COMPARATIVE EFFICACY STUDY OF A POLYHERBAL FORMULATION WITH OTHER AVAILABLE DRUGS IN *PROPIOBACTERIUM ACNES* INDUCED RAT MODEL

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ABSTRACT

Introduction: *Propionibacterium acnes* (*P. acnes*) is a commensal anaerobic, Gram positive bacteria playing an important role in the pathogenesis of various skin infections and diseases. The study evaluated the efficacy of a polyherbal formulation and compared with various marketed anti acne formulation in *P. acnes* induced acne in albino wistar female rats. Biochemical and enzymatic parameters were studied and histopathology of ear pinna tissue was done in acne exposed rats. **Materials and Methods:** 48 rats were distributed in eight groups containing six rats in each group. Group I: Control (saline treated), Group II: *P. acne* induced group (diseases control), Group III: *P. acne* induced group (polyherbal formulation treated) and group IV, V, VI, VII, VIII: *P. acne* induced group (marketed drug A, B, C, D, and E treated). **Results:** Present finding showed that activities of antioxidant enzymes were significantly decreased along with increased level of MDA in untreated group at 10th day (induction period) and 30th day (treatment period) of the study. Similarly, the level of total protein, Albumin and cholesterol were also found to be significantly increased along with decreased level of triglyceride in untreated group at 10th day. These biochemical parameters were significantly improved at 30thday. **Conclusion:** After treatment antioxidant enzymes (SOD, Catalase) along with MDA, improved significantly and normalized intestinally, we observed that polyherbal formulation have an antiseptic and antibacterial property that may be beneficial for human who suffer from chronic Acne Vulgaris.

KEYWORDS: Polyherbal; topical drug; antimicrobial; anti-inflammatory; antiseptic properties.

INTRODUCTION

Acne vulgaris is a chronic inflammatory skin problem that presents with blackheads, whiteheads, and inflamed spots. It is a common condition, which affect 54% of adult women and 40% of adult men but mostly affected in adolescents (80%).[1] Acne develops as a result of increased sebum production; hyperkeratinisation and inflammation. According to the lesion type, acne can be classified into four main categories: non-inflammatory (purely comedone acne), mild papular, scarring papular, and nodular; the latter three are inflammatory acne lesions.[2] The evaluation of lesions and their complications are important to assess the severity of acne. The psychosocial impact, failure to respond to previous therapies and occupational disability are three additional factors, which are used in grading of acne.

The treatment of acnes aims to lessen the inflammatory or non-inflammatory acne lesions, improve appearance, prevent or minimise potential adverse effects, and minimise any scarring.[3] The use of oral and topical treatments have limited approaches due to ineffectiveness, inconvenience, poor tolerability or side-effects. So, anti-acne remedies must possess properties to reduce the number of inflammatory lesions, suppress the growth of *Propionibacterium acne*, or reduce sebaceous gland size and secretory activity.[4] The Improvement of clinical signs assessed through skin lesion counts (total of inflamed and non-inflamed counted separately) and acne severity scores. In many studies it is unclear whether acne induction and severity can be assessed by histological lesion count and inflammation grading at the site of acne. Furthermore, the connection between lesson count, inflammation, the disease severity and its localization is unknown. There are very scant literatures available on herbal medicine which explains the treatment of acne by effective and more convenience way with better tolerability and lesser side-effects.

Polyherbal drug is a new micro emulsion containing the herbal extracts of *Melaleucaalternifolia* oil, *Rosmarinusofficinalis* oil, *Menthaarvensis* oil along with *Citrus limon*, developed as an anti-acne herbal nano-emulsion for topical use. Whereas Drug A is a retinoid activity containing 0.1% topical gel, Drug B is *lignans* containing, Drug C is salicylic containing keratolytic creams, Drug D and Drug E is corticosteroid and Salicylic acid containing (akeratolytic agents) with different composition in an ointment formulations.

As animal skin is quite sensitive for most of the chemicals so an allergic, irritant contact dermatitis and phytophoto dermatitis can be the topical adverse effects of natural products. Thus this polyherbal formulation has been evaluated for the acute dermal irritation and corrosion potential on adult *Albino* rabbit's skin for a specified period of time in our previous study.[5]

MATERIALS AND METHODS
Chemicals
All biochemical such as thiobarbituric acid (TBA), Sodium Docedyl Sulphate (SDS), Hydrogen Peroxide and other bio chemicals are procured from Hi media Laboratories Ltd. (Mumbai India) and Sigma, St. Louis MO, U.S.A. Other chemicals were purchased locally for measurements of liver enzymes for estimation of SOD, Catalase and MDA parameters, were purchased from Transasia Diagnostics India Ltd. Bombay. The Elisa kits were procured from Invitrogen & USCN Bio life sciences. A new drug polyherbal formulation was obtained from research and development of Venus Medicine Research Centre, Baddi, HP.

Animals
Albino Wistar female rats (150-200 gm) were allowed to acclimatize for 3-4 weeks. They were maintained in hygienic laboratory condition at controlled temperature and humidity in alternating 12-hours light and dark and feed with standard pellet feed and filtered & UV treated water and libitum. The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee ((IAEC/CS/11/2011) of Venus remedies.

Induction of acne via *P. acne* bacterial strain
O.2 ml of Propionibacterium acnes bacterial strain (MTCC No. 1951) were grown by adding 2.5 ml blood sample in 10 ml MH broth at 35-37 degree Celsius and kept for 3 hrs for the growth of the sample. It was further centrifuged, washed and suspended in saline phosphate buffer (0.2 M, pH 7.0) to obtain the culture with final desired concentration. *P.acne* bacteria induced skin inflammation was evoked by intradermal injection of 20ml of 1×10^6 CFU/ml using 30-gauge needle in the central, ventral portion of the left ear of rats belonging to all groups except the control group. A ten day induction time is taken and an increase in ear diameter of the injected ear confirms the induction of acne.

Grouping of animals
Forty-eight rats procured as mentioned above were distributed in eight groups each containing six rats as shown below:
Group I: Control (normal saline treated)
Group II: *P.acne* induced group (disease control)
Group III: *P.acne* induced group (treated with polyherbal formulation)
Group IV: *P.acne* induced group (treated with marketed drug A)
Group V: *P.acne* induced group (treated with marketed drug B)
Group VI: *P.acne* induced group (treated with marketed drug C)
Group VII: *P.acne* induced group (treated with marketed drug D)
Group VIII: *P.acne* induced group (treated with marketed drug E)

Treatments
Drug selected for each group was topically applied twice a day according to the body surface area for 20 days (Treatment period). Physical observations (Body weight, Body temp., Ear diameters) as well as blood, plasma and tissue samples were taken periodically at 20th day of drug treatment in order to monitor the progress of treatment and to compare the efficacy of these drugs in acne treatment. Ear skin was taken for all the groups for the measurement of histological analysis.

