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Sprague Dawley Rats' Haematological Parameters Are Not Affected by Cocoa Pod Husk Pectin Used as a Pharmaceutical Excipient

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1. Introduction

The development of pharmaceutical excipients is crucial for optimizing drug delivery and pharmaceutical formulation [1]. The performance of excipients, rather than just the active ingredients, determines the quality of medications [2]. In contemporary pharmacological dose formulations, excipients frequently serve many purposes [3]. Research on natural polymer materials shows promise as a path toward new excipients. Natural products are affordable, easily accessible, adaptable, biocompatible, biodegradable, and environmentally benign [1, 4].

There has been a lot of interest in the creation of natural polymers as excipients because of their special qualities that can be customized for a variety of uses thanks to their extensive chains and functional groups. Furthermore, they can be altered to create novel materials with a variety of physicochemical characteristics using both low and high molecular weight materials [1, 5]. Many applications, such as matrix controlled systems, film coating agents, buccal films, microspheres, nanoparticles, implants, viscosity enhancers, stabilisers, disintegrants, solubilisers, emulsifiers, suspending agents, gelling agents, and binders in drug formulation, have resulted from the successful development of these polymers as excipients [6]. In Ghana, West Africa, cocoa is a significant crop for both agriculture and the economy. One way to add value to the cocoa pod husks (CPHs) is to process them into useful compounds like pectin after the cocoa beans are removed.

waste production [7, 8]. Pectins are naturally occurring polymers that contain methyl esters of uronic acid and linear chains of (1, 4)-linked α-d-galacturonic acid residues [9]. Following the processing of cocoa beans, CPH pectin is recovered from pod husk waste. According to a prior study, CPH pectin possesses the necessary physicochemical traits to be employed as a multipurpose medicinal excipient with exceptional qualities [10]. The moisture content, ash value, and degree of esterification of the CPH pectin are 0.19 ± 0.06%, 1.0%, and 26.8%, respectively.

The swelling index was 357.3 ± 4.6, 274.7 ± 4.6, and 360.0 ± 0.0 in 0.1 M HCl, phosphate buffer pH 6.8, and distilled water, respectively, and ± 2.5% (low-methoxyl pectin) [10]. According to a study by Yapo et al., under the given extraction circumstances, CPH pectin has an average molecular weight of 43–82 kDa and an intrinsic viscosity of 162–304 mL/g [11].

The need for such appropriate controlled release formulations for optimal drug delivery and improvement of patient compliance stems from the fact that controlled release formulations are superior to conventional formulations in managing chronic diseases, such as those influenced by the circadian rhythm, which result in less than ideal treatment outcomes. Pectin has been studied as a matrix-controlled system [6, 12]. The effectiveness of CPH pectin as a rate-controlling polymer in controlled release formulations for chronic

illnesses and ailments impacted by the circadian rhythm was shown by preliminary exploratory studies. Comorbidities and reports of negative haematological effects, including macrocytosis, iron deficiency, poor utilization of red cell iron, decreased red cell formation, decreased saturation of iron binding protein [13], anemia [14], and thrombocytopenia [15], are linked to certain chronic liver, kidney, and adrenal insufficiency conditions. Cardiovascular disease, which involves problems with the heart and blood vessels, is influenced by white blood cell count and hemostatic factors [16]. Chronic airway illness was also impacted by aberrant granulocytic numbers [17]. Additionally, using some herbal remedies to treat such chronic illnesses has led to some negative side effects, like haematuria [18]. Therefore, excipient-induced synergistic or additive effects and increased susceptibility to disease should not be linked to the treatment of such chronic disorders. In this sense, the identification of other hematological illnesses shouldn't be complicated by the effects of excipients.

The drug is given, absorbed, and then delivered to the site of action by the blood [19]. It is crucial that the excipients used in the medicine formulation do not in any way damage the operation of the blood coagulation system, disease-fighting cellular components (such as leucocytes), or haematological indicators like hemoglobin.

