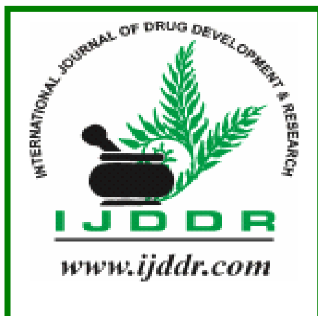

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Original Research Manuscript

PROTECTIVE ROLE OF ACNANO DRUG IN *ACNE VULGARIS*

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ABSTRACT

To evaluate the effect of Acnano drug on status of antioxidant enzymes and other biochemical parameters and its comparison with clearasil drug in the plasma of acne induced rat model. Twenty four rats were selected and divided into three groups. Group I was control treated group, whereas group II and group III were infected plus clearasil as well as infected plus acnano treated group. Acne was induced by the topical administration of cyproterone acetate drug for three weeks and *determine the antioxidant enzymes along with free radical mediated damage (malonaldialdehyde and myloperoxidase activity) as well as hepatic and renal enzymes was done in the plasma of all groups. Our results showed a significant decrease in the enzyme activities along with increased free radical mediated damage and hepatic and renal enzymes in both acne induced infected group as compared with control group. After treatment with clearasil and acnano drug for 7 days, the enzymes activities along with free radical mediated damage as well as hepatic and renal enzymes, were improved in both infected plus treated groups. When acnano treated group was compared with clearasil treated group, these enzyme activities along with free radical mediated damage and other biochemical parameters were increased significantly in acnano treated group after 7 days treatment. Our findings concluded that acnano drug was found to be effective and equivalent to clearasil drug because of their ability to reduced oxidative stress along with increased the enzymes activity and improved liver and kidney function tests due to hormonal imbalance during administration of anti-androgenic drug .*

Key words: *Clearasil, acanano, antioxidant enzyme, hepatic and renal enzymes*

Introduction

Acne vulgaris is one of the most common dermatological diseases frequently found in the late childhood and adolescence[1]. Sebaceous hyperplasia, follicular hyperkeratinization and bacterial hypercolonization, as well as immune reactions and inflammations may lead to acne, which has quite complex pathogenesis[2]. It is a common inflammatory condition affecting the pilosebaceous unit. Proprionibacterium acnes produce follicular lipases, proteases, hyaluronidases and several enzymes which may play an important role in the inflammatory process[3]. In acne, sebum is produced by sebaceous glands, content changes and reactive oxygen species (ROS) may released from the impacted damaged follicular walls; at the same time it is thought that this may be the reason for the progress of the inflammation in the pathogenesis of the disease [4]. It is well known that most of the drugs which is commonly used in the treatment of acne act by decreasing reactive oxygen species [5].

Oxygen is a vital component for life, which can produce oxy free radicals such as superoxide anion, H_2O_2 and hydroxyl radicals. These radicals are formed with the reduction of oxygen to the water.

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Normally, the production of these radicals is slow and these radicals are removed by the antioxidant enzymes in the cell. In normal cell, there is proper balance between free radical generating and free radical scavenging enzymes ratio. Any imbalance between these ratios can lead to damage several kinds of macromolecules such as lipid, protein and nucleic acids. There are some of the important antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and oxidized glutathione etc. When these enzymes are insufficient for oxidative stress, then oxy free radical donates its impact by starting the lipid per oxidation on the membranes of organs and cell. Malonaldialdehyde (MDA) is the end product of lipid per-oxidation and one of the indicators of free radical mediated damage (oxidative stress). Although acne vulgaris is the most frequent disease of the young population, only a few studies on antioxidative system in acne pathophysiology have been performed up to now[6-7]. In the present study, the authors have tried to determine the effect of acnano drug on status of antioxidant enzymes and other biochemical parameters and its comparison with clearasil drug in the plasma of acne induced rat model.

Materials and Methods

Chemicals & drug

Thiobarbituric acid (TBA), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), 5.5 -Dithio bis 2- nitrobenzoic acid (DTNB), tetra nicotinamide adenine dinucleotide phosphate (NADPH) tricholoroacetic acid (TCA), epinephrine and reduced glutathione (GSH), were purchased from Sigma Chemical Co., St Louis, MO, USA. Biochemical kits for estimation of Creatinine, SGOT, SGPT and Uric acid was obtained from Bayer Diagnostics India Ltd.,Baroda, Gujrat India. All other reagents used were of high quality and analytical grade. Acnano drug was obtained from Venus remedies Ltd, and clearasil drug was purchased locally from a pharmacy (Chandigarh).

Animals

Twenty four rats (weighing 100 to 150 g) were used in the experiment. The rats were fed standard pelleted diet and water *ad libitum.* The test room was air conditioned with temperature 23 ± 2°C, humidity 65 ± 5%, and with artificial fluorescent light (10 and 14 hours of light and dark, respectively). The study was approved by the institutional animal ethical committee.