Parameters
Various parameters- i.e. physiological monitoring, haematological parameters by (Sysmax Model XT2000i), biochemical parameters by (Erba Manheim; Model EM200), antioxidant enzymatic activity by the Method of Misra and Fradovich,[6] catalase by the method of Luck,[7] free radicle (MDA) in tissue sample using paraffin fix ethanol staining method,[8] and Cytokines levels by using ELISA Reader (Merck, Serial No. 21041098, MIOS Junior) were assayed to evaluate the comparative efficacy of various marketed formulation with polyherbal formulation drugs.(Fig. 1)
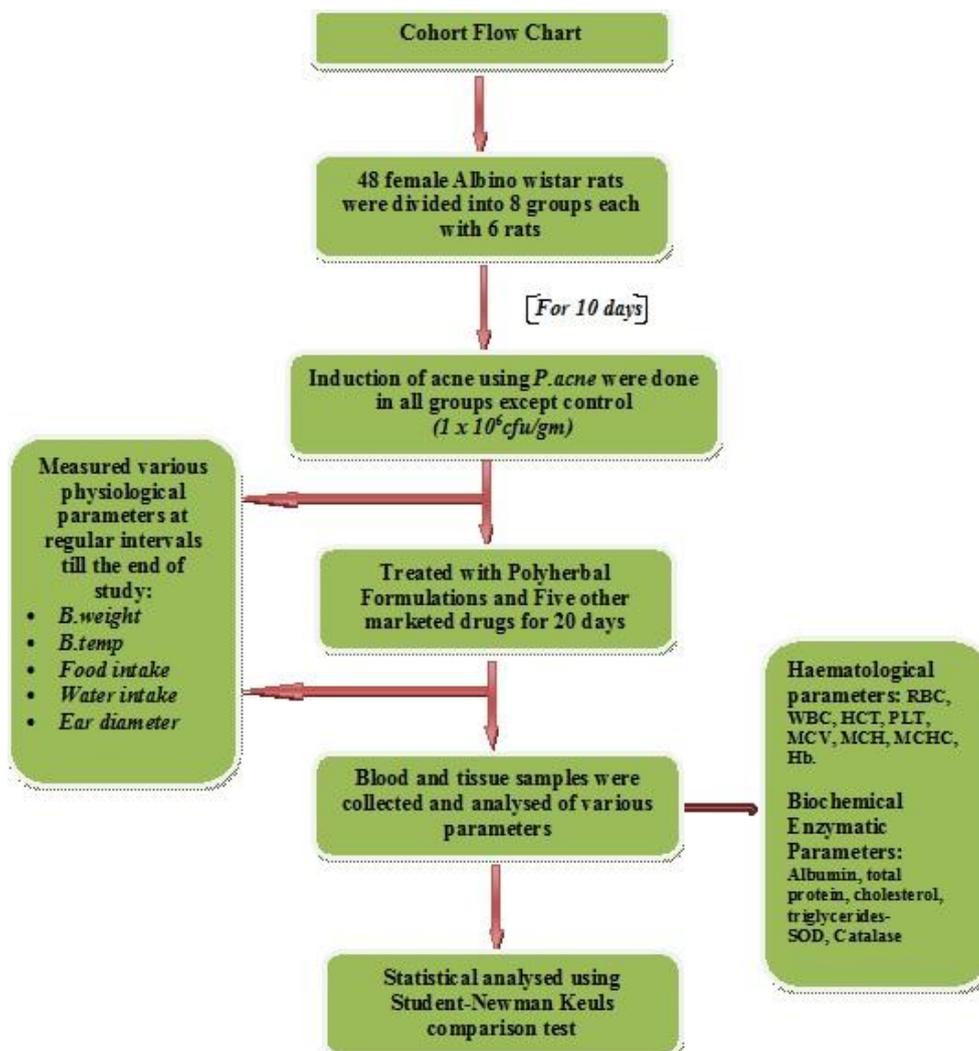
Fig. 1: work plan.

Histological parameters
The rats were anaesthetised by using Xylazine-Ketamine combination anaesthesia and ear pinna tissue samples were collected in 10% buffered formalin saline for histological studies. The formalin fixed tissues were washed overnight in running tap water, dehydrated in ascending grades of alcohol, and cleared in benzene. The 4-5 micron thick tissue sections were cut from the paraffin embedded tissues and were stained with haematoxylin and eosin stain (H&E) for routine histopathology (Luna 1968). Morphological changes (lesion count, sebaceous gland hyperkeratinisation) were assessed by using routine light microscopy.

Statistical Analysis
The data obtained was analysed statistically. All values were expressed as Mean+ SD. One Way Analysis of Variance (ANOVA) with Student-Newman Keuls comparison test was used to determine statistical difference between controls vs. infected groups & infected vs. treated groups. P values<0.05 were considered statistically significant and P values<0.001 were considered highly statistically significant.

RESULTS
In the present study the physical parameters such as body weight, food intake, water intake and temperature on daily basis till the end of the study (30th days).Author observed a significant (p<0.05) decrease in body weight in acne induced groups as compared to the control group. After treatment with polyherbal formulation, Drug A, Drug B, Drug C, Drug D and Drug E for 20 days, the body weight was slightly increased in all treated groups. When compared between induced groups versus all treated groups, the body weight was found significantly increased (p<0.01) in all treated groups. (Fig-2).
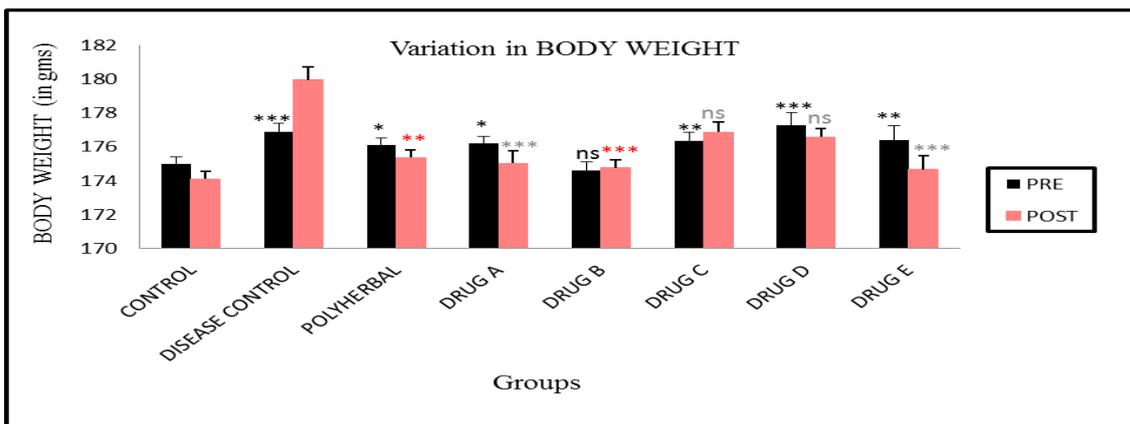
Fig. 2: Status of body weight in acne induced groups before and after treatment with Polyherbal formulation, Drug A, Drug B, Drug C, Drug D and Drug E.

