inflammatory cells were observed. *MyP* water extract treated rats showed the normal lobular architecture of liver with the normal central portal vein, radiating plates of hepatocytes and peripheral portal tracts composed of the hepatic artery, bile ductule and, distal portal vein. In the detailed histopathological analyses, there were no findings suggestive of toxic effects (Figure 2 – Figure 8). These results proved to be consistent with biochemical analyses, confirming the safety of using the *MyP* water extract.

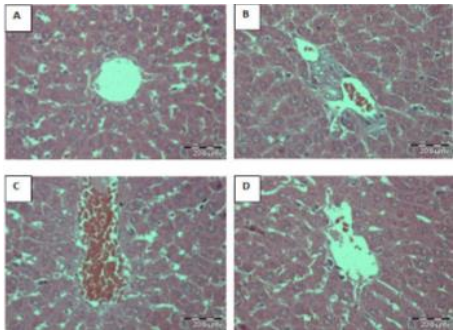

Figure 2: Liver

There were no adverse histopathological conditions observed in the control (A) and *MyP* water extract (B, 100 mg/kg; C, 200 mg/kg; D, 400 mg/kg) treatment groups. There was no sign of congestion was observed in the liver at doses of 200 and 400 mg/kg. The normal size of sinusoidal spaces indicated no vascular congestion.

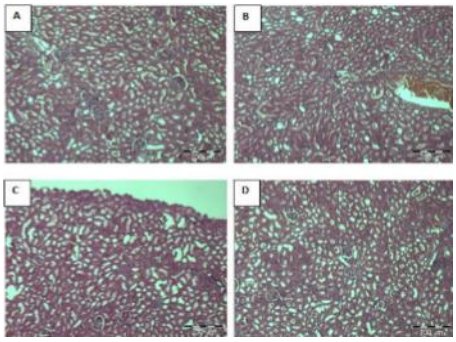

Figure 3: Kidney

There were no adverse histopathological conditions observed in all the *MyP* water extract treatment groups. There were no clumping or pyknosis of nucleus detected in the highest treatment of *MyP* water extract. Background containing tubules in the group of *MyP* water extract treated a rat demonstrated normocellular glomerular clusters.

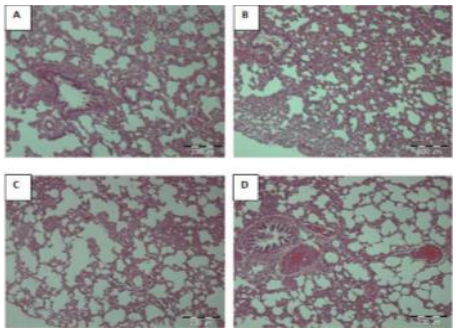

Figure 4: Lungs

The lungs were normal in all the *MyP* water extract treatment groups compared with control. The interstitium containing few blood vessels surrounding the alveolar air spaces. No inflammatory cells detected which determined that there were no inflammations occurred during treatment.

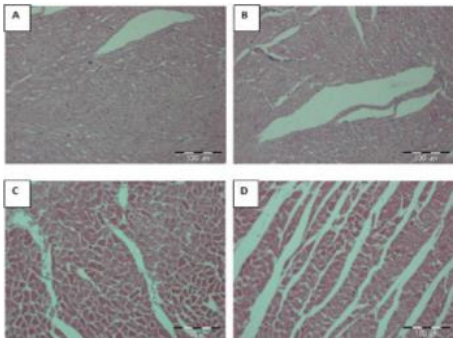

Figure 5: Heart

The heart was normal in all the *MyP* water extract treatment groups. The cardiac cells were arranged in interlacing and parallel array as shown in picture D. Their nuclei were spindle-shaped and elongated as can be observed in picture B. Treatment of *MyP* water extract did not cause any damage to the heart cell.

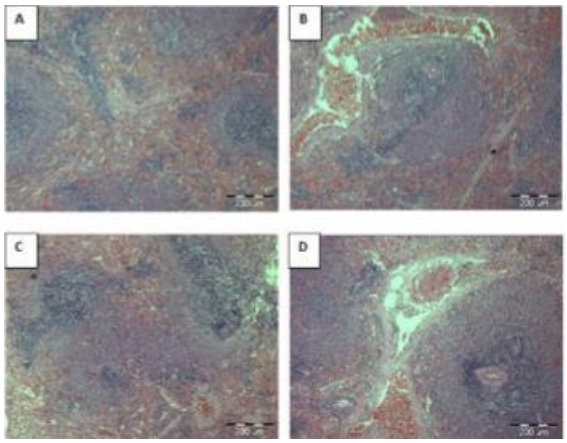

Figure 6: Spleen

The control group showed a normal cell observation. There was also no congested sign found in all the *MyP* water extract treatment groups. Picture B and D show the background of splenic stroma indicating engorgement of sinusoids by red blood cells which is considered normal condition.

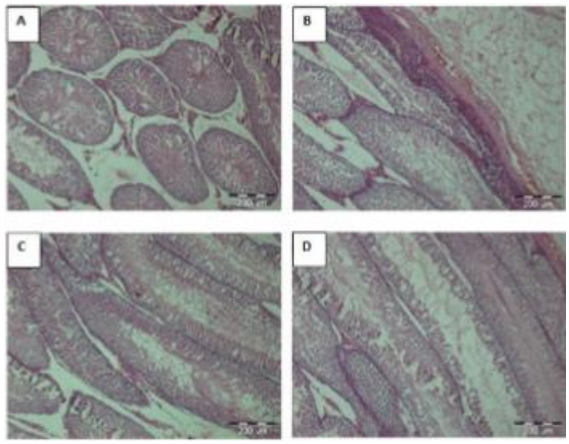

Figure 7: Testes

All the *MyP* water extract treatment groups displayed as normal histopathological conditions. Besides that, the interstitium showed no abnormal structures, or any damage of Leydig cells.

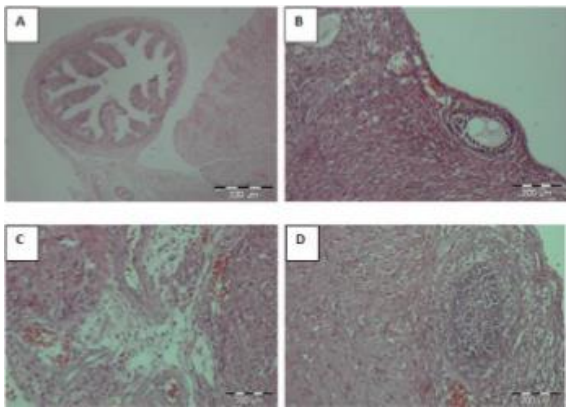

Figure 8: Ovaries

Control group only displayed normal histopathological structures. In the *MyP* water extract treatment groups, picture B, C and D did not show any changes in cell structure and *MyP* water extract treatment- related cell damage.

CONCLUSION

In conclusion, preparation of for first and second batch of *MyP* water extract showed high antioxidant activity. The extract has no observable subchronic toxic effect on *Sprague Dawley* rats especially for female rats administered with *MyP* water extract at a concentration of 400 mg/kg BW, 200 mg/kg BW and 100 mg/kg BW.

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