Acne induced model

Acne was induced by the *inhibition of sebum secretion* in animals according to method of *Neumann*[8]. For induction of acne, total sixteen rats were selected and divided in to group II & group III. Group I received 20% ethanol solution only. The interscapular area of group II and III animals were shaved and after 24 hours cyproterone acetate drug was applied locally to shaved area with dose 6mg/cm^2 in 20% ethanol solution. The drug was applied for 3 weeks for induction of acne. After induction of acne, blood sample was collected in 3.8% sodium citrate solution containing vial for preparation of plasma and analyzed the enzyme activities and biochemical parameters.

Treatments

The drugs treatments were given topically two times a day for 7 days. The rats were divided into three groups of eight rats each as given below.

Group I (n=8) control; ethanol treated group (20%)
Group II (n=8) acne induced + Clearasil treated group
Group III (n=8) acne induced + Acnano treated group

After 7 days treatments of both drug topically, again blood sample was collected from all groups and prepared the plasma at 6500 rpm for 15 minutes and further analyzed all the biochemical

parameters and antioxidant enzymes activities in the plasma of acne induced rat model.

Enzyme Assay

All the enzymes were carried out at 0-4 °C

Assay of superoxide dismutase activity (EC 1.15.1.1)

SOD activity was determined by the Method of Misra and Fradovich[9].The reaction mixture consisted of 1.0 ml carbonate buffer (0.2 M, pH 10.2), 0.8 ml KCl (0.015M), 0.075 ml of plasma preparation and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml of epinephrine (0.025M). The change in absorbance was recorded at 480 nm at 15 second interval for one minute at 25^0C. Suitable control lacking enzyme preparation was run simultaneously.

One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine.

Assay of Catalase activity (EC 1.11.1.6)

Catalase activity was measured by the method of Luck[10]. The reaction mixture consisted of 0.3 ml phosphate buffer, (0.2 M; pH 6.8), 0.1ml H_2O_2 (1M) and water to make the final volume to 3.0ml. The reaction was started by adding the 0.05ml of plasma preparation. The change in the absorbance was recorded at 15 second interval for one minute at 240 nm at 25°C. Suitable control was run simultaneously. One Unit of enzyme activity was defined as the amount of enzyme that liberates half of the peroxide oxygen from H_2O_2 in 100 sec at 25°C.

Estimation of Myloperoxidase activity

MPO activity was determined by a modification of the O-dianisidine method [11]. The assay mixture, in a cuvette with a path length of 1 cm, contained a 0.3 ml 0.1M phosphate buffer (pH 6.0), 0.3 ml (0.01 M) H_2O_2, 0.5 ml (0.02 M) O-dianisidine (freshly prepared) in deionized water and 25 µl plasma preparation in a final volume of 3 ml. The plasma preparation was added in last and the change in absorbance was recorded at 460 nm for 10 min. All measurements were carried out in duplicate. One unit of MPO is defined as that giving an increase in absorbance of 0.001 per min and specific activity is given as IU/mg protein.

Estimation of reduced glutathione

The reduced glutathione was estimated by the method of Hissin and Hilf[12]. Plasma preparation 0.1 ml was mixed with 3.0 ml of 10% (w/v) TCA reagent and kept for 10 min, proteins were precipitate and filter out. After filtration, 2.0 ml filtrate was taken and added to 4.0ml of phosphate buffer (0.3 M; pH 7.4) and 1.0 ml of DTMB (1.0% w/v aqueous sodium citrate). A blank sample was prepared in similar manner using double distilled water in place of the filtrate. An appropriate standard solution of 0.1 ml GSH (10 µmole) was also run simultaneously. The pale yellow color was developed and optical density was measured at 412 nm by spectrophotometer.

Estimation of Lipid peroxidation level

Lipid peroxidation level in the plasma sample was expressed in MDA. It was determined by the procedure of Ohkawa et al., [13]. The reaction mixture consisted of 250 µl of plasma prepartion, 0.20 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid, 1.5 ml of 0.8% thio barbituric acid (TBA) and water to make up the volume to 4.0 ml. The tubes were boiled in water bath at 95°C for one hour and cooled immediately under running tap water. Added 1.0 ml of water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) and vortexed. The tubes were centrifuged at 3500 rpm for 30 minutes. The upper layer was aspirated out and optical density was measured at 532 nm. The reference standard used was 1,1, 3,3 tetra ethoxy propane.

Protein estimation

Protein was measured by the method of Lowery et al.,[14], using Folin phenol reagent. Bovine serum albumin was used as standard.

Measurement of SGOT, SGPT, Uric acid and Creatinine

These biochemical parameters were analyzed by using commercially available kits (Bayer Diagnostics India Ltd.,Baroda, Gujarat India).