Certain excipients have been linked to erythrocyte destruction and the possibility of hemolysis, leucopenia and lymphocytopenia [20], leukemia [21], and a decrease in hemoglobin and hemocrit following the administration of some solubilizing enhancing agents [22]. Furthermore, the method of administration affects the excipient's toxicity profile [22].For the treatment of various chronic illnesses, it is crucial that the enabling excipients used in the drug formulation have the appropriate safety profile for continuous long-term administration. Apart from the active ingredient, which was regarded as "inert," excipients were once thought of as parts of a pharmaceutical dosage form. Since excipients are widely recognized as useful ingredients in medications, the idea of them has changed throughout time [2, 23]. Excipients may come from plants, animals, or minerals. Because natural excipients come from a variety of sources, proper quality control and safety assessment must be carried out. According to earlier studies, pectin's biological actions include lowering blood cholesterol levels, preventing blood heavy metal poisoning, and possessing hemostatic and antidiarrheal properties [24, 25].

Furthermore, excipients' biological behavior does not always indicate inertness. Natural excipients have the potential to display biological activity [26], which could inevitably impact their safety profile [21]. The extraction of these natural polymers, which may include additional trace phytochemical and biological components, is another issue [23].

An assessment of the main indicators of haematological toxicity in Sprague Dawley rats (SDRs) following long-term oral continuous dosing is necessary due to the paucity of data on the impact of CPH pectin on the haematological profile [27]. Therefore, the study looked into how CPH pectin affected bilirubin, a few haematological markers, and the spleen's histology.

Materials And Methods

1.1. Resources. As previously reported [10], the study used freeze-dried CPH pectin with a degree of esterification of 26.8% extracted with hot water (hot water soluble pectin, HWSP) and 4% w/v hot aqueous citric acid (citric acid soluble pectin, CASP).

1.2. Maintenance and Husbandry of Animals. About six-week-old, healthy male and female Sprague Dawley rats were acquired from the Centre for Plant Medicine Research's (CPMR) Animal House in Mampong-Akwapim, Ghana's eastern area. Under experimental settings (temperature 22 ± 2°C, relative humidity 60–70%, and 12-hour light-dark cycle), the animals were kept in stainless steel wire mesh cages with soft wood shavings and fed with

feed from Ghana's Accra-based Agricare Co., Ltd. The animals had unlimited access to pure water. The

study was carried out in compliance with the National Institute of Health standards for the Care and Use of Laboratory Animals, which are included in the US standards, and the Institutional Research Committee of the CPMR that is in charge of Animal Care and Use [28].

1.3. Treatment and Grouping of Animals. Based on weights, twenty-four male Sprague Dawley rats were divided into four experimental groups (n = 6) at random. For ninety days, the control group (Group A) received five milliliters of distilled water orally every day. The second group of test animals

Oral gavage was used to administer 0.714 mg/kg of CPH pectin to Group B, 7.14 mg/kg to Group C, and 71.4 mg/kg to Group D. Twenty-four female rats divided into four groups (n = 6) participated in the studies again. The female rats' nulliparity and lack of pregnancy were guaranteed. During the ninety days, all of the animals received treatment. Every day, the animals were inspected and checked for any signs of abnormality or toxicity, as well as for mortality.

Body Weights (1.1). Weighings were taken of each rat in the experimental groups on day 0 and then every week for the duration of the 90-day study period for every dose level.

1.2. Consumption of Food and Water. Over the course of the ninety days, the amount of food and water that each rat ingested was tracked.

1.3. Hematology. On the same day, each rat's blood was extracted by tail bleeding and placed in Eppendorf tubes with EDTA for hematological examination. An automated haematology analyzer (KX-2IN, Sysmex Corporation, Japan) was used to measure the RBC, WBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, and RDW-CV%. Over the course of the ninety days, this was carried out every thirty days.

1.4. Biochemistry of Serum. Before blood was drawn the following day, the animals in each group were fasted for the whole night. Blood samples were taken into BD-serum separator tubes for the biochemical examination. After that, the blood samples were centrifuged for ten minutes at 3000 rpm. After that, the serum was kept at 4°C and used the next day for both direct and total bilirubin assays. Over the course of the 90-day period, the analysis was carried out every 30 days.

1.5. Sacrificing animals. On the day of the experiment, a skilled specialist put the rats to death by cervical dislocation.

Histopathology (1.6). 10% formalin was used to preserve the splenic tissue. After processing, the spleen tissue was sectioned at 3–5 um and fixed in paraffin. For the microscopic analysis, the slices were then stained with hematoxylin-eosin [29].