Body temperature was significantly ($p<0.001$) increased in *P.acne* induced groups as compared with control group. After treatment with respective drugs for 20 day, the temperature was reduced significantly in Polyherbal formulation, Drug B,C,D and E treated groups but didn't showed any difference in Drug A treated group.(Fig-3).
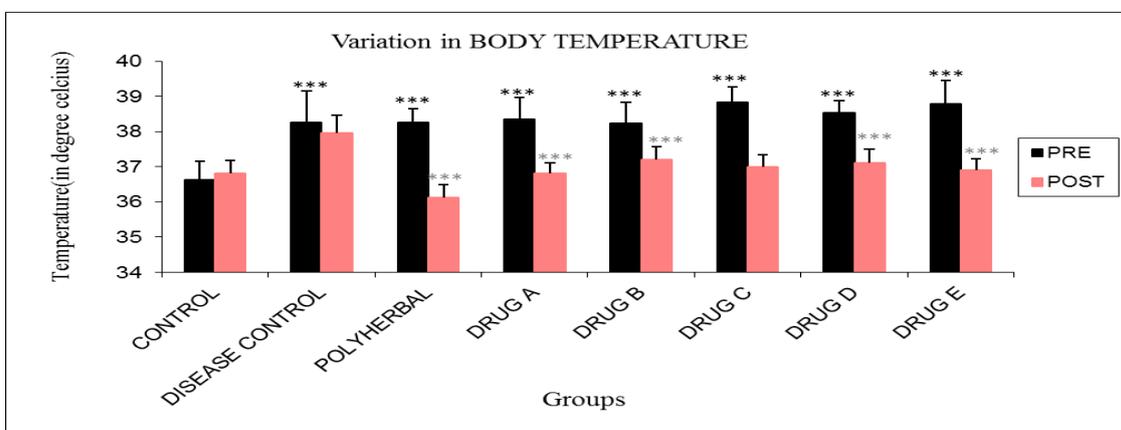

Fig. 3: Status of body temperature in acne induced groups before and after treatment with Polyherbal formulation, Drug A, Drug B, Drug C, Drug D and Drug E.

The food & water intake were slightly altered in acne induced groups as compared with control group. When acne induced group was compared with treated groups, there was significant ($p<0.05$) changes in food intake and water intake. (Table-1).

Table 1: Status Food and Water intake in acne induced groups before and after treatment with Polyherbal formulation, Drug A, Drug B, Drug C, Drug D and Drug E.

Parameters	Result	Normal Control	Disease Control	Polyherbal	Drug A	Drug B	Drug C	Drug D	Drug E
Food Intake (gm/kg)	Pre	66.5±2.12	46.5±2.12	6.0±2.83	45.0±2.83	44.5±3.54	46.5±2.12	45.5±4.95	44.5±2.12
	Post	64.0±1.41	41.5±2.12	62.0±1.41	53.0±1.41	57.5±2.12	55.5V4.95	57.0±2.83	56.0±4.24
Water Intake (ml/l)	Pre	73.5±2.12	50.0±2.83	59.0±4.24	60.5±2.12	50.0±1.41	56.5±2.12	60.0±2.83	56.5±2.12
	Post	74.0±2.83	43.5±2.12	71.0±1.41	60.2±1.41	66.0±5.66	63.5±2.12	63.0±1.41	62.5±4.95

Ear Diameter
Ear diameter was measured on daily basis up to30[th] day of the study. Author observed significantly ($p<0.001$) increased in acne induced group as compared to control group. After treatment with respective drugs for 20 days treatment, the ear diameters were significantly decreased in all groups. As compared to the groups (A-E), polyherbal formulation showed comparatively reduction in the inflammation of ear diameter (Fig-4).
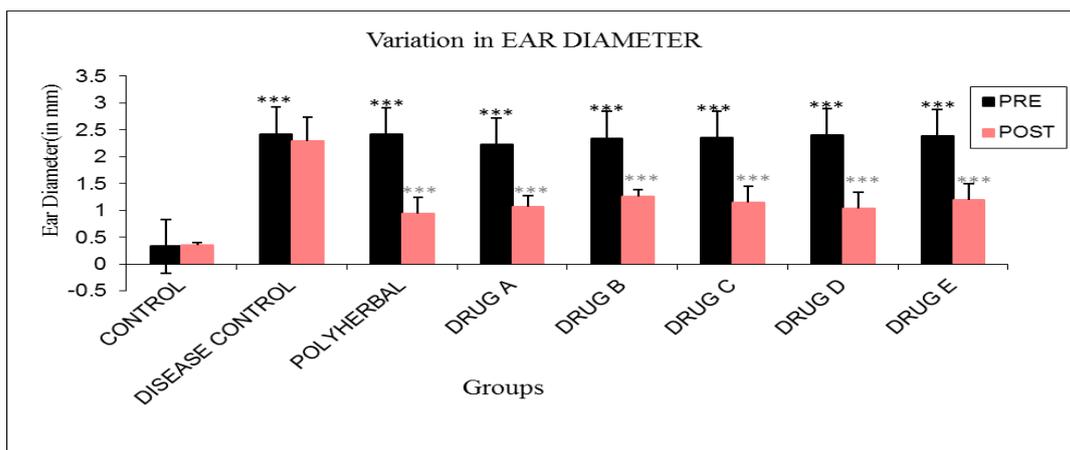
Fig. 4: Status of ear diameter in acne induced groups before and after treatment with Polyherbal formulation, Drug A, Drug B, Drug C, Drug D and Drug E.

Haematological parameters
There was slightly significant increase in the Haematological parameters such as RBC count, HCT, MCV, MCH, MCHC, Hb at 10th days of induction and at 30th days after treatment and Author finds significant increase in WBC count in acne induced groups as compared to control groups. In case of treated groups there is slightly decrease in the RBC parameters for

Polyherbal formulation, Drug A, Drug B and Drug E and a significant decrease in the WBC parameter. Drug C and Drug D showed non-significant change for all haematological parameters. PLT count was found slightly decreased in induced groups, which on treatment increased significantly close to the control values in Polyherbal formulation and Drug A (Table 2).

Table 2: Status of hematological parameters in acne induced groups before and after treatment with Polyherbal formulation, Drug A, Drug B, Drug C Drug D and Drug E.

