



Antioxidant Properties and Effect of Ethanolic Extract of *Pulcria crispa* on Biochemical and Hematological Parameters of Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author HMD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RAS and AAE managed the analyses of the study. Author AAE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Pulcria crispa* (*P. crispa*) is an herbal plant traditionally used to treat common ailments.

Objective: In this study, we investigated *P. crispa* for its phytochemical constituents, antioxidant properties and effects on biochemical and hematological parameters as well as safety in albino rats.

Methods: Phytochemical analysis of ethanolic extract of *P. crispa* was conducted using standard procedures. *In vitro* 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and reducing power assay were used for the investigation of antioxidant activity of extract. Acute effects on physical and behavioral changes and mortality were monitored up to 72 h after administration of different doses of *C. crispa*. Chronic effects on body to organ ratio, biochemical and hematological parameters were measured after administration of rats with different doses of *P. crispa* extract for 30 days.

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Results: Alkaloids, flavonoids, phenols and tannins were the most abundant constituents found in *P. crispera* extract, which displayed a significant antioxidant activity measured by reducing power and DPPH assays. No physical, behavioral changes and mortality were noted following the acute treatment of rats with the extract. Similarly, no significant change in body to organ weight ratio was observed after chronic treatment. Hematological parameters including RBCs, Hb, PCV, MCV, MCH and MCHC values were unaltered while W.B.C count was elevated in *P.crispa* administered rats compared to control.

P. crispera extract significantly reduced glucose, urea, creatinine, cholesterol, bilirubin, AST and ALT levels, whereas, triglycerides and total protein levels were increased in response to *P.crispa* treatment.

Conclusions: This study demonstrates that *P. crispera* extract is rich in bioactive compounds and possesses significant antioxidant properties. Extract was also found to be safe and had no significant adverse effects on hematological parameters and exerted beneficial effects on biochemical parameters.

Keywords: *Pulcria crispera*; biochemical; antioxidant; acute; subacute toxicity; rats.

1. INTRODUCTION

Natural plant based products are well known for their beneficial effects to human health due to their numerous pharmacological properties [1]. These natural products are rich sources of secondary metabolites with antioxidant properties and shown to be beneficial against cancer, diabetes, atherosclerosis, alzheimer's disease, diabetes, infections [2]. According to the recent World Health Organization (WHO) report, about 25% of current drugs are plant-based and a large world population depend on medicinal plants for basic medical requirements [3]. Despite their relatively lower side effects, WHO emphasizes that the safety of all herbal medicine should be the overriding criterion in their selection and usage [3].

The Plant *P.crispa* offamily Asteracea, is widely grown all over the world. *P. crispera* is a traditional medicinal plant used for many years to cure various ailments, particularly heart and gastrointestinal disease due to its antioxidant properties [4-6]. Additionally, some of *Pulcicia* species are used as galactagogues, antiepileptics, antimicrobial, antifungal and antioxidants due to their active phytochemical constituents including monoterpenes, diterpenes and sesquiterpenes [7-9].

Due to the well-established role of antioxidants in disease prevention due to their ability to blunt oxidative stress, it is important to examine the antioxidant potential of *P. crispera* as well as to assess its safety in acute and chronic use. Therefore, in this study we measured antioxidant activity of *P. crispera* by reducing power power and DPPH assays. Possible toxic effects of *P. crispera*

was evaluated by measuring hematological and biochemical parameters in albino rats after acute and chronic administration of varying doses of ethanolic extracts of *P. crispera*.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

Ascorbic acid was purchased from Merck Co. (Darmstadt, Germany) and DPPH from Sigma Aldrich Co. (St. Louis, USA). All assay kits for biochemical analysis were obtained from either Randox Laboratories (Ardmore, UK) or Agappe Diagnostics (Kerala, India). Other chemicals used were of analytical grades and obtained locally.

2.2 Plant Material Collection

Aerial parts of *P. crispera* were collected during its flowering season, from Al-Madinah Al-Munawara region in Saudi Arabia. The *P. crispera*, arial parts were washed thoroughly with water, dried under shade for two weeks and powdered. The powdered material (300 g) was mixed with 1 L of 70% ethanol for 48 hours and centrifuged at 2500×g. Obtained supernatant was concentrated by evaporation and lyophilized. Obtained residue was dissolved in water and used to administer the rats.