1.7. Analysis of Statistics. The data was statistically analyzed using GraphPad Prism version 5.03. The mean ± standard error of mean (SEM) (n = 6) was used to express the results. Two-way and one-way analyses of variance (ANOVA) were used to assess any significant changes between dosage groups and controls. In the event that the test revealed significant differences, either the Bonferroni or Dunnett's post hoc analysis was carried out. A significant P value was defined as less than 0.05.

2. Results

2.1. Mortality. There was no mortality associated with the study.

TABLE 1: Results showing the effect of CPH pectin on body weight change in SDRs.

Week	Control	0.714 mg/kg	7.14 mg/kg	71.4 mg/kg
		Male		
0	161.00±4.62	163.00±4.70	161.67±3.39	171.30±4.13
4	209.50±6.46	230.83±6.17	229.33±4.05	227.50±4.80
8	234.17±4.48	260.00±7.90	256.87±8.65	249.50±5.50
13	267.67±16.87	272.50±12.26	266.17±12.36	270.30± 10.00
		Female		
0	133.00±1.24	162.67±0.88	153.17±0.98	117.83±0.79
4	173.70±5.29	179.17±1.49	173.83±2.17	161.50±2.88
8	186.83±6.93	184.50±3.37	182.00±0.45	180.67±4.24
13	200.50±7.84	190.83±1.49	185.50±2.26	189.00±4.40

2.2. Food and Water Consumption. There was no significant variation in the food and water consumption among the groups under study.

2.3. Effect of CPH Pectin on Weight Change in Male and Female Sprague Dawley Rats. There was a consistent weight gain (P>0.05) in male rats. There were, however, no dose-specific patterns in body weight related to treatment. Female rats demonstrated rapid weight gain during the first four weeks and a slower rate thereafter. There were slight changes in female body weight ($P < 0.05$) at week 13 prior to termination. There were no dose-specific patterns in body weight related to treatment (Table 1).

2.4. Effect of CPH Pectin on RBC Indices of Male and Female Sprague Dawley Rats. Table 2 shows the influence of the administration of CPH pectin on haematological indices for the male rats after a 90-day subchronic toxicity study. In comparison to the control, treated animals showed no remarkable changes with $P > 0.05$ except for the MCV value for male rats treated with medium dose (7.14 mg/kg) after day 30 ($P < 0.01$). All other parameters showed similar results at 30 and 60 days except for MCV. Values for MCV indicated an isolated statistically significant reduc- tion with the medium dose group after 30 days. Table 3 shows the influence of the administration of CPH pectin on haematological indices for the female rats after a 90- day subchronic toxicity study. In comparison to the control, treated animals showed no remarkable changes. All other parameters showed similar results at 30 and 60 days except for MCV.

2.5. Effect of CPH Pectin on WBC Indices of Male and Female Sprague Dawley Rats. Table4 shows the effect of CPH pectin on the levels of leucocytes, lymphocytes, and neutrophils in male SDRs for the 90-day subchronic toxicity study. Minor fluctuations were observed in both control and treated groups during the 90-day administration of CPH pectin with no marked effects ($P > 0.05$) on these white blood cell indices. Similarly, there were minor fluctuations observed for the female rats in both control and treated groups during the 90- day administration of CPH pectin (Table 5).

TABLE 2: Results showing the effect of CPH pectin on red blood cell indices of male rats.

