



HEALTH SCIENCES

Pharmacological and toxicological evaluation of two anti-asthmatic polyherbal formulations

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Abstract: The study aim was to evaluate the toxic potential of two polyherbal formulation i.e. " PH 1 & PH 2" and scientific validation of their anti-asthmatic use. Acute oral toxicity study as per OECD 425 TG was conducted. For validation of anti-asthmatic claim, *in vivo* assay named Ovalbumin (OVA)-induced murine method in Wistar rats was used. Eosinophils and IgE antibody were quantified post-administration of low and high doses of the formulations. No mortality was observed in acute toxicity study. Elevated levels of alkaline phosphatase and damaged liver structure indicating the hepatotoxicity were more pronounced in PH 2 treated rats. Congestion in kidney tissue and increased urea level were evident of the nephrotoxic nature of PH 2 in animals. Treatment with selected polyherbal products decreased the MDA level while increasing the SOD and GSH levels in lung tissue homogenates. The maximum decrease in IgE load (3.18 ± 0.08 IU/mL) was found in rats treated with 12 mg/kg dose of PH 1 followed by 100 mg/kg dose of PH 2 (3.44 ± 0.06 IU/mL). It was concluded that both polyherbal formulations had anti-asthmatic activities, however, PH 1 exhibited the liver and kidney toxicity and should be cautiously used.

Key words: Acute toxicity, polyherbal, IgE, OECD 425, anti-asthmatic.

INTRODUCTION

Asthma is generally regarded as the most common chronic inflammatory disorder characterized by the presence of inflammation and bronchial hyperresponsiveness that reversibly obstruct airflow (National & Prevention 2007). The aggravated response to airway-narrowing is generally triggered by changes in weather, viral respiratory infections, allergens or exposure to irritants and exercise that leads to repeated symptoms of chest tightness, breathlessness, wheezing or coughing and tend to vary in intensity over time. Asthma can affect individuals of all ages and ethnicity (Alhassan et al. 2016, Quirt et al. 2018).

Asthma being a heterogeneous condition in children and adults shows quite complex

phenotypes under multifaceted host-environment interactions. Precisely, both allergic (e.g., molds, dust mites, etc.) and non-allergic stimuli (e.g. tobacco smoke, cold air and exercise, etc.) can trigger asthma (Kim & Mazza 2011). Previously, it was thought that the stimulus then triggers an intricate web of immune responses through activation of type 2 T-helper lymphocytes (Th2) which produces interleukins (IL-4, IL-5, IL-9 and IL-13) that leads to the recruitment of eosinophils and production of immunoglobulin E (IgE) causing inflammation of the airways (Lemanske & Busse 2010).

Many polyherbal products are recognized globally to provide symptomatic relief and ameliorate the disease progression. Various target specific biological activities shown by these herbal

and poly-herbal products include bronchodilation, anti-anaphylactic, mast cell stabilization, anti-allergic, anti-spasmodic, anti-inflammatory and inhibition of mediators such as lipoxygenase, cyclooxygenase, leukotrienes, phosphodiesterase, cytokine and platelet activating factor in the treatment of asthma (Mali & Dhake 2011). In sub-continent, several industries are involved in the production of Ayurvedic or Unani herbal formulations. There are more than six thousand medicinal herbs that are used in treating various medical conditions in India (Seth & Sharma 2004). Among these formulations, “Kushta Abrak Kalan (PH 1)” of Hamdard laboratories and “Qars Dama Ajmali (PH 2)” of Ajmal Dawa Khana are used for their anti-asthmatic claims. PH 1 is used to treat cough, asthma, spermatorrhoea and leucorrhoea, and is prepared from white talc, *Aloe vera* mucilage and potassium nitrate. PH 2 is comprised of *Piper longum*, *Glycyrrhizaglabra*, *Acacia arabica*, *Papaver somniferum* and *Viola odorata*. It is used for the management of asthma and productive cough. There is no scientific study carried out on the anti-asthmatic evaluation of these polyherbal formulations. In 21st century, toxicity evaluation of pharmaceuticals, chemicals and food substances etc. become imperative to ensure safety of testing substances (Parasuraman 2011). Therefore, the current study was designed to assess the medicinal safety profile and toxicity index of selected polyherbal products and validate product claim as an anti-asthmatic agents.

MATERIALS AND METHODS

Materials

PH 1 was tablet manufactured by Ajmal Dawa Khana, Pakistan (Batch # 009, MFG: 01-18, EXP: 01- 21) while PH 2 was tablet manufactured by Hamdard Laboratories, Pakistan (Batch # 01, MFG: 02- 16, EXP: 02-21).