2.3 Animal Experiment

Male albino rats weighing about 180 g were allowed to acclimatize to animal house facility for 2 weeks. Rats were maintained under 12 hr light-dark cycles and had free access to diet and water. To study the possible acute effects of *P. crispera*, rats were randomly segregated into five

groups with each group containing 5 rats. Rats in each of four groups were orally administered with a single aqueous extract dose of *P. crispera* at 250, 500, 1000, or 2000 mg/kg body weight concentration. Group of rats not administered with the extract served as control. Rats were placed under observation for up to 72 h for behavioral, physical changes and for the mortality. To investigate chronic effects of *P. crispera*, rats were orally administered with a single dose of aqueous extract of *P. crispera* at 50, 100, 200 mg/kg body weight concentration daily for 30 days. At the end of the experimental duration, rats from all groups were sacrificed under mild chloroform anesthesia followed by cervical dislocation. Blood samples (5 ml) were collected in tubes with or without anticoagulant. Serum was separated and used for biochemical analysis, while whole blood was used for the analysis of hematological parameters. Organs including pancreas, kidney, liver, heart and spleen were excised, washed in PBS and weighed [10].

2.4 Phytochemical Analysis

Phytochemical analysis of ethanolic extract was carried out to screen the presence of quinones, glycosinolates, tannins, terpenoids, flavonoids, saponins, glycosides, anthroquinones and alkaloids, following the standard protocol [11,12].

2.5 The DPPH Assay

Antioxidant activity of ethanolic extract of *P. crispera* was measured by DPPH assay, which relies on the reduction of DPPH free radical in the presence of antioxidant molecules. The method employed for DPPH assay was reported by Bracca et al., 2001 [13]. Five ml DPPH methanolic solution was mixed with 10, 20, 40, 80, or 160 µg/ml concentration of plant extract or ascorbic acid standards at matching concentrations. Absorbance was measured at 517 nm at 0, 1, 15 and 30 minutes subsequent to DPPH addition. The change in absorbance at above time intervals was calculated and scavenging activity was expressed as:

$$\% \text{ radical scavenging} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control Absorbance}} \times 100$$

2.6 Reducing Power Activity (RPA?) Determination

The RPA relies on the fact that substances, with reducing potential, react with potassium

ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which in turn reacts with ferric chloride to form ferric-ferrous complex. The method documented by (Oyaizu) was employed in this study [14]. Plant extract at 1, 2, 4, 8 or 16 µg/ml concentrations or ascorbic acid standards at identical concentrations were mixed with 2.5 ml potassium ferricyanide and 2.5 ml phosphate buffer. The mixture was incubated at 50°C for 20 minutes and following this 2.5 ml of TCA solution was added. Contents were centrifuged at 6000 rpm for 10 minutes and the clear supernatant was obtained. To the obtained supernatant, 0.5 ml of ferric chloride was added and the absorbance was measured at 700 nm.

2.7 Relative Organ Weights

Following excision pancreas, liver, spleen, kidney, and heart were weighed and their relative weights were calculated as below.

$$\text{Relative organ weight} = \frac{\text{absolute organ weight (g)}}{\text{Body weight of rat at the time of sacrifice day (g)}} \times 100$$

2.8 Hematological Parameters

Hematological parameters including red and white blood cell counts were determined using *Neubauer counting Chamber* as described by [15]. Hemoglobin (Hb) concentration was measured by cyanmethemoglobin method [16]. Packed cell volume (PCV) and hematocrit (Hct) was determined by obtaining packed cell percentage using microhematocrit capillary tubes [17].

2.9 Biochemical Estimations

Serum levels of cholesterol, glucose, uric acid, urea, triglycerides, total bilirubin, creatinine, total proteins, serum activity of, alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) were measured using an automated biochemical analyzer (Vitalab Selectra E Mod. 6002-160, Vital Scientific, EU).

2.10 Statistical Analysis

Statistical analysis was carried out by SPSS software (version 17, Chicago, IL, USA). Comparisons between groups were carried out by ANOVA followed by Post hoc Dunnett's test. A $p < 0.05$ was considered significant.

3. RESULTS

3.1 Phytochemical Constituents of *P. crispera*

The quantitative analysis revealed alkaloids to be the most abundant phytochemical component *P. crispera* extract, followed by saponins, phenols and tannins, flavonoids, reducing compound, glycosides, triterpenoids, while proteins & acids were the least abundant phytochemical contents (Table 1).

3.2 Acute Toxicity Study

The rat that receives high doses of the drug seems to be hyperactive, which persisted for first 1 1/2 h and then they resume their normal activity without any change in any of their activities. Along with high locomotor activity, they also found to scratch around the mouth for about 10 min. In this study, no mortality was observed till the end of the study (Table 2).