Red blood cell indices	Day	Control	Low dose 0.714 mg/kg	Medium dose 7.14 mg/kg	High dose 71.4 mg/kg
RBC (x 10^6/ul)	30	8.72±0.09	9.16±0.23	8.93±0.14	8.91±0.13
	60	8.78±0.07	8.68±0.12	8.58±0.18	8.60±0.11
	90	9.03±0.11	8.90±0.12	8.77±0.15	8.91±0.14
HGB (g/dl)	30	15.00±0.18	15.52±0.36	15.63±0.43	15.48±0.16
	60	15.37±0.20	15.18±0.34	15.03±0.22	15.08±0.12
	90	15.50±0.13	15.38±0.34	15.35±0.27	15.35±0.18
HCT (%)	30	49.47±0.59	49.68±0.68	49.87±1.07	48.52±0.61
	60	47.48±0.67	47.10±1.21	45.87±0.83	46.88±0.68
	90	48.82±0.44	49.97±0.97	47.62±0.88	50.30±0.78
MCV (fl)	30	55.60±0.19	54.92±0.62	52.95±0.26∗∗	53.73±0.35
	60	53.20±0.31	52.65±0.57	53.50±0.36	54.23±0.40
	90	54.08±0.70	53.50±1.11	54.83±0.53	55.88±0.49
MCH (pg)	30	17.08±0.18	16.93±0.26	16.98±0.22	17.15±0.13
	60	17.32±0.11	17.10±0.26	17.52±0.16	17.55±0.12
	90	17.05±0.11	16.92±0.21	17.47±0.39	17.47±0.11
MCHC (g/dl)	30	30.63±0.25	30.53±0.18	31.90±0.19	31.63±0.12
	60	32.37±0.13	32.23±0.19	32.80±0.16	32.18±0.29
	90	31.83±0.12	31.22±0.59	31.60±0.85	30.90±0.18
RDW-SD (fl)	30	29.55±0.26	29.55±0.30	28.00±0.39	27.75±0.40
	60	27.50±0.24	27.03±0.27	27.52±0.07	27.68±0.31
	90	28.55±0.91	29.87±1.28	28.23±0.76	29.83±0.45
RDW-CV (%)	30	13.55±0.56	14.70±0.77	14.57±0.38	12.53±0.39
	60	13.03±0.63	13.08±0.58	13.12±0.33	13.03±0.36
	90	14.30±1.14	15.33±1.40	13.57±0.57	13.58±0.50

∗∗Significantly different ($P < 0.01$)

2.6. Effect of CPH Pectin on Platelet Indices of Male and Female Sprague Dawley Rats. Analysis of platelet indices (Table 6) showed no significant changes ($P > 0.05$) in platelet count (PLT), though there were insignificant minor reductions after day 30 in male rats. Similarly, there were no remarkable changes in the ratio (P-LCR) and size (PDW and MPV) after the 90-day continuous administration of CPH pectin. Again, analysis of platelet indices in female rats (Table 7) showed no significant changes ($P > 0.05$) in platelet count (PLT). There was minor reduction of no significance after day 30 in the female rats. Similarly, there were no remarkable changes in the ratio (P-LCR) and size (PDW and MPV) after the 90-day continuous administration of CPH pectin

2.7. Effect of CPH Pectin on Bilirubin Levels of Male and Female Sprague Dawley Rats. Table 8 shows the influence of CPH pectin on direct and indirect bilirubin levels after a 90-day continuous administration of CPH pectin in male SDRs. In comparison to the control, there were insignificant elevations in bilirubin at day 30 ($P > 0.05$) in the treated groups. There were no remarkable changes observed in bilirubin levels for days 60 and 90 ($P > 0.05$). The effect of CPH pectin on indirect bilirubin levels showed insignificant elevations in bilirubin at day 30 ($P > 0.05$) in the treated groups. There

were no remarkable changes observed in the levels for days 60 and 90 ($P > 0.05$).

Similarly, in the female rats (Table 9), there were generally insignificant reductions in direct bilirubin levels with no dose-specific pattern. There were no remarkable changes observed in bilirubin levels for the 90-day study period ($P > 0.05$). In comparison to the control, there were no remarkable changes observed in indirect bilirubin levels for the 90-day study period in female rats as well ($P > 0.05$).

Table 10 shows the effect of CPH pectin on total bilirubin levels after a 90-day continuous administration of CPH pectin in male SDRs. In comparison to the control, there were insignificant elevations in total bilirubin at day 30 ($P > 0.05$) in the treated groups. Generally, there was a reduction in total bilirubin levels observed for days 60 and 90 ($P > 0.05$) with no dose-specific pattern. There were no remarkable changes observed in total bilirubin levels. Again there were generally insignificant reductions in total bilirubin levels ($P > 0.05$) with no dose-specific pattern in female rats.

2.8. Effects of CPH Pectin on the Spleen of the Female SDR Spleen. Figure 1 shows tissues from the spleen with normal white pulp, consisting of lymphocytes, and normal red pulp

2.9. ,

TABLE 3: Results showing the effect of CPH pectin on red blood cell indices for female rats.