Quantitative phytochemical analysis

Flavonoid content

Sample solution 0.3 mL, 0.5 mL of 0.5M NaNO₂, 3.4 mL of 30% methanol and 0.5 mL of 0.3M AlCl₃ were added to a test tube and mixed well. After 5 min, 1 mL of 1N NaOH was added. Rutin was used as reference standard. Absorbance was measured at a wavelength of 506 nm. A standard curve was drawn by plotting absorbance vs different concentrations of reference standard and the linear regression equation was obtained. The total flavonoids content was expressed as mg of rutin equivalents (RE)/ g of sample by linear regression curve, obtained from the calibration curve of rutin (Saeed et al. 2012).

Phenolic content

Folin-Ciocalteu reagent method was followed for determining total phenolic content. To 1 mL sample, 10% Folin-Ciocalteu reagent (2.5 mL) and 2% Na₂CO₃ (2 mL) were added, mixed and incubated for 15 min at room temperature. As reference standard different concentrations of gallic acid were used. Absorbance was recorded at 765 nm. The total phenolic content was expressed as mg equivalents of Gallic acid (Yadav & Agarwala 2011).

DPPH free radical scavenging activity

Sample solution was made by dissolving the powdered drug in 95% methanol at a concentration of 1 mg/mL. To prepare a stock solution, 3.94 mg of DPPH was dissolved in 100 mL of methanol that was added to different concentrations of sample (10-500 µg/mL). Sample solutions were shaken vigorously, and incubation was done in the dark for 15 min. After incubation, the absorbance was taken at 517 nm. Control solution was prepared by following the same procedure without the test sample. For reference standard, different

concentrations of rutin were prepared (Saeed et al. 2012). DPPH scavenging effect (percentage of inhibition) was calculated by using the following formula:

Animals

Albino healthy rats of either sex were obtained from Veterinary Research Institute (VRI) Lahore and placed in animal house of Riphah International University Lahore. Animals were provided standard international conditions of maintaining animal house i.e. room temperature $23 \pm 2^\circ\text{C}$, humidity 45-55%, 12 h day and night cycle. Animals had free access to food and water and were kept in the animal house fourteen days before the start of experiments in order to acclimatize them.

Ethical approval

Studies were performed after getting approval from research ethical committee (REC) of Riphah International University. Study reference no. was REC/RIPSLHR/2018/25. REC is ruled under the regulation of the Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (1996).

Acute oral toxicity study

Female, non-pregnant, nulliparous rats were selected for this study that was carried out by following OECD TG 425 guidelines (Gribaldo et al. 2005). Rats were divided into control and treatment groups (n=5). Limit dose 2000 mg/kg dose was given to single rat and observed for one day when animal survived then four more rats were administered the same dose. Animals in control group were monitored in the same way as that of treatment groups. All the rats were observed daily till 14 days for any behavioral changes or signs of toxicity. Body weights were recorded on weekly basis. On 15th day, blood samples were collected via cardiac puncture for biochemical analysis and animals were

humanely killed under anesthesia (anesthesia was given with 3-5% isoflurane with oxygen) and vital organs were removed for histopathological analysis.

Validation of anti-asthmatic activity

In vivo assay called as Ovalbumin (OVA)-induced murine method was used to validate anti-asthmatic activity of both polyherbal formulations. Thirty-five mice were divided into seven groups (n=5). Group 1 was normal control, receiving vehicle only. Group 2 was disease control. Group 3 served as standard control receiving 12 mg/kg of salbutamol. Groups 4 -5 were treated with PH 1 at doses 6 and 12 mg/kg respectively. Groups 6-7 were given PH 2 at doses 50 and 100 mg/kg respectively. Treatments were given to groups 4-7 on days 23-27th via oral gavage 1 h before OVA administration.

All the doses were calculated from human dose.

For sensitization (asthma induction), groups 2-7 were administered 50 μg OVA and 1 mg alum along with 100 μL of Phosphate buffer saline (PBS) on 0, 7th and the 14th day intraperitoneally. Next, these animals were challenged with 100 μg OVA in 50 μL PBS on days 12th, 25th, 26th and 27th via the intranasal route. On 28th day, after 24 h of last challenge, rats were humanely killed after taking a blood sample through cardiac puncture and lungs were excised and preserved in 10% formalin for histopathology (Shamshuddin & Zohdi 2018).

$$\text{DPPH \% scavenging activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Biochemical analysis

Malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH) levels were quantified in tissue homogenate of the lungs. Tissue homogenate was prepared by mixing 1g

of lung tissue in 10 mL of 0.1M PBS (pH 7.4) by tissue homogenizer followed by centrifugation at 3000 rpm for 15 min and supernatant of tissue homogenate was used for biochemical analysis.