Parameters	Result	Normal Control	Disease Control	Polyherbal	Drug B	Drug C	Drug D	Drug E
RBC	Pre	73.28±4.26	76±2.41	75.01±4.52	76.01±5.71	72.01±5.36	71.01±3.21	75.01±2.21
$(10\times10^{6}/\mu l)$	Post	72.26±2.16	75±3.11	76.09±5.20	75.15±4.15	72.5±4.42	71.10±4.39	74.56±1.19
Hb	Pre	192.14±25.88	189.84±44.23	190.27±13.71	188.73±9.11	190.45±8.12	189.73±7.12	188.73±6.54
(g/dl)	Post	191.10±10.88	190.44±8.45	188.12±4.51	189.25±5.10	190.0±5.50	188.0±5.10	188.01±5.50
HCT(□)	Pre	5.01±0.28	5.93±0.15	5.41±0.10	5.30±0.15	5.82±0.29	5.75±0.20	5.50±0.14
	Post	5.12±0.18	6.39±0.52	5.98±0.14	5.99±0.35	6.0±0.21	5.97±0.22	5.98±0.24
MCV	Pre	67.22±6.21	69.36±8.25	62.2±315	64.41±1.59	62.29±2.15	66.8±3.53	67.2±4.21
(μm^{3})	Post	67.0±5.22	68.12±4.52	62.0±4.23	65.49±2.58	62.85±5.36	65±4.54	66.42±5.10
MCH	Pre	0.52±0.003	0.54±0.030	0.57±0.02	0.57±0.03	0.50±0.02	0.59±0.03	0.6±0.04
(pg)	Post	0.5±0.01	0.56±0.02	0.57±0.01	0.58±0.01	0.52±0.01	0.57±0.03	0.59±0.002
MCHC(□)	Pre	73.28±4.26	76±2.41	75.01±4.52	76.01±5.71	72.01±5.36	71.01±3.21	75.01±2.21
	Post	72.26±2.16	75±3.11	76.09±5.20	75.15±4.15	72.5±4.42	71.01±4.39	74.56±1.19
WBC	Pre	192.14±25.88	189.84±44.23	190.27±13.71	188.75±9.11	190.45±8.12	189.73±7.12	188.73±6.54
$(10\times10^{3}/\mu l)$	Post	191.10±10.88	190.44±8.45	188.12±4.51	189.25±5.10	190±5.50	188±5.10	188.01±5.50
PLT	Pre	5.01±0.28	5.93±0.15	5.41±0.10	5.30±0.15	5.82±0.29	5.75±0.20	5.50±0.14
$(10\times3/\mu l)$	Post	5.12±0.10.248	6.39±0.52	5.98±0.14	5.99±0.35	6.0±0.21	5.97±0.22	5.98±0.24

Biochemical parameters
Total protein
The level of total protein was significantly increased (p<0.001) in acne induced group at 10th day as compared to control group. After treatment with respective drug for 20 days, the level was significantly reduced in all treated groups as compared to acne induced groups. When Polyherbal formulation treated group was compared among Drug A, Drug B, Drug C, Drug D and Drug E treated group, the level of total protein was much closer to control value in Polyherbal formulation and Drug A(p<0.001) as compared to other drug groups(p<0.05)

(Table3).

Albumin
The level of albumin was significantly increased (p<0.001) in acne induced group at 10th day as compared to control group. After treatment with respective drug for 20 days, the level was significantly reduced in all treated groups as compared to acne induced groups. When Polyherbal formulation treated group was compared among Drug A, Drug B, Drug C, Drug D and Drug E treated group, the level of albumin was much closer to control value in Polyherbal formulation and Drug C as

compared to other drug groups (Table 3).

Triglycerides
The level of triglycerides was significantly decreased ($p<0.001$) at 10th day in acne induced group as compared to control group. After treatment with respective drug for 20 days, the level was significantly increased in all treated groups as compared to acne induced groups. When Polyherbal formulation treated group was compared among Drug A, Drug B, Drug C, Drug D and Drug E treated group, the level of triglycerides was much closer to control value in Polyherbal formulation and Drug E as compared to other drug groups (Table 3).

Cholesterol
The level of cholesterol was significantly increased ($p<0.001$) in acne induced group at 10th day as compared to control group. After treatment with respective drug for 20 days, the level was significantly reduced in all treated groups as compared to acne induced groups. When Polyherbal formulation treated group was compared among Drug A, Drug B, Drug C, Drug D and Drug E treated group, the level of cholesterol was much closer to control value in Polyherbal formulation and Drug D as compared to other drug groups which didn't showed much variation (Table 3).

Antioxidant enzymes
Superoxide dismutase
Superoxide dismutase activity was found to be significantly decreased ($p<0.001$) in plasma as well as in ear tissue of group–III when compared to control group after 10 days induction period. This enzyme activity was significantly increased($p<0.001$) and improved in plasma as well as ear tissue of infected + treated groups and reached almost near to control level after administration especially in case of Polyherbal formulation and Drug A after 20 days treatment. Drug E also showed slight significant ($p<0.01$) increase but other groups didn't showed much alteration (Table 3).

Catalase
Catalase activity was found to be significantly decreased in plasma as well as in ear tissue of Group –III after 10th day induction period .When compared to control group. This enzyme activity was significantly increased ($p<0.001$)and improved in plasma as well as ear tissue of infected + treated groups and reached almost near to control level after administration especially in case of Polyherbal formulation for 20 days treatment. Drug B and D also showed significant increase ($p<0.01$) while Drug C and Drug E showed slightly significant increase ($p<0.05$) (Table 3).

Free radical mediated damage level
Malonaldialdehyde (MDA*)*
Malonaldialdehyde (MDA) level was significantly increased ($p<0.001$) in *P.acne* induced groups at 10th day as compared to control group. After treatment with

Polyherbal formulation, Drug A, Drug B, Drug C, Drug D and Drug E for 20 days, the level of MDA (as marker of lipid peroxidation) was significantly reduced($p<0.001$) in all treated groups (Table 3).

Cytokines
TNF-α
TNF- α was increased very significantly ($p<0.001$) at 10th day in infected group. The level of TNF- α was statistically significantly decreased ($p<0.001$) in Drug A, Drug B and Polyherbal formulation treated group after treatment for 20 days with respective drugs. Drug C, Drug D and Drug E showed slightly significant change after treatment ($p<0.05$) (Table 3).

IL-6
IL-6 also showed very significant change ($p<0.001$) at 10th day in infected group. The level of IL-6 was statistically significantly changed in all treated groups after treatment for 20 days with respective drugs. When the level of IL-6 was compared in all treated groups, the level was found to be significantly altered in all the treated groups after 20 days treatment as compared to control (Table 3).

Table 3: Status of Biochemical parameters in acne induced groups before and after treatment with Polyherbal formulation, Drug A, Drug B, Drug C, Drug D and Drug E.