Red blood cell indices	Day	Control	Low dose 0.714 mg/kg	Medium dose 7.14 mg/kg	High dose 71.4 mg/kg
RBC (x 10^6/ul)	30	8.13±0.10	8.36±0.18	7.72±0.19	8.25±0.11
	60	7.77±0.06	8.15±0.15	7.96±0.14	7.88±0.14
	90	8.08±0.10	8.14±0.21	8.14±0.17	8.26±0.12
HGB (g/dl)	30	15.40±0.12	14.57±0.23	14.20±0.26	14.98±0.08
	60	14.95±0.13	14.87±0.27	14.27±0.29	14.62±0.15
	90	15.18±0.19	14.72±0.28	14.82±0.23	15.18±0.19
HCT (%)	30	43.80±0.47	42.88±0.40	41.42±0.86	43.48±0.40
	60	42.75±0.39	43.95±0.69	41.97±0.60	42.83±0.54
	90	44.30±0.94	43.87±0.87	44.22±0.68	44.97±0.65
MCV (fl)	30	53.85±0.61	52.35±0.35	53.70±0.54	52.73±0.39
	60	54.97±0.26	53.93±0.36	54.23±0.44	54.37±0.50
	90	54.72±0.61	53.97±0.68	54.32±0.40	54.42±0.38
MCH (g/dl)	30	18.95±0.14	17.78±0.15	18.38±0.18	18.15±0.17
	60	19.23±0.10	18.23±0.21	17.96±0.50	18.58±0.21
	90	18.82±0.12	18.10±0.26	18.20±0.16	18.38±0.13
MCHC (g/dl)	30	34.97±0.30	34.48±0.26	34.67±0.18	34.80±0.18
	60	34.55±0.12	34.00±0.14	34.32±0.20	34.12±0.16
	90	34.37±0.20	33.63±0.09	33.82±0.22	33.83±0.07
RDW-SD (fl)	30	26.00±0.18	26.22±0.23	25.98±0.21	25.98±0.13
	60	26.68±0.16	26.72±0.22	26.62±0.23	26.62±0.23
	90	26.58±0.20	26.58±0.12	26.75±0.59	26.16±0.10
RDW-CV (%)	30	11.53±0.25	11.37±0.24	11.50±0.79	10.95±0.15
	60	11.50±0.26	11.70±0.25	12.25±0.92	11.37±0.21
	90	11.35±0.31	11.67±0.09	11.93±0.89	11.17±0.44

TABLE 4: The influence of CPH pectin administration on white blood cell indices for male rats.

White blood cell indices	Day	Control	Low dose 0.714 mg/kg	Medium dose 7.14 mg/kg	High dose 71.4 mg/kg
WBC (x10^3 ul)	30	14.38±0.56	13.43±0.78	13.03±0.49	13.80±0.59
	60	14.47±1.48	12.60±1.09	13.68±0.80	12.50±1.18
	90	10.63±0.65	10.65±0.67	10.28±0.56	11.13±0.69
LYM (%)	30	76.57±4.80	78.03±3.39	79.28±1.65	75.13±1.38
	60	78.12±2.12	75.20±2.19	78.58±4.23	74.72±7.42
	90	71.95±2.54	67.13±0.63	68.98±4.19	68.60±3.81
NEUT (%)	30	17.33±1.90	17.07±1.05	17.50±1.40	17.52±2.08
	60	15.77±1.58	16.65±1.45	16.45±1.91	14.13±2.88
	90	21.42±2.20	20.00±0.76	19.96±2.80	20.40±2.46

consisting of vessels with red blood cells. There are regular lymphoid aggregates.

3. Discussion

Essential oils, extracted from the seeds of *Simmondsia chi- nensis,* have been reported to be beneficial in formulation as enhancers in matrix transdermal patches [30], emulgels [31], and microemulsions [32]. However, there seem to be adverse reports associated with suppression of bone marrow

with severe anaemia, profound weight loss, and death with a high dose of a constituent of the plant *S. chinensis* in chronic feeding studies in rats [33]. Data from preclinical toxicity studies are highly beneficial for the determination and evaluation of the effects of potential excipients on body weight and haematology for formulation development.