MDA level

Thiobarbituric acid (TBA) reagent (0.38 % TBA, 15% Trichloroacetic acid (TCA), 0.25 N HCl) 3 mL, was added to 1 mL supernatant of tissue homogenate, mixed together and cooled in ice bath for 15 min followed by centrifugation at 3500 rpm for 10 min. Absorbance was measured at 532 nm (Hira et al. 2019). Concentration of MDA was calculated as below.

$$\text{MDA} = \text{Abs}_{532} \times 100 \times V_T / (1.56 \times 10^5) \times W_T \times V_U$$

Whereas, Abs_{532} was the absorbance, V_T was the total volume of the mixture (4 mL), 1.56×10^5 is the molar extinction coefficient, W_T was the weight of lungs, and V_U was the volume of tissue homogenate.

SOD activity

SOD activity was determined through reaction mixture, which was made by adding 0.1 mL pyrogallol solution, 2.8 mL of 0.1 M potassium phosphate buffer (pH 7.4) and 0.1 mL of supernatant of tissue homogenate. Absorbance was taken at 525 nm. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation under the assay conditions (Hira et al. 2018).

GSH level

Phosphate solution (4 mL), DTNB reagent (0.5 mL) and 10% TCA (1 mL) were added to 1 mL of supernatant of tissue homogenate. Absorbance was measured at 412 nm (Mir et al. 2019). Concentration of GSH level was calculated as: $\text{GSH} = Y - 0.00314 / 0.0314 \times \text{DF} / \text{LT} \times V_U$ Whereas, Y= absorbance at 412 nm, DF= dilution

factor (0.1 mL), LT= lungs tissue homogenate, V_U = volume of supernatant of tissue homogenate.

Quantification of IgE antibody

Serum level of allergen-specific IgE antibody was measured by solid-phase enzyme-linked immunosorbent assay (ELISA) through the mouse Anti-OVA IgE Antibody (Catalog # 3004) assay Kit (Pykalainen et al. 2005).

Statistical analysis

Data were presented as mean \pm S.D. For statistical analysis, two-way ANOVA followed by Bonferroni test was applied by using GraphPad Prism® version 5.0. $p < 0.05$ value was set as the statistical significance level.

RESULTS

Quantitative phytochemical analysis

Flavonoid content

Flavonoid contents were calculated by using the linear regression equation $y = 0.319x + 0.024$ ($R^2 = 0.996$). PH 2 contained 0.53 mg RE/mg of sample flavonoid content that was higher than that of quantified in PH 1 i.e. 0.49 mg RE/mg of sample.

Phenolic content

Linear regression equation $y = 0.323x + 0.008$ ($R^2 = 0.921$) was used to quantify the phenolic content. Phenolic content found in PH 1 (1.047 mg GAE/mg of sample) were more than three times greater than value quantified in PH 2 (0.33 mg GAE/mg of sample).

DPPH free radical scavenging activity

Samples showed a concentration dependent increase in % scavenging effect. Rutin was used as standard antioxidant compound and it showed 94.73% inhibition at 500 $\mu\text{g/mL}$

concentration. On the same concentration polyherbal products had very low % scavenging effect which was 28.95% with PH 2 and 20.65% with PH 1 (Table I). IC_{50} value of rutin was 3.18 $\mu\text{g/mL}$. IC_{50} of PH 2 and PH 1 had 974 $\mu\text{g/mL}$ and 2334 $\mu\text{g/mL}$ respectively. It was calculated from linear regression equations. IC_{50} had an inverse relation with potency, in this study, rutin was the most potent antioxidant and in the samples, PH 2 possessed more antioxidant potential as compared with that of PH 1.

Acute oral toxicity study

Behavioral observations displayed normal fur and skin, urine color and feces consistency, respiration and sleep in the treatment groups. No convulsions and mortality were recorded in 14 days study (Table II). Body weights of control and treatment groups were increased progressively in both control and treatment groups (Figure 1).

Biochemical analysis

The collected blood samples were centrifuged for 10 min at 3000 rpm for collection of blood plasma, which was used for determination of

blood sugar, lipid profile, liver function tests, renal function tests and complete blood count.

Blood sugar

Random and fasting blood sugar of control and both treatment groups were checked. No significant variations were observed between control and both treatment groups. Results are summarized in Figure 2.

Renal function test

No change in serum creatinine was observed between control and both treatment groups while serum urea was higher in PH 1 treated group when compared with control group (Figure 3).

Lipid profile

No variations were observed in levels of cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), very low-density lipoproteins (vLDL), total lipids, and cholesterol/HDL ratio among control and both treatment groups (Table III).

Table I. The percentage DPPH scavenging activities of samples and rutin at different concentrations.