3.3 DPPH Assay

DPPH assay was used to determine the antioxidant properties of *P. crispera*. The data are presented in Table 4. As can be seen antioxidant activities of plant extract at indicated concentrations significantly and dose dependently matched to the antioxidant activities measured with the ascorbic acid. Thus, it may be postulated that *P. crispera* has the potential to reduce the free radicals.

3.4 RPA Assay

The reducing power of plant extract and ascorbic acid standard are provided in Table 3. Plant extract at different doses demonstrated a significant reducing power which was comparable to ascorbic acid standard at identical concentrations. Importantly reducing power of extract was dose dependent as was the case with the standard.

3.5 Behavioral and Physical Changes and Mortality

The rats that received high doses of the drug were hyperactive with higher locomotor activity for the initial 90 minutes after the administration of extract. All the rats administered with the different doses of extract were also found to scratch around their mouths for first for about initial 10 minutes. These rats gradually reverted back to their normal activity. No other physical or behavioral changes were observed in any of the rats administered with the extract. Likewise, no mortality was observed.

3.6 Relative Organ Weight

Effects of *P. crispera* extract on different organs of rats are presented in Table 5. The organ to body weight ratios of rat kidney, liver, pancreas, heart and spleen, were comparable with controls. No significant variation was noted in any of the tested organs with the doses of the extracts examined.

Table 1. Preliminary qualitative phytochemical analysis of *P. crispera* aerial portions

Phytoconstituent	Water	Ethanol	Methanol	Ethyl acetate	Hexane
Quinones	+++	+++	+++	+	+
Anthroquinone	-	-	-	-	-
Tannins	+	+++	+++	-	-
Coumarins	++	+++	+++	-	-
Saponins	++	+	+	+	+
Glycosides	+	++	++	+	-
Flavonoids	+	+++	+++	-	-
Alkaloids	-	+++	+	+	+
Triterpenoids	+	++	++	++	++
Phenolic	+	++	+++	-	-

Table 2. Acute toxicity profile of *P. crispera* extract

Group	Dose (mg/kg)	No of animals	Mortality
Group 1	250	5	0/5
Group 2	500	5	0/5
Group 3	1000	5	0/5
Group 4	2000	5	0/5

Table 3. *In vitro* antioxidant activity: DPPH assay

Sample	Conc. ($\mu\text{g/ml}$)	% DPPH radical scavenging activity
Ascorbic acid	10	80.32 \pm 0.23
	20	82.48 \pm 0.16
	40	84.76 \pm 0.12
	80	87.79 \pm 0.13
	160	89.37 \pm 0.17
Plant extract	10	75.83 \pm 0.12
	20	75.91 \pm 0.11
	40	76.07 \pm 0.03
	80	76.18 \pm 0.02
	160	76.29 \pm 0.01

All values are expressed as mean \pm SEM, $p < 0.05$ compared to standard. The data was analyzed by one-way analysis of variance (ANOVA) followed by Post hoc Dunnett's test by using the software Graph pad prism 5

Table 4. Reducing power assay

Tested Material	Concentrations ($\mu\text{g/ml}$)	Reducing Power in % (\pm SEM)	IC50 ($\mu\text{g/ml}$)
Ascorbic acid	1	19.48 + 0.16	2.35
	2	43.65 \pm 0.12	
	4	68.26 \pm 0.02	
	8	81.97 \pm 0.05	
	16	97.25 \pm 0.06	
Plant extract	1	9.68 \pm 0.16	4.64
	2	22.29 \pm 0.13	
	4	28.78 \pm 0.19	
	8	36.29 \pm 0.18	
	16	78.58 \pm 0.12	

All values are expressed as mean \pm SEM, $p < 0.05$ compared to standard

Table 5. Effect of *P. crispera* ethanolic arial parts extract on the organ–body weight ratios

Organs	Control	<i>P. crispera</i> concentration (mg/kg body weight)		
		50	100	200
liver	4.47 \pm 0.02	4.49 \pm 0.06	4.48 \pm 0.03	4.46 \pm 0.07
kidney	1.49 \pm 0.06	1.49 \pm 0.02	1.49 \pm 0.07	1.50 \pm 0.03
Pancreas	0.76 \pm 0.07	0.75 \pm 0.08	0.75 \pm 0.03	0.75 \pm 0.02
spleen	0.91 \pm 0.03	0.91 \pm 0.02	0.92 \pm 0.04	0.92 \pm 0.06
Heart	0.71 \pm 0.04	0.72 \pm 0.01	0.72 \pm 0.08	0.71 \pm 0.02

Values are expressed as Mean \pm S.D. Significant at ($P < 0.05$) as compared to control Group

3.7 Hematological Parameters

The effects of *P. crispera* extract on the hematological parameters are shown in Table 6. A significant increase in WBC count was noticed in rats treated with 50, 100 or 200 mg/kg body weight concentrations of *P. crispera* extract. However, no significant difference in RBC, Hb, MCH, HCT and MCV values were observed in rats administered with the indicated concentrations of *P. crispera* as compared to control.