Statistical analysis

The resulting data were analyzed statistically. All values are expressed mean ± SD. One -way Analysis of variance (ANOVA) with student Newman Keuls test was used to determine statistical difference between before and after treatment of all groups and $p<0.05$ were considered statistically significant.

RESULTS

The results of present study showed that there was significantly ($p<0.001$) decrease in the superoxide dismutase activity in the plasma of acne induced group II and group III as compared to control group (group I). The enzyme activity was found to be statistically significantly increased in the plasma of clearasil treated group II ($p<0.001$) as well as acnano treated group III ($p<0.001$) as compared to both infected groups, after treatment of both drugs for 7 days. When clearasil treated group II was compared to acnano treated group III, the superoxide dismutase activity was significantly increased ($p<0.05$) in the plasma of acnano treated group after 7 days treatment. After treatment of both infected groups with respective drugs, the superoxide dismutase activity was increased in both treated groups in comparison to control group (Fig. 1).

Catalase activity was also lowered significantly ($p<0.001$) in the plasma of both acne induced groups (II and III) as compared to control group. After treatment of both acne induced groups by clearasil and acnano drug for 7days, the enzyme activity was significantly ($p<0.001$) increased in the plasma of clearasil as well as in acnano treated group as compared with both infected groups. When clearasil treated group was compared with acnano treated group after 7 days treatment, the enzyme activity was significantly ($p<0.01$) elevated in acnano treated group (Fig.2).

Reduced glutathione level was significantly ($p<0.001$) decreased in the plasma of acne induced group III. Whereas in case of group II, this level was decreased but did not change significant as compared to control group. The level of GSH was increased but did altered significantly in clearasil treated group when compared with infected group II. In case of acnano treated group, the level was found to be statistically significant ($p<0.001$) increased in the plasma of acnano treated group as compared with infected group III after 7 days treatment. When clearasil treated group was compared to acnano treated group, the GSH level was found to be increased but insignificant in the plasma of acnano treated group after treatment (Fig.3).

Myloperoxidase (MPO) activity was significantly increased ($p<0.001$) in the plasma of acne induced groups as compared with control group. The MPO activity was found lowered and statistically significant ($p<0.001$) in the plasma of both treated groups after topically administration of clearasil and acnano drug for 7 days. This enzyme activity was lowered but did not altered significant in the plasma of acnano treated group when compared with clearasil treated group (Tab.1)

MDA level (as a marker of free radical mediated damage) was significantly increased ($p<0.001$) in the plasma of both acne induced groups (group II &III) as compared with control group. This level was found to be decreased ($p<0.001$)

significantly in both respective treated groups after 7 days treatment as compared with both infected groups. When clearasil treated group was compared with acnano treated group, this level was found to be reduced significantly ($p<0.01$) in acnano treated group after seven days treatment (Tab.1) .

Serum glutamyl oxaloacetic transaminase (SGOT) and serum glutamyl pyruvic transaminase (SGPT) levels were also increased in the plasma of both acne induced groups as compared with control group. These levels were found to be decreased insignificantly in the plasma of clearasil treated groups as compared with infected group after 7 days treatment. Whereas in case of acnano treated group, these level were decreased ($p<0.05$; $p<0.001$) significantly in the plasma of acnano treated group after 7 days treatment. When clearasil treated group was compared with acnano treated group, the level of SGOT was ($p<0.001$) significantly decreased in the plasma of acnano treated group while in case of SGPT, the level was found to be decreased but in significant in the plasma of acnano treated group (Tab.2).

Creatinine and uric acid levels were also found to be increased ($p<0.001$) significantly in the plasma of both acne induced groups as compared to control group. These levels were reduced ($p<0.001$) significantly in the plasma of clearasil as well as acnano treated group after 7 days treatment when compared with both infected groups. When clearasil treated group was compared with acnano treated group, the creatinine level was found to be decreased ($p<0.001$) significantly in the plasma of acnano treated group while in case of uric acid, the level was reduced but insignificant in the plasma of acnano treated group after 7 days treatment (Tab.2).

Discussion

Acne is a pleomorphic disease[15]. It is caused due to hormonal imbalance. Androgens are the main cause in the development of acne. Anti androgenic drug may causes liver and kidney toxicity during treatment of acne. In the present study, authors have also determined the status of hepatic and renal enzymes in the plasma of acne induced rat by topical application anti-androgenic drug (cyproterone acetate). There are several reports suggested that cyproterone acetate (CPA) drug induced hepatic[16], and renal toxicity [17].

Free radicals are toxic molecules that play a significant role in the inflammatory skin diseases[4,18]. 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[19]. 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[4, 20-21]. 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 to comedogenic effects, and they have been specified in open or closed comedones as highly concentrated [4].