Sr. No.	Concentrations ($\mu\text{g/mL}$)	Rutin	PH 1	PH 2
1	15.62	44.18 \pm 0.963	11.76 \pm 0.050	5.070 \pm 0.800
2	31.25	50.67 \pm 1.948	13.61 \pm 0.259	10.75 \pm 0.025
3	62.50	57.34 \pm 2.099	14.18 \pm 0.309	11.62 \pm 0.090
4	125	67.56 \pm 2.250	16.14 \pm 0.725	16.94 \pm 0.595
5	250	81.49 \pm 0.996	16.55 \pm 0.204	18.93 \pm 0.720
6	500	94.73 \pm 0.644	20.65 \pm 0.304	28.95 \pm 0.415

Values are presented as mean \pm SEM; n=3, PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

Table II. Behavioral patterns of rats at a limit dose (2000mg/kg P.O.) in acute toxicity study.

Parameters	Time																	
	30 min			4 h			24 h			48 h			7 th day			14 th day		
	Control	PH 1	PH 2	C	H	PH 2	C	PH 1	PH 2	C	PH 1	PH 2	C	PH 1	PH 2	C	PH 1	PH 2
Fur & skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urine(color)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Feces	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N
Convulsions	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Itching	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mortality	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Respiration	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N=Normal, P = Present, A = Absent. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

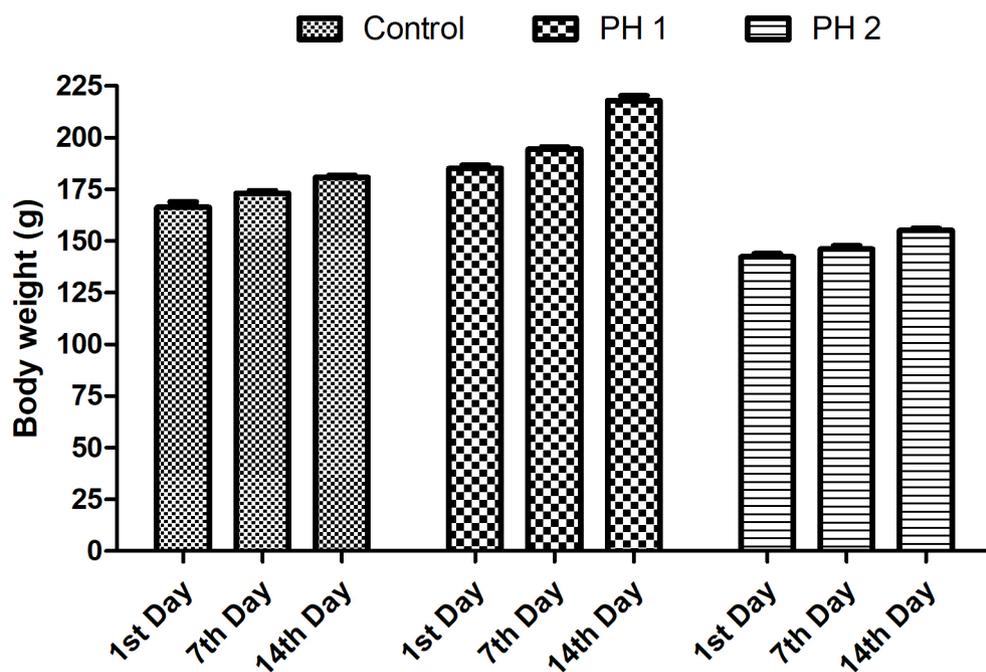


Figure 1. Effect of polyherbal products on body weight of rats in acute toxicity study Values are presented as mean ± SEM. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