Parameters	Result	Normal Control	Disease Control	Polyherbal	Drug A	Drug B	Drug C	Drug D	Drug E
T. Protien (g/dL)	Pre	5.01±0.28	5.93±0.15	5.41±0.10	5.19±0.24	5.30±0.15	5.82±0.29	5.75±0.20	5.50±0.14
	Post	5.12±0.18	6.39±0.52	5.98±0.14	5.58±0.12	5.99±0.35	6.0±0.21	5.97±0.22	5.98±0.24
ALB (g/dL)	Pre	2.7±.35	4.8±0.42	4.8±0.71	4.5±0.21	4.7±0.21	4.7±0.14	4.6±0.14	4.6±0.07
	Post	2.6±0.28	5.5±0.14	3.0±0.14	4.0±0.14	3.7±0.14	4.0±0.28	3.8±0.21	3.9±0.14
TGA (mg/dL)	Pre	66.5±2.12	142.5±3.54	71.0±1.14	94.0±5.66	91.0±5.66	83.0±4.24	85.5±0.71	89.5±6.36
	Post	67.5±3.54	137.5±3.54	134.0±2.8	129.0±1.4	138.5±4.9	136.5±2.1	138.5±3.54	133.5±2.1
Cholesterol (mg/dL)	Pre	57.5±3.54	94.5±3.54	93.0±2.8	97.0±2.8	93.0±2.8	95.0±4.2	97.0±2.8	92.5±3.5
	Post	56.0±2.83	98.0±1.41	61.0±2.12	76.0±2.83	79.5±2.12	78.5±4.95	57.5±3.54	74.5±3.54
SOD (mg/dL)	Pre	0.061±0.004	0.033±0.004	0.027±0.001	0.030±0.001	0.031±0.003	0.029±0.001	0.030±0.002	0.028±0.002
	Post	0.062±0.005	0.027±0.001	0.061±0.002	0.037±0.003	0.035±0.006	0.041±0.002	0.048±0.004	0.046±0.006
Catalase (mg/dL)	Pre	0.043±0.004	0.021±0.001	0.018±0.001	0.019±0.001	0.017±0.002	0.019±0.001	0.020±0.002	0.021±0.002
	Post	0.042±0.003	0.017±0.003	0.036±0.001	0.025±0.003	0.026±0.001	0.021±0.002	0.031±0.006	0.027±0.001
MDA (mg/dL)	Pre	0.074±0.004	0.016±0.008	0.175±0.003	0.172±0.004	0.174±0.008	0.172±0.004	0.177±0.002	0.171±0.001
	Post	0.075±0.007	0.175±0.006	0.072±0.001	0.048±0.002	0.065±0.006	0.065±0.004	0.055±0.004	0.027±0.001
TN-α (pg/dl)	Pre	3.4±0.21	6.2±0.4	6.4±0.7	5.4±0.3	6.0±0.3	5.7±0.4	5.3±0.1	5.4±0.4
	Post	3.5±0.49	6.5±0.4	3.3±0.14	5.0±0.28	4.7±0.14	4.4±0.14	4.3±0.28	4.6±0.14
IL-6 (pg/dl)	Pre	18.4±0.21	18.3±0.42	18.3±0.21	18.1±0.21	18.3±0.21	18.0±0.21	18.3±0.14	18.2±0.35
	Post	18.1±0.14	18.2±0.07	17.9±0.42	17.9±0.07	18.0±0.14	17.9±0.21	17.9±0.42	17.9±0.14

Histological observations

The microscopic examinations of the tissue sections of untreated acne lesions revealed infiltration of mixed population of inflammatory cells. Severe focal infiltration of lymphocytes, neutrophils, and plasma cells were seen in perivascular and reticular region of dermis. Sebaceous gland showed pyknotic nuclei. The acne lesions treated with Polyherbal formulation and Drug D treated groups showed normal histology after 20 days treatment while Drug A, Drug B, Drug C, and Drug E treated group showed mild perivascular and perifollicular lymphocytic infiltration. Few eosinophils were seen within the reticular region of dermis. (Figure 5-12).Lesion count was performed for different groups and graded in all groups after treatment. (Table 4).

Table 4: Histology Lesion Count grading in all groups after 20 days treatment with respective drugs.

Groups	Treatment	Observatory events			Overall lesion count
		Inflammation	Sebaceous gland clogging	Hyper-keratinization	
Group I	Normal saline	--	--	--	--
Group II	--	++++	+++	++++	++++
Group III	Polyherbal	+	--	--	+
Group IV	Drug A	+++	++	+++	+++
Group V	Drug B	++	+	++	++
Group VI	Drug C	++	++	+	++
Group VII	Drug D	++	++	+	++
Group VIII	Drug E	+++	+++	+++	+++

Reference grading index for lesion count
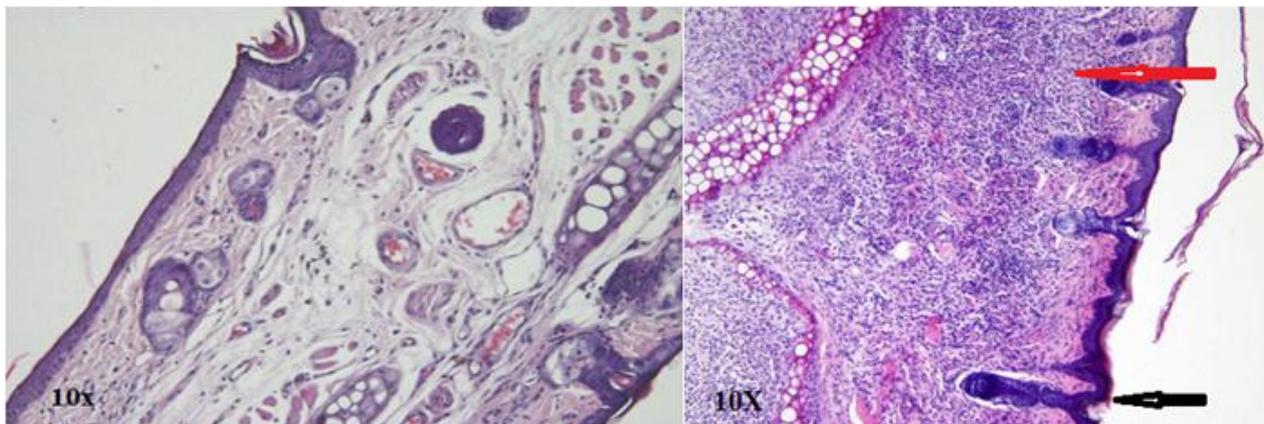
++++   Very high
+++   High
++   Normal
+   Low
--   Not present
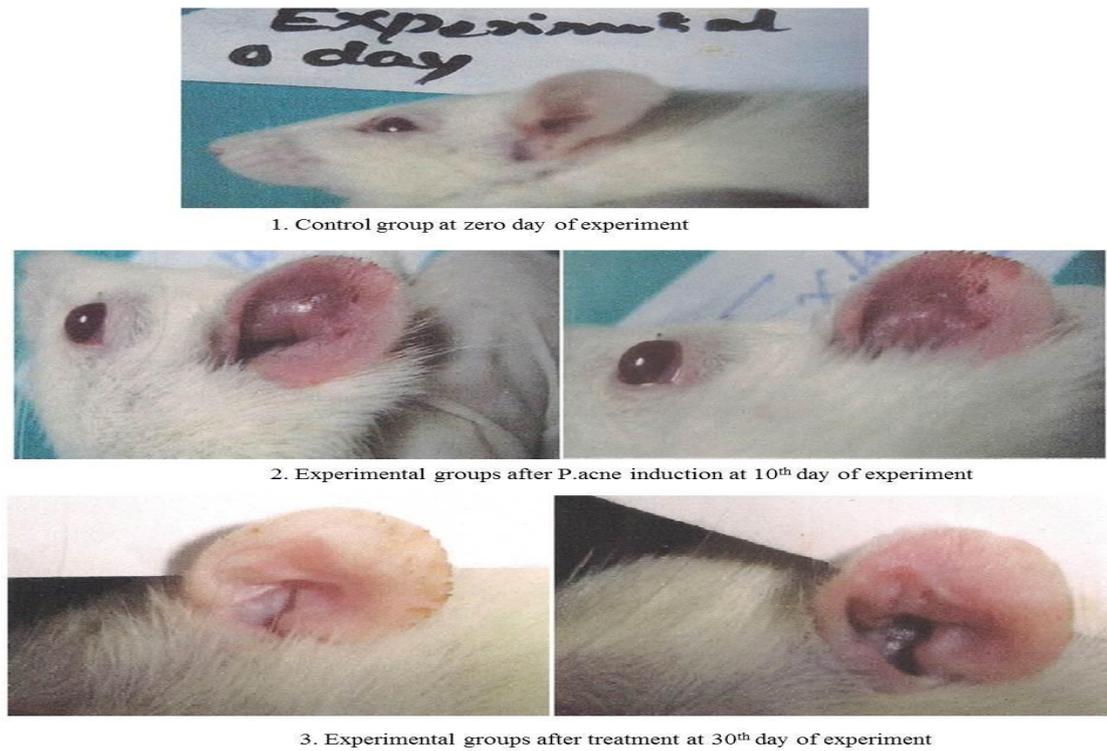
Fig. 5: Gross observatory changes in acne induced rat model.