3.8 Biochemical Estimations

Effects of *P. crispera* on biochemical parameters are given in Table 6. There was a significant decline in glucose, urea, creatinine, total cholesterol, bilirubin, AST and ALT levels in rats treated with *P. crispera* extract compared to those in control rats. Whereas, rats treated with extract demonstrated a significant increase in triglycerides and total protein was noted in extract treated rats as matched to control. No significant change was observed in other studied

parameters, which were comparable to those in control.

4. DISCUSSION

Recently there is an increased interest in research aimed at discovering safe nutraceutical drugs for the treatment of various human ailments. The traditional medicinal plants remained an important source of raw materials to the pharmaceutical drug industry due to their numerous beneficial effects. On the other hand, less studied wild plants are recently shown to possess antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties [18]. *P. crispa* is extensively used in traditional medicine and is known to possess a number of phytochemicals. In the present study phytochemical analysis showed that *P. crispa* is rich in various compounds including flavonoids, steroids, glycosides phenols, glucosinolates, alkaloids, coumarins and terpenes. These data are consistent with the previous studies which have shown *p. crispa* extract to contain similar active compounds [19-21]. Importantly, these bioactive agents are considered potential mediators of health benefits of medicinal plant.

These phytochemicals are well-known for their free radical scavenging activities and therefore, promoted as antibacterial, anti-inflammatory, and antitumor activities [22-24]. In this study, *P. crispa* extract exhibited a significant DPPH scavenging antioxidant potential and also reducing power as evident from reduction of potassium ferricyanide to form potassium ferrocyanide. Moreover, the effects were concentration dependent as increased antioxidant activity was noticed with the increased concentration of extract. These findings point out that the *P. crispa* extract can be a major source of natural antioxidant and can be utilized in the prevention and treatment of chronic diseases [25]. The antioxidant activity of *P. crispa* found in the study could be attributed to its major components such as phenols, flavonoids, glucosinolates, alkaloids, glycosides, steroids, coumarins, and terpenes. Besides, other minor constituents such as s glycosides and aponins. *P. crispa* could also display antioxidant activities and therefore individual component of *P. crispus* need further investigation for their, involvement in the antioxidant activity [26].

Table 6. Effect of *P. crispa* extract on haematological composition of all experimental rat groups after 28 days

Parameters	Control	<i>P. crispa</i> concentration (mg/kg body weight)		
		50	100	200
W.B.C. ($\times 10^3/\mu\text{L}$)	7.29 \pm 1.43	7.34 \pm 1.68*	7.73 \pm 1.44**	8.21 \pm 1.67**
R.B.C. ($\times 10^6/\mu\text{L}$)	6.55 \pm 1.55	6.39 \pm 1.17	6.08 \pm 1.57	5.88 \pm 1.62
Hb (g/dl)	14.89 \pm 0.24	14.66 \pm 1.07	14.53 \pm 1.35	14.48 \pm 0.24
PCV (%)	43.84 \pm 0.62	43.63 \pm 1.14	43.18 \pm 1.23	42.93 \pm 1.37
MCV (fl)	46.57 \pm 1.42	45.68 \pm 1.42	45.12 \pm 1.28	44.85 \pm 2.15
MCH (pg)	14.46 \pm 0.66	14.24 \pm 1.11	14.07 \pm 0.42	14.51 \pm 0.58
MCHC (g/dl)	40.54 \pm 1.18	37.48 \pm 1.83	39.76 \pm 2.24	41.27 \pm 2.56

A compared to control, * $p < 0.05$, ** $p < 0.01$ Values are expressed as mean \pm SEM