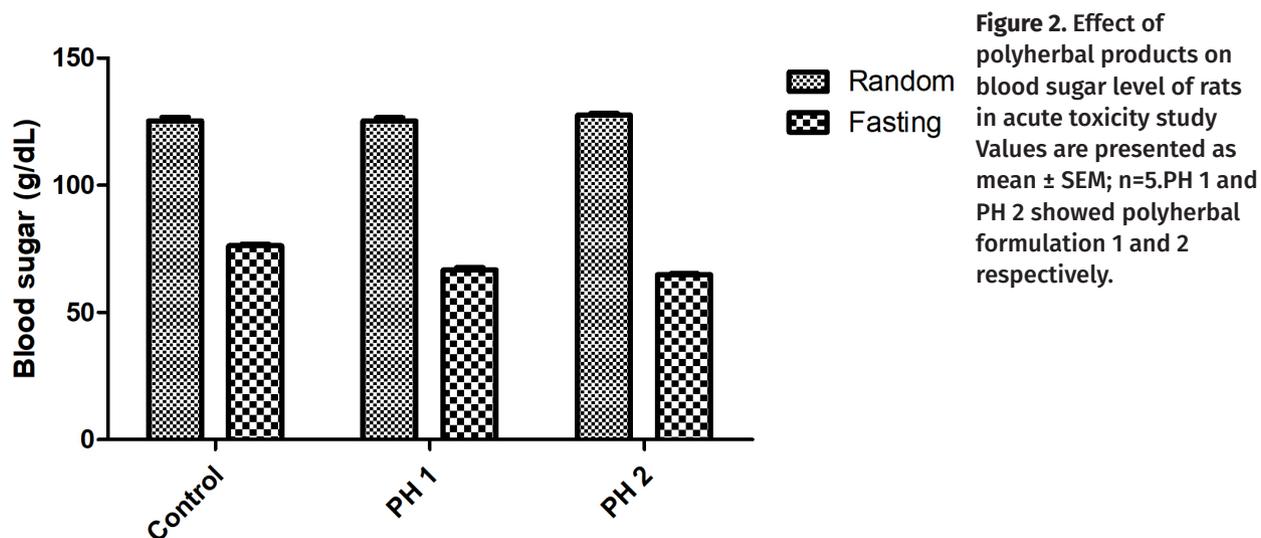


Figure 2. Effect of polyherbal products on blood sugar level of rats in acute toxicity study. Values are presented as mean ± SEM; n=5. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

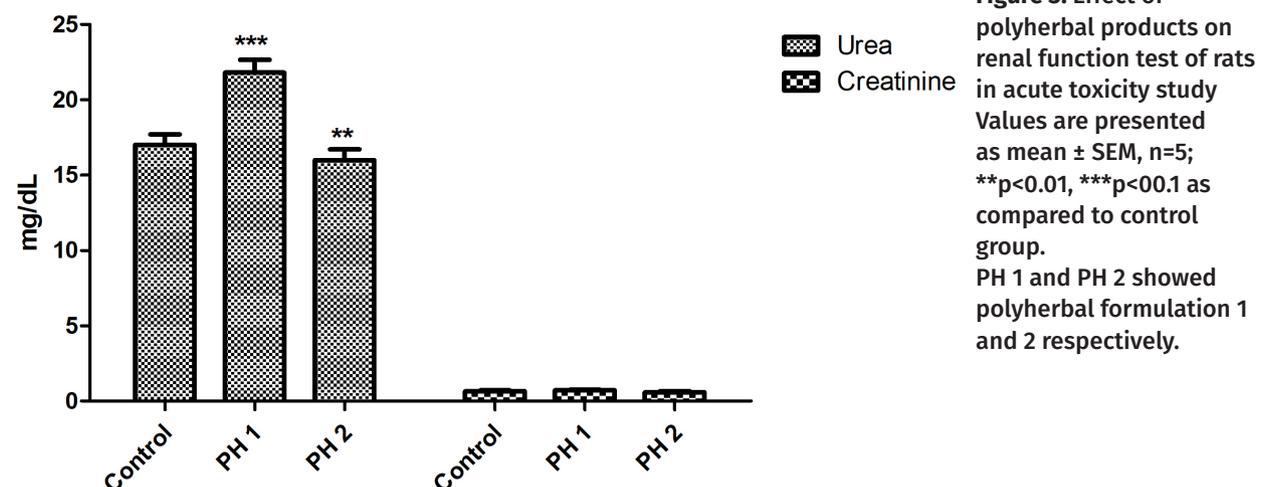


Figure 3. Effect of polyherbal products on renal function test of rats in acute toxicity study. Values are presented as mean ± SEM, n=5; **p<0.01, ***p<0.001 as compared to control group. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

Table III. Effect of polyherbal products on lipid profile of rats in acute toxicity study.

Parameters (mg/dL)	Control	PH 1	PH 2
Cholesterol	62.0 ± 0.7	59.0 ± 2.4	67.0 ± 3.3
Triglycerides	54.4 ± 1.0	52.2 ± 2.1	54.4 ± 2.1
HDL	18.8 ± 0.5	19.4 ± 1.0	20.6 ± 0.5
LDL	32.3 ± 1.5	30.8 ± 0.9	33.8 ± 2.8
VLDL	11.2 ± 0.2	10.6 ± 0.4	10.9 ± 0.4
Total Lipids	380 ± 1.4	378 ± 7.7	381 ± 9.7
Cholesterol/HDL Ratio	3.31 ± 0.12	3.07 ± 0.1	3.21 ± 0.1

Values are presented as mean ± SEM, n=5, as compared to control group. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

Liver function test

There were significant changes in biomarkers of liver function such as an increase in alkaline phosphatase levels in both treatment groups was observed. It was found that a decrease in alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels in PH 2 treated animals while no significant changes in ALT and AST levels in PH 1 treated group was revealed. No significant changes were observed in total bilirubin, total protein, globulin, albumin and the A/G ratio among groups (Table IV).