1. Gross observation shows normal Ear pinna in control group at day zero. 2. In experimental group- ear pinna gets inflamed with whiteheads.3. After 20 days treatment at 30th day, ear pinna turns into normal by reducing inflammation and whiteheads.

Fig. 6: Histological observation in Group I (Control; normal saline treated) Histological changes in Group II (Disease Control) and after 20 days of treatment.

Group-1 There is no infiltration of lymphocytes, neutrophils and monocytic cells and sebaceous glands were also found intact.

Group-2The microscopic examination of tissue sections of un-treated acne lesions revealed infiltration of mixed population of inflammatory cells (Red arrow). Severe focal infiltration of lymphocytes, neutrophils and plasma cells were seen in perivascular and reticular region of the dermis. Sebaceous gland showed pyknotic nuclei and clogging (black arrow).
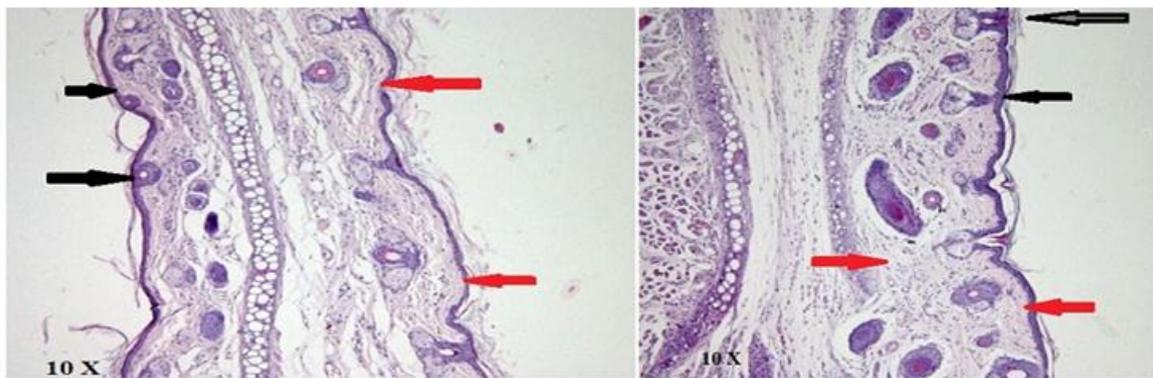
Fig. 7: Histological changes in Group III (Polyherbal formulation treated) after 20 days of treatment.

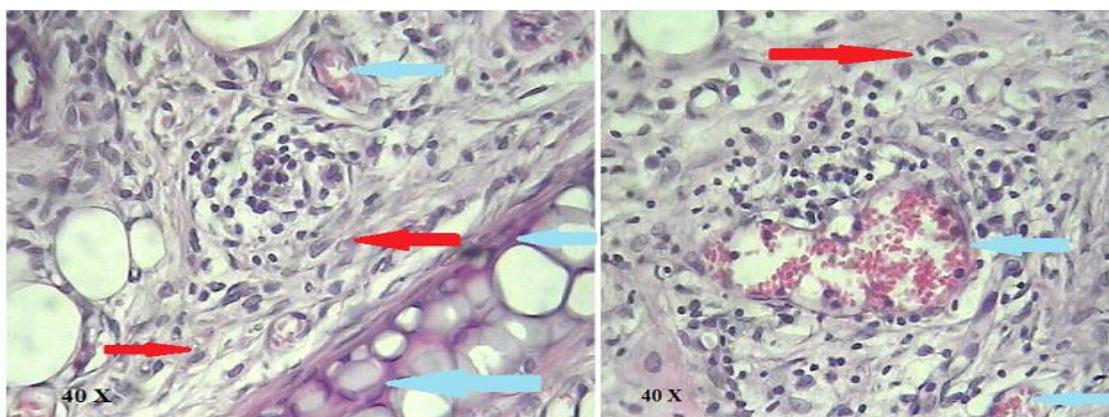The microscopic examination of tissue sections of Polyherbal formulation treated acne lesions revealed almost clear and reduced level of infiltration of inflammatory cells (Red arrow) Intact epitheliums with proper non-clogged sebaceous gland attached to hair follicle were present (black arrow).
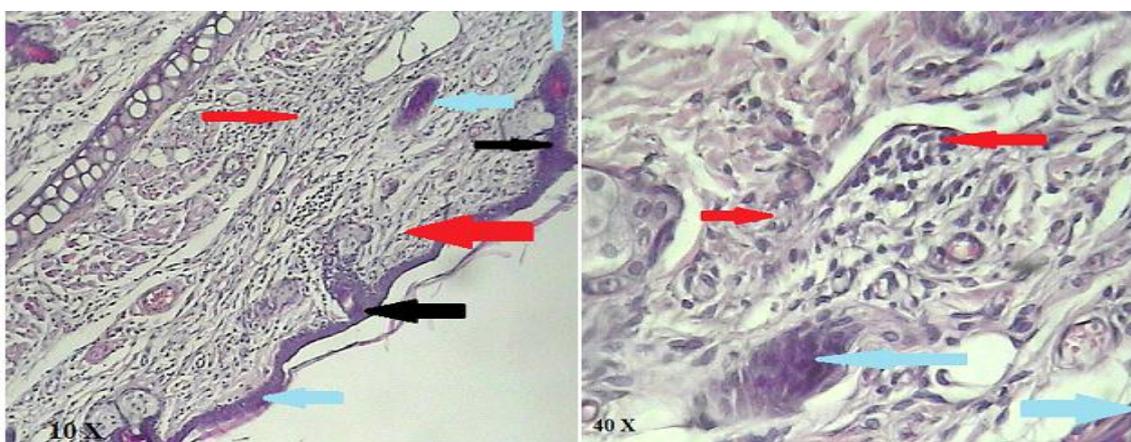
Fig. 8: Histological changes in Group IV (Drug A treated) after 20 days of treatment.