Table 7. Effects of *P. crispa* extract on serum biochemical parameters of rats

Group	Control	50 mg/kg	100 mg/kg	200 mg/kg
glucose level (mg/dl)	97.75 \pm 1.32	91.58 \pm 1.36 *	88.93 \pm 1.21*	81.00 \pm .74**
Urea (mg/dl)	23.25 \pm 0.03	22.94 \pm 0.02	22.87 \pm 0.02	22.72 \pm 0.08*
Creatinine (mg/dl)	0.76 \pm 0.04	0.72 \pm 0.05a*	0.73 \pm 0.07a*	0.74 \pm 0.08*
Cholesterol (mg/dl)	58.45 \pm 1.23	51.44 \pm 2.52*	48.58 \pm 2.38**	46.17 \pm .64**
Triglycerides (mg/dl)	73.62 \pm 1.21	97.41 \pm 1.28**	86.18 \pm 1.87**	83.74 \pm 2.57**
Protein (mg/dl)	76.15 \pm 3.18	81.45 \pm 1.68*	87.37 \pm 4.33**	92.34 \pm 0.19**
Bilirubin (mg/dl)	0.72 \pm 0.03	0.67 \pm 0.05*	0.38 \pm 0.01***	0.25 \pm 0.04***
AST (U/l)	56.76 \pm 0.21	54.65 \pm 0.11*	52.29 \pm 0.15*	49.88 \pm 0.08**
ALT (U/l)	51.13 \pm 0.21	35.18 \pm 0.02**	37.13 \pm 0.12**	38.87 \pm 0.15**
ALP (U/l)	64.53 \pm 0.12	64.31 \pm 0.07	63.92 \pm 0.13	64.76 \pm 0.11

A compared to control; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Values are expressed as mean \pm SEM

The antioxidants react with free radicals such as DPPH and negate their potential [27]. Moreover, scavenging effects of antioxidants on the DPPH are dose dependent to certain extent [28].

In this study, the safety of different doses *P. crispa* extract was established by physical, and behavioral changes and the mortality. It is shown that oral LD50 of any drug or drug >1000 mg/kg is considered safe [29]. This correlates in the case of *P. crispa* extract that the dose of 2000 mg/kg could be safe. However, LD50 is not a standard way of measuring safety as many variables such as gender, age, animal species, diet, strain, bedding, caging conditions, and ambient temperature, can affect the LD50 value. [30].

The reaction of the plant extract with the tissues may elicit inflammation, and cellular constriction which may alter organ/body ratio. In the present study, no significant change in body to organ weight ratios in *P. crispa* administered rats compared to those in control rats supporting the nontoxic nature of *P. crispa* [31].

P. crispa extract also exerted no effect on Hb and RBCs which shows that the extract treatment did not led to anemia [32]. The decrease in HCT was also within normal range in the extract treated rats. Packed cell volume, WBC and Hb are important indicators of pathophysiological change [15]. The observed increase in WBC count in animals treated with extract underscores the ability of *P. crispa* to augment immunity [33]. This is supported by the fact that change in WBC count upward correlates with the functional and intact immune system and its ability to fight off infection [34].

The liver and kidney functional markers are important and useful parameters to measure for the toxicological assessment of pharmacological agents as it is well-known that both are vital organs in the metabolism and clearance of drugs. Moreover, biochemical changes result in the organ dysfunction [35,36]. In the present study, investigated biochemical markers either unaltered compared to control or favorably changed indicating that there are no adverse effects of *P. crispa* extract and in fact there are health promoting effects. These data also corroborate with the lack of any detrimental effects of *P. crispa* extract on hematological parameters and organ to body weight ratio in the present study.

Nutraceuticals are naturally derived bioactive compounds that are found in foods, dietary supplements and herbal products. Numerous prospective benefits for health are offered by Nutraceuticals, such as improve health, treat and prevent many diseases as cancer, inflammation, cardiovascular diseases, obesity, diabetes and others [37]. So, the use of natural antioxidants, as that found in the extract of *Pulcra crispa*, in the food industry and nutraceutical products is recommended.

Since oral administration of *P. crispa* up to 2000 mg/kg, dose had no major effect on organ weight, biochemical and hematological parameters and organ to body weight ratio, it could be considered safe., Further, *P. crispa* at 50, 100 and 200 mg/kg exerted no adverse effects on lungs, kidney, spleen, liver, and heart after 30-day chronic treatment period. Therefore, it could be suggested that the *P. crispa* plant extract is safe and could be promoted for its beneficial health effects.

5. CONCLUSION

Phytochemical analysis revealed that *P. crispa* is an excellent source of bioactive metabolites including phenolic and flavonoids compounds. Consistent with the well-established properties of these bioactive compounds, *P. crispa* extract exhibited a significant antioxidant activity. Besides, this study has demonstrated that there are no acute and chronic adverse effects of *P. crispa* on vital organs, hematological parameters, liver and kidney functions and other biochemical indices. In fact *P. crispa* extract displayed several favorable effects on biochemical parameters. Therefore, *P. crispa* appears to be an important medicinal plant with multiple health promoting properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval to carry out animal work was obtained from the Ethical Committee of Biomedical and Medical Research, University of Taibah (No. 040 - 1439, dated 10/04/2018). Also, the Laboratory Animal use and Care Principles, Canadian Council on Animal Care Guidelines and Protocol Review were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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