Hematological analysis

No remarkable alterations in the levels of Hb, total leukocyte count (TLC), total red blood cells (RBC), hematocrit (HCT), platelets, neutrophils, lymphocytes, monocytes, eosinophil and other parameters were observed in both groups as compared to control group. Results are summarized in Table V.

Histopathological analysis

Kidney

The normal proximal and distal tubules and glomeruli were observed in the control group. PH 2 treated groups showed intact glomeruli with normal morphology. No changes were observed in any type of tubules. No evidence of inflammation or necrosis was seen. Whereas, PH 1 showed the same symmetry of cells as that of the control group, however, a mild vascular congestion was noted (Figure 4).

Liver

The histopathological examination of liver of the control group showed normal structure. In PH 2 treated groups the liver architecture was maintained. Hepatic cords and hepatocytes were normal. No degenerative changes or necrosis were seen. No inflammatory cells in the sections were observed. The only change observed was mild to moderate dilatation of some central veins. Whereas, PH 1 treated group had disturbed liver architecture. The central veins were dilated. Hepatic cords were disorganized and disrupted at places. Cytoplasmic vacuoles were seen in some hepatocytes. At a few places mononuclear cell aggregates were also seen (Figure 4).

Table IV. Effects of polyherbal products on liver function test of rats in acute toxicity study.

Parameters (mg/dL)	Control	PH 1	PH 2
Total Bilirubin (mg/dl)	0.320 ± 0.03	0.260 ± 0.02	0.300 ± 0.05
ALT (U/L)	117.0 ± 1.04	114.0 ± 1.87	82.66 ± 2.33*
AST (U/L)	252.4 ± 9.02	225.4 ± 2.65	186.0 ± 9.4***
Alk. Phosphatase (U/L)	461.2 ± 0.58	855.0 ± 30.1***	789.3 ± 12.2***
Total Protein (g/dl)	6.760 ± 0.05	6.520 ± 0.12	6.600 ± 0.05
Albumin (g/dl)	4.200 ± 0.05	4.140 ± 0.09	3.833 ± 0.08
Globulin (g/dl)	2.540 ± 0.05	2.360 ± 0.10	2.766 ± 0.03
A/G Ratio	1.680 ± 0.07	1.750 ± 0.09	1.400 ± 0.05

Values are presented as mean ± SEM, n=5; *p<0.05, ***p<0.001 as compared to control group.

Table V. Effects of polyherbal products on CBC of rats in acute toxicity study.

Parameters	Control	PH 1	PH 2
Hb (g/dL)	12.5 ± 0.17	12.9 ± 0.15	13.3 ± 0.32
TLC (10 ⁹ /L)	7.78 ± 0.07	7.44 ± 0.21	7.04 ± 0.35
Total RBC (10 ¹² /L)	6.67 ± 0.31	6.67 ± 0.29	7.18 ± 0.36
PCV (%)	37.2 ± 1.88	44.1 ± 2.32	42.6 ± 2.38
MCV (fl)	56.8 ± 0.36	68.3 ± 3.30	61.8 ± 3.76
MCH (pg)	19.0 ± 0.67	19.8 ± 0.76	19.1 ± 0.62
MCHC (%)	32.8 ± 1.05	29.3 ± 1.48	30.6 ± 1.33
Platelets (10 ⁹ /L)	882 ± 30.8	906 ± 24.8	881 ± 41.2
Neutrophils (%)	3.60 ± 0.40	4.00 ± 0.55	4.00 ± 0.55
Lymphocytes (%)	88.4 ± 0.24	87.4 ± 0.51	87.6 ± 0.51
Monocytes (%)	5.20 ± 0.20	6.60 ± 0.60	7.20 ± 0.49
Eosinophils (%)	1.60 ± 0.24	2.00 ± 0.01	1.80 ± 0.20

Values are presented as mean ± SEM, n=5, as compared to control group. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