The microscopic examination of tissue sections of Drug A treated acne lesions revealed high rate of infilterated neutrophils, monocytes and lymphocytes. Presence of fibroblasts was 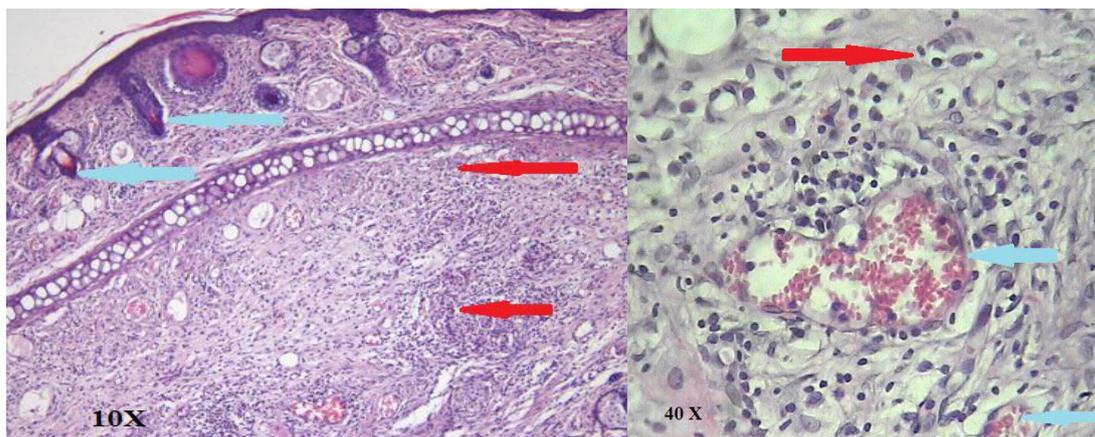also seen at higher magnification (Red arrow) mild clogging at the sebaceous gland was also seen (black arrow), keratinisation was also observed (blue arrow)

Fig. 9: Histological changes in Group V (Drug B treated) after 20 days of treatment.

The microscopic examination of tissue sections of Drug B treated acne lesions revealed infilterated neutrophils, monocytes and lymphocytes lesser than Drug A but more th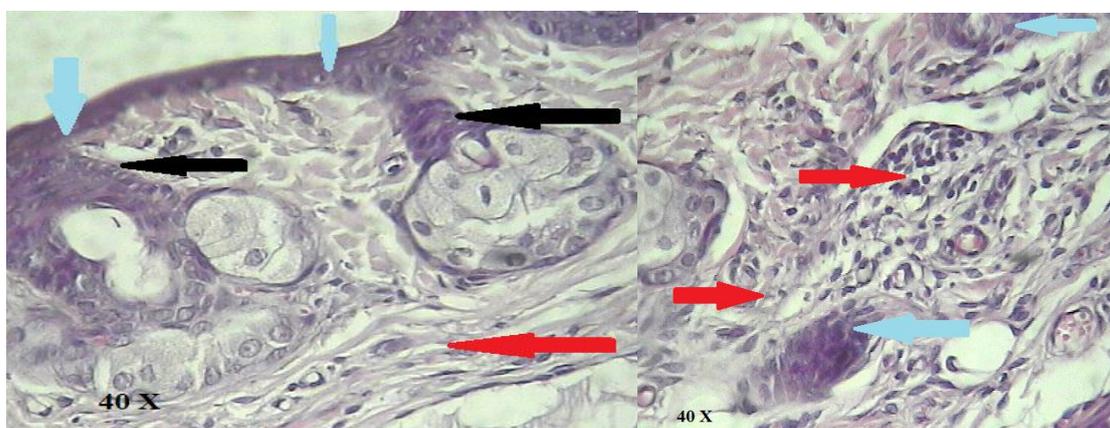an Polyherbal group. Presence of fibroblasts was also noticed at higher magnification (Red arrow), hyper-keratinisation was also observed (blue arrow) at some regions.

Fig. 10: Histological changes in Group VI (Drug C treated) after 20 days of treatment.

The microscopic examination of tissue sections of Drug B treated acne lesions revealed infiltrated neutrophils, monocytes and lymphocytes lesser than Drug A but more th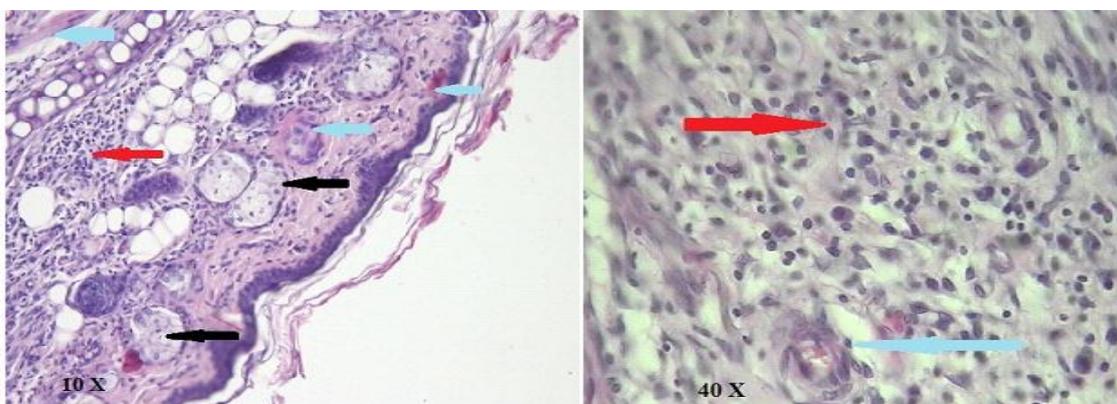an Polyherbal group. Presence of fibroblasts was also noticed at higher magnification **(Red arrow)**, hyper-keratinisation was also observed **(blue arrow)** at some regions.

Fig. 11: Histological changes in Group VII (Drug D treated) after 20 days of treatment.

The microscopic examination of tissue sections of Drug D treated acne lesions revealed infiltrated inflammatory cells at various locations in the reticular and perivascular region of the dermis. Presence of fibroblasts was also noticed at higher magnification **(Red arrow)**, hyperplasia and clogged status of the sebaceous gland also observed **(black arrow)** slight keratinisation was also observed **(blue arrow)** at some regions.

Fig. 12: Histological changes in Group VIII (Drug E treated) after 20 days of treatment.

The microscopic examination of tissue sections of Drug E treated acne lesions revealed infilterated inflammatory cells in the reticular and perivascular region of the dermis. Presence of fibroblasts was also noticed at higher magnification (Red arrow), hyperplasia of the sebaceous

gland also observed (black arrow) slight keratinisation was also observed (blue arrow) at some regions.