Eisele et al. reported on an associated low weight gain with the administration of a copolymer excipient, due to the swelling behaviour of the test material [34]. CPH pectin had similar swelling capacity and a viscous nature useful for

TABLE 5: The influence of CPH pectin administration on white blood cell indices for female rats.

White blood cell indices	Day	Control	Low dose 0.714 mg/kg	Medium dose 7.14 mg/kg	High dose 71.4 mg/kg
WBC (x10³ ul)	30	12.47±1.00	13.13±1.98	15.20±1.40	13.73±1.16
	60	14.98±1.05	15.55±2.10	16.72±1.21	14.40±1.20
	90	13.80±1.45	13.85±0.55	15.17±1.52	12.77±0.97
LYM (%)	30	70.57±3.22	67.32±2.63	70.50±4.21	71.58±2.10
	60	68.23±3.17	70.07±2.96	65.85±6.92	74.78±3.29
	90	69.20±3.22	70.37±3.93	66.00±2.84	67.17±2.16
NEUT (%)	30	20.42±1.17	20.47±1.97	21.30 ±2.19	21.78±1.90
	60	20.62±2.53	23.27±2.95	21.93±2.97	18.72± 1.45
	90	22.27±2.58	24.45±3.50	22.33±1.68	22.48±2.12

TABLE 6: The influence of CPH pectin administration on platelet indices for male rats.

Platelet indices	Day	Control	Low dose 0.714 mg/kg	Medium dose 7.14 mg/kg	High dose 71.4 mg/kg
PLT (x10³/ul)	30	1019.83±42.53	1033.50±38.47	1013.17±47.30	1046.83±48.54
	60	837.33±23.61	884.83±63.93	924.67±43.98	969.50±41.36
	90	881.83±49.09	923.67±63.82	972.83±54.03	1015.50±45.94
PDW (fl)	30	8.65±0.26	8.83±0.24	8.88±0.11	9.00±0.27
	60	9.12±0.14	9.62±0.36	9.57±0.11	9.47±0.35
	90	9.05±0.27	9.20±0.29	9.25±0.13	9.00±0.25
MPV (fl)	30	7.33±0.13	7.40±0.15	7.40±0.06	7.47±0.16
	60	7.47±0.14	7.68±0.17	7.77±0.10	7.73±0.21
	90	7.40±0.13	7.45±0.12	7.60±0.06	7.55±0.14
P-LCR (%)	30	6.62±1.32	7.78±0.86	7.57±0.30	8.42±0.89
	60	9.10±0.62	10.35±1.03	9.90±0.43	10.02±1.30
	90	8.18±0.74	8.37±0.74	9.28±0.39	8.63±0.94

its gelling ability and application in formulation [10] which could create a feeling of satiety. However, the 90-day oral continuous administration of CPH pectin did not influence the male rat body weight.

Female rat weight gain was rapid for all dose levels within the first four weeks and at a slower rate thereafter. There were no toxicologically relevant changes in body weight. This suggests that CPH pectin did not impair carbohydrate or fat metabolism as well [35]. It is also plausible that the presence of certain bioactives from cocoa pod husk has a positive influence on carbohydrate and lipid metabolism. Cocoa polyphenols are present in the cocoa pod husk. As many phenolic compounds are water-soluble, minimal quantities could be extracted together with CPH pectin [10, 36]. Cocoa polyphenols were reported to improve the cholesterol profile of patients. In this study, body weight, inflammatory markers, insulin resistance, and glycaemic control were not affected [37].

Moreover, there was no indication of any abnormal signs of toxicity in the rats under investigation, such as piloerection, cyanosis, paralysis, drowsiness, sedation, or gastrointestinal problems such as diarrhea.

Haematological preclinical toxicity studies are essential to identify test compounds that can exert toxic effects on the cellular constituents of blood (e.g., red blood cells (erythrocytes), white cells (leucocytes), and platelets). Any observed toxic responses could result from the direct effect of the test compound on the circulating cells. Haemolytic anaemias have been reported after subsequent red blood cell destruction or an interference with their production and/or their development. Data from such important studies is able to detect the development of anaemia and gain some insight into the mechanism leading to toxicity [29]. The haematopoietic system consisting of blood cells presents as a peculiar target organ as it is susceptible to damage on exposure to potential toxicants [38].