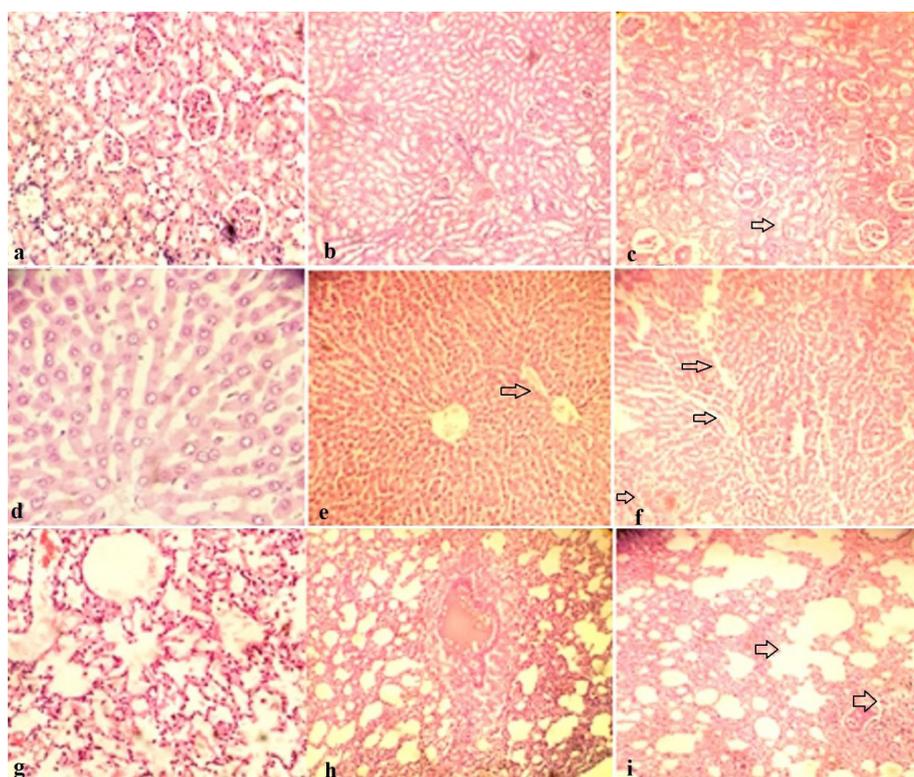


Figure 4. Histopathological pictures of rats exposed to single oral dose of 2000 mg/kg of polyherbal formulations PH 1 and PH 2 at 40X magnification

Kidney sections of rats are shown in (a) Control (b) PH 2 that exhibited normal kidney histology and (c) PH 1 that presented mild vascular congestion. Liver sections of rats are shown in (d) Control (e) PH 2 that presented dilated central vein and (f) PH 1 that presented damaged liver architecture, dilated central vein and infiltration of inflammatory cells. Lungs sections of rats are shown in (g) Control (h) PH 2 that presented vascular congestion and sparse lymphocytic aggregates and (i) PH 1 that exhibited lymphocyte aggregation and alveolar distension and destruction.

Lungs

The control group showed the normal structure with intact alveoli. No hemorrhage or necrosis was seen. PH 2 treated groups had mild vascular congestion and sparse lymphocytic aggregates and PH 1 treated group showed marked alveolar distension with wall destruction and collapse. Focal areas of hemorrhages were seen. Vascular congestion was moderate. Several aggregates of lymphocytes were randomly seen (Figure 4)

Validation of anti-asthmatic activity

Disease control showed a decrease in SOD and GSH levels, whereas MDA level increased in it. Treatment with standard, low and high doses of selected polyherbal products increased

SOD and GSH levels and decreased MDA level. These results are supporting the validation of anti-asthmatic activity of polyherbal products. Table VI showed the effect of low and high doses of both polyherbal products, standard (Salbutamol) and disease control on SOD, GSH and MDA levels.

IgE and eosinophil's count

IgE level and eosinophil count increased in disease control as compared to control group values and standard treatment showed decrease in these values. Dose dependent decrease in IgE value and eosinophil count was noted with polyherbal products which indicated effectiveness of polyherbal formulations in asthma (Table VII).

Table VI. Effect of polyherbal products on oxidative stress parameters in the lungs of asthmatic rats.

Tests (ug/mg)	Control	Standard	Disease control	PH 1 6 mg/kg	PH 1 12 mg/kg	PH 2 50 mg/kg	PH 2 100 mg/kg
SOD	16.8 ± 0.015	29.1 ± 0.013***	15.2 ± 0.038***	28.6 ± 0.003***	20.7 ± 0.015***	18.6 ± 0.006***	28.6 ± 0.026***
GSH	0.63 ± 0.001	0.78 ± 0.002***	0.55 ± 0.004***	0.43 ± 0.004***	0.95 ± 0.001***	0.71 ± 0.001***	0.63 ± 0.002
MDA	0.17 ± 0.001	0.19 ± 0.001	0.20 ± 0.004	0.17 ± 0.001	0.21 ± 0.001	0.19 ± 0.001	0.20 ± 0.001

Values are presented as mean ± SEM, n=5; ***p<0.001 as compared to control group. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

Table VII. Effects of polyherbal products on IgE and eosinophil's count of mice in control and treatment groups.