DISCUSSION

Acne is a pleomorphic disease. It is caused due to hormonal imbalance. It is commonly accepted that *P.acne (PA)* plays a central role in acne pathogenesis. On the other hand, *PA* is not pathogenic by normal standards, because no correlation between the number of bacteria and the severity and type of acne has been found. Nevertheless, this bacteria produces a variety of extracellular products, such as lipases, proteases, and chemotactic factors, which are responsible for the initiation and maintenance of the inflammatory response.[9] Thus, *PA* directly contributes to the development of acne via its effects on humoral and cell-mediated immunity, complement activation, and pro-inflammatory mediator production by phagocytic cells. It has been reported that specific antibodies against *PA* are involved in the pathogenesis of acne.[10] In order to develop an animal model of inflammation that would be relevant to acne vulgaris, killed *PA* bacteria were injected intradermally into the ears of rats. In our model, we injected the ears of rats with *PA* to stimulate a local, chronic inflammation. The main difference between the development of acne lesions in humans and the development of skin lesions in the inflamed rat's ears is that these animal models essentially bypass the gradual formation of comedonesand they develop changes partially equivalent to the stage of comedo rupture. *PA* (bacteria isolated from acne lesions), however, induced local inflammation with papules and pastules formation.

Androgens are the main cause in the development of acne. Anti-androgenic drug may causes liver and kidney toxicity during treatment of acne. Free radicals are toxic molecules that play a significant role in the inflammatory skin diseases. Pro-inflammatory lipids and cytokines seem to act as mediators for the beginning of the acne lesions. *Propionibacterium acnes (P.acnes)*, a Gram positive microaerophile bacteria is responsible for the local inflammatory response of acne, with the activation of monocytes and production of cytokines.[11] *Propionibacterium* acnes taking part in acne pathogenesis cause the release of some chemotactic factors leading to neutrophils accumulation, and this situation causes damages to follicular epithelia after the release of some inflammatory factors such as lysosome enzymes as a result of phagocytosis. The attracted neutrophils, after phagocytosis, are thought to release lysosomal enzymes and produce free radical, with resultant damage to the follicular epithelium.[12]

In our study, during the microscopic examination of tissue sections of un-treated acne lesions it revealed infiltration of mixed population of inflammatory cells. Severe focal infiltration of lymphocytes, neutrophils and plasma cells were seen in perivascular and reticular region of the dermis. Sebaceous gland showed pyknotic nuclei. For Polyherbal formulation, the microscopic

observations revealed normal histological findings while other drugs A, B, C, D, E showed mild lymphocytic infiltration in the reticular region of dermis after 20 days treatment. This strongly supported the efficacious role of Polyherbal formulation in treating of inflammatory acne lesions. Free radical is released from the active neutrophils in the inflammatory tissue. These free radicals are attack on DNA and/or membrane lipids and cause chemical damage to them, including the healthy tissue. The control of free radical production is essential for normal physiological cell function. Squalene, which is specific to human sebum, protects skin surface from lipid peroxidation, while its lipid peroxidation products lead tocomedogenic effects, and they have been specified in open or closed comedones as highly concentrated. The increased level of MDA in the acne infected groups and its decreased level in treated groups showed the level of lipid peroxidation in acne. It also confirms and supports the finding of other authors about role of oxidative stress in acne induced models. [13] One another study also.[14] reported that antioxidant enzymes such as superoxide dismutase and myloperoxidase activities were decreased in polymorphonuclear leukocytes of acne induced patients. In our study, it was clear that free radical mediated causes acne induction due to bacterial infection by administration of *P. acne* culture in the rats. Because the MDA level was found higher in acne induced group in comparison to control group. Other workers have published similar results.[15] Superoxide dismutase-catalase (SOD-CAT) system consists of antioxidant an enzyme, which plays a significant role in the defence against Oxygen toxicity. [16] Various studies have reported that superoxide radical can damage surrounding healthy epidermal cells.[17]

After topical application of Drug A and Polyherbal formulation drug for 20 days treatment, all the antioxidant enzymes activities were significantly increased along with significantly reduced MDA level as well as biochemical parameters in the plasma of both drug treated groups as compared to infected groups. When other drugs treated group was compared to Polyherbal formulation treated group, the activities of antioxidant enzymes were increased along with decreased MDA level.

In order to assess the biochemical changes in acne we took the biochemical analysis and found elevated levels of lipids and proteins in plasma. In our study, the levels of biochemical parameters (Cholesterol, Albumin, and Total Protein) were increased and level of triglycerides (TG) decreased in the plasma of acne induced groups as compared to control group. But this level reached to normal range on treatment with Polyherbal formulation drug and other drugs. This observation made us to conclude that there is still some undiscovered part of the involvement of the biochemical parameters in acne vulgaris pathophysiology and treatment. It is also established by authors that sebum provides Triglycerides (TG) as the substrate for *P.acnes* growth, which is acted

upon by *P. acnes* lipase to form diglycerides, monoglycerides and free fatty acids from which glycerol, and the utilizable moiety for *P. acnes* metabolism is formed.[18] This statement provides strong affirmation to our finding of reduced level of TG in induced groups as compared to control. This may be due to its usage as substrate by the *P.acne* bacteria for growth. The level on treatment with Polyherbal formulation increased significantly and reached close to control group establishing the destruction of *P.acne* by polyherbal formulation.

While assessing the haematological parameters it was found that the levels of RBC and WBC parameters decreased during course of infection and increased after treatment. The level of platelets also increased significantly in the infected group and decreased after the treatment. This provides an account for the systemic effect of Polyherbal formulation and other drugs and provided a point in their mechanism of action for the treatment of acne vulgaris. As far as physiological parameters such as increased ear diameter is considered, it confirmed the induction of inflammatory acne in rats and supports similar findings by other authors.[19,20] Also the increased body temperature in infected groups and its decrease to normal range provides further affirmation to the diseased condition of animals. Food and water however showed slight alteration which showed that there is no or lesser effect of acne on diet.[21]

Cytokines are present in normal sebaceous glands, and they are affected by many factors. IL-1a, tumor necrosis factor (TNF)-a, IL-6 and IL-8 are released into supernatant in unstressed sebocyte culture. In a stressed environment, the amounts of released cytokines increase significantly. The treatment of cultured sebocytes with *P. acne* and LPS significantly unregulated the expression of pro inflammatory cytokines.[22] While LPS stimulated TNF-α and IL-1a, P. acnes stimulated CXCL8 and TNF-alpha only. *Propionibacterium acnes* also had slight effect on IL-1a. There was also a difference in the cytokine production curve over time after treatment between *P. acnes* and LPS.

In our study, the level of IL-6 and TNF-alpha increased significantly in the induced group which is supported by the finding of above authors and highlighting the role of *P.acne* in cytokine activation. Following the treatment with Polyherbal formulation the levels of these cytokines decreased significantly showing reduction in the inflammation.

CONCLUSIONS
From the result of this study, it is concluded that the a polyherbal formulation has most effective role for the management of acne disease that reduced inflammation along with increased antioxidant level and reduced free radical mediated damage during acne condition and possess the antibacterial, antimicrobial, anti-inflammatory and antiseptic properties.

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