An evaluation of haematological indices after a 90-day administration of CPH pectin revealed no adverse effects on the red blood cell indices like RBC, HGB, MCH, HCT, and MCHC and was unaffected in both male and female SDRs. However, there was an incidental decrease in the MCV of the male rats after day 30 when rats received the medium dose of the CPH pectin. Balan and Veretiuc reported on the haemolytic activities of some polymer excipients [39], while other investigators reported excipient-induced haemagglu- tination due to the formation of polyelectrolyte complexes [40, 41]. Fernandes et al. also reported the deleterious effect of the degradation products of polymeric excipients on human

Drug- or excipient-induced white cell toxicity could be assessed by the measurement of the total number of circulating leucocytes (i.e., WBC count together with an estimation of the percentage of each of the different types of leucocytes, e.g., neutrophils) and lymphocytes present in the sample. A toxicant may cause leucopenia or leukocytosis. Such responses may be associated with one or more cell types (e.g., neutrophilia associated with certain inflammatory reactions) [29]. In this study, the administration of CPH pectin did not cause changes in white blood cells in both male and female SDRs. Similarly, the continuous administration of CPH pectin showed no deleterious effects on neutrophils, suggesting that CPH pectin did not inhibit granulopoiesis or neutrophil function. It also did not cause neutropenia. This also suggests that CPH pectin over the period did not cause neutrophil damage [46–48]. It may be inferred then that CPH pectin did not induce leucopenia, lymphocytosis, or lymphocytopenia [37].

Owing to the importance of various leucocytes in fighting infection and their involvement in immune responses, itcomes as a relief that CPH pectin did not induce adverse effects on these white blood cells. Moreover, administration of CPH pectin during the 90-day study showed no adverse immunological implications, unlike other excipients or drugs that exhibit immunotoxic effects [49, 50].

Platelets are susceptible to a variety of toxicants. Drug- or excipient-induced thrombocytopenia results from periph- eral destruction or bone marrow suppression. Increased peripheral destruction can result from immune mediated mechanisms or from platelets having a shortened survival time. A drug or

excipient may not affect platelet number but could impair platelet function. In this case, platelet counts are normal, but animals may exhibit abnormal bleeding tendencies [29].

There have been reports of polymer excipients that have thrombogenic activity subsequent to adhesion and activation of platelets and blood coagulation [51]. In this study, the platelet count was unaffected by the administration of CPH pectin over the 90-day period. This suggests that CPH pectin had no thrombocytopenic effect and did not exhibit any abnormal bleeding tendencies [29]. There were also no abnormal levels in the biomarkers PDW and MPV and P- LCR to suggest thromboembolic disease after the continuous administration of CPH pectin [52]. The findings also suggest

the compliance to good manufacturing practice with respect to the efficiency of extraction, suitable processing, and storage of CPH pectin required for pharmaceutical grade excipients, as there was no indication of the presence of harmful contaminants from adulteration, heavy metal, or microbial contaminants which could result in haematotoxicity [23, 53]. CPH pectin, belonging to the class of polysaccharides, was administered orally throughout the study. There have been previous reports indicative of the negligible absorption of pectin in the gastrointestinal tract when administered orally [54, 55]. Pectin is a soluble dietary fibre and is generally regarded as safe (GRAS) by the FDA, with a wide margin of safety [56]. This suggests that there is limited systemic exposure of CPH pectin, which may account for its observed safety profile even after continuous long-term administration.

The spleen is known to play an important role in the clearance of damaged and aging blood cells as well as enhancing the immune system of the host [37]. There were no treatment- or dose-related microscopic pathological changes in the spleen after administration of CPH pectin over the 90- day study period.

These findings, although in their early days, are signif- icant. This is because CPH pectin has demonstrated safety as natural polymer and thus its future approval and incor- poration into pharmaceutical formulation demonstrate the immense benefit of natural polymers as excipients as has been mentioned previously.

4. Conclusion

The findings suggest that CPH pectin administered up to 71.4 mg/kg for 90 days showed no evidence of toxicity on the major haematological indices in male and female SDRs.

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