Tests	Control	Standard	Disease Control	PH 1 6mg/kg	PH 1 12mg/kg	PH 2 50 mg/kg	PH 2 100 mg/kg
IgE (IU/mL)	1.12 ± 0.05	1.46 ± 0.05	6.84 ± 0.1***	5.46 ± 0.05***	3.18 ± 0.08***	5.04 ± 0.05***	3.44 ± 0.06***
Eosinophil's count %	1.80 ± 0.20	2.10 ± 0.2***	2.80 ± 0.2***	2.40 ± 0.24**	2.20 ± 0.20	2.60 ± 0.24*	2.20 ± 0.20

Values are presented as mean ± SEM, n=5; *p<0.05, **p<0.01, ***p<0.001 as compared to control group. PH 1 and PH 2 showed polyherbal formulation 1 and 2 respectively.

DISCUSSION

Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world. It causes airway narrowing associated with changes in the levels of eosinophil's, mast cells, lymphocytes, cytokines and other inflammatory cell mediators (Holgate & Polosa 2008, Pavord et al. 2012). In this study two products were chosen which are already used by the population in South Asia. Anti-asthmatic potential of polyherbal products was assessed by efficacy test which includes IgE and eosinophil's count. A recent study found that a high level of IgE directly indicated the severity of asthma (Liou et al. 2014). In the present study, the IgE value of the disease control group was high and upon treating the standard group with Salbutamol, a considerable decrease in IgE was noted when compared with disease control group. It was previously concluded that an oral dose of the β 2-agonist Salbutamol, was sufficient to provide clinical benefit at therapeutic levels and did not accentuate the seasonal increase of IgE in the standard treatment group (Hong et al. 2013). PH 1 had more pronounced anti-asthmatic effect as compared PH 2. The Eosinophil's count was shown to be markedly decreased in patient group who had taken steroids during a research study (Cowan et al. 2010). Both polyherbal products expressed non-significant decrease in eosinophil count in treated groups as compared to disease control group. IgE antibodies are responsible for mast cell degranulation after activating specific transmembrane receptors on mast cells. Anti-IgE drugs or antibodies stabilize mast cells by inhibiting the production or neutralizing the IgE antibodies and are therefore useful in treating different allergic disorders (Siebenhaar et al. 2018, Chang & Shiung 2006). The present study showed that both polyherbal formulations not only reduced eosinophil count

but also stabilized mast cells through inhibition of IgE antibody formation.

Biochemical analysis revealed some unique outcomes as PH 2 had exerted a minor increase in the level of SOD upon its low dose, however, the level of SOD elevated remarkably at high dose. On the other side, PH 1 illustrated a considerable increase in SOD level, however, on higher dose the level decreased. This could be questionable elevations as a study approved that SOD has significant effects on airway responsiveness (Ofori-Amoah et al. 2016). Another biochemical parameter is the GSH level. PH 2 showed a rise in value as compared to diseased group, but high doses showed a minor decrease and PH 1 resulted vice versa. It was claimed previously that the oxidative state of glutathione had been raised with the severity of asthma. A significant decrease in MDA level was indicative of ameliorating the severity of asthmatic condition (Deng et al. 2013). However, polyherbal formulations did not exhibit a significant fall in MDA level except low dose of PH 1.

After efficacy analysis, a study related to toxicity must be conducted so the proportion of therapeutic uses and adverse effects must be known. The effect of either of the polyherbal formulations on renal function test was an increase in urea the group treated with PH 1 that could lead to the development of uremia and may progress to hyperuricemia so the PH 1 may have the potential of nephrotoxicity (Engen et al. 2017). Creatinine level was not increased upon treating with any polyherbal formulation. Results of liver function tests could show safety because lower levels of ALT and AST in treated groups mainly pointed towards safe use of polyherbal formulations. As increased level of ALT and AST in the serum are the markers of damage of liver, however, a noticeable increase in Alkaline Phosphatase pointed towards the

toxicity of both polyherbal formulations as the enzyme was implicated in the liver and biliary duct associated damage (Fatima et al. 2019, Saleem et al. 2019). Therefore, both polyherbal formulations might have the potential to cause liver damage, bile stones, biliary obstruction and nephrotoxicity. An acute toxicity study conducted female rats showed a normal lipid profile, blood sugar (both random and fasting) and complete cell count showing the safety of treated both polyherbal formulations. Histopathology of rats treated with PH 2 showed no significant changes, but the tissues treated with PH 1 exhibited histopathological evidence of damage to both liver and lungs.

CONCLUSIONS

This study concluded that PH 2 might be more efficacious in asthma as it decreased IgE load to a greater extent as compared to PH 1. Both products are safe at human consumable dose i.e. one tablet daily but it may cause damage in liver and kidney when dose levels are parallel to 2000mg/Kg. Future studies must be directed to standardization of these polyherbal formulations for their optimum and consistent response in diseased individuals.

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Uzma Saleem drafted the manuscript, Maryam Usman performed the experiments, Fareeha Anwar did graphical work, Muhammad Furqan Akhtar contributed in drafting the manuscript particularly discussion section and Bashir Ahmad critically reviewed the manuscript.