In the study, the all the antioxidant enzymes activities (SOD, CAT and GSH) were significantly lowered along with increased in the levels of biochemical parameters (creatinine, uric acid, SGOT and SGPT), MPO as well as MDA level in the plasma of acne induced groups as compared to control group. Kurutas et al reported that antioxidant enzymes such as superoxide dismutase and myloperoxidase actvities were decreased in polymorphonuclear leukocytes of acne induced patients [7]. In our study, it was clear that

free radical mediated causes acne induction due to hormonal imbalance by administration of anti-androgenic drug in the rats. Because the myloperoxidase activity as well as MDA level was found higher in acne induced group in comparison to control group. Other workers have published similar results[6, 22]. Superoxide dismutase- catalase (SOD-CAT) system consists of antioxidant enzymes, which plays a significant role in the defense against oxygen toxicity [23]. SOD is an enzyme existing in cytoplasm and providing the formation of hydrogen peroxide. However, CAT destroys hydrogen peroxide[24- 25]. The function of SOD is to convert superoxide anion free radicals that are detrimental to all living cells, to H_20_2 and molecular oxygen [26]. Various studies have reported that superoxide radical can damage surrounding healthy epidermal cells [27].

After topical application of clearasil and acnano drug for 7 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 clearasil treated group was compared to acnano treated group, the activities of antioxidant enzymes were increased along with decreased MDA level and all biochemical parameters in the plasma of acnano treated group. Acnano is a topical drug which contains tea tree oil as active ingredient along with other oils such as rosemary oil, mentha oil. These ingredients have an antibacterial, antimicrobial, anti-inflammatory and antiseptic properties[28-30].

Status of superoxide dismutase activity in acne induced rat before and after treatment

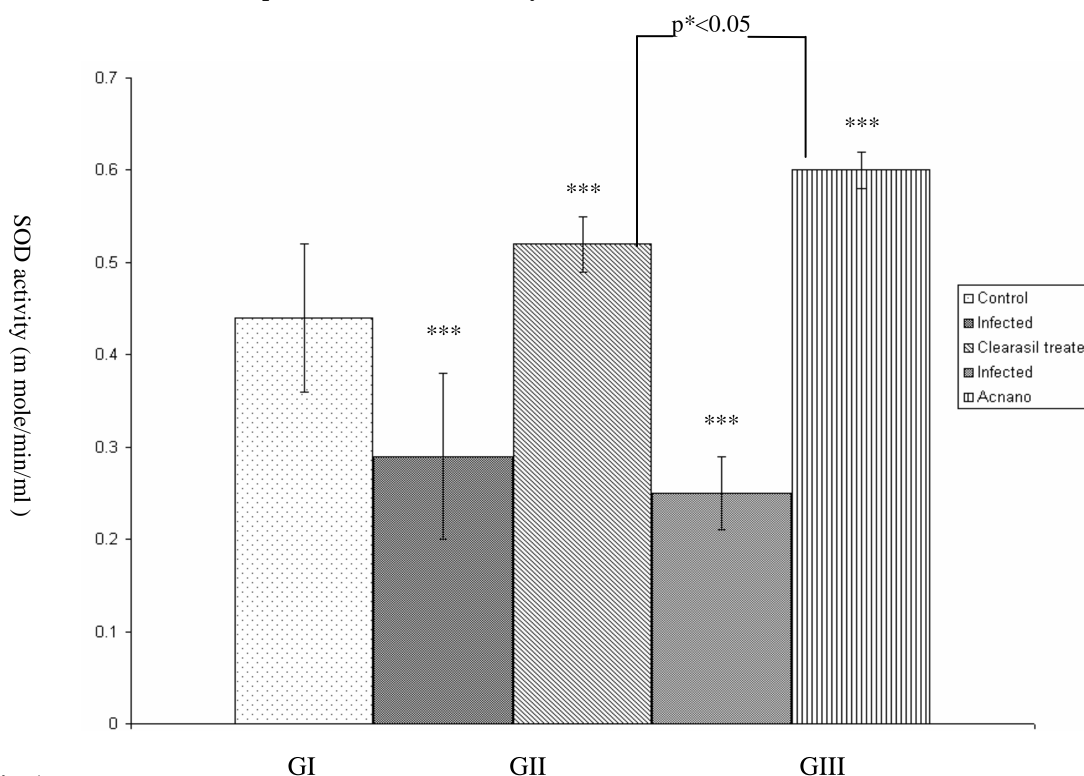

Fig.1. Values are expressed in Mean ± SD. Where G is group. Statistical analysis was determined between control vs infected group and infected vs treated groups. Where $P^{***}<0.001$ (highly significant), $P^{**}<0.0$(significant), $P^{*}<0.05$(significant) and $P>0.05$(not significant)

Status of catalase activity in acne induced rat before and after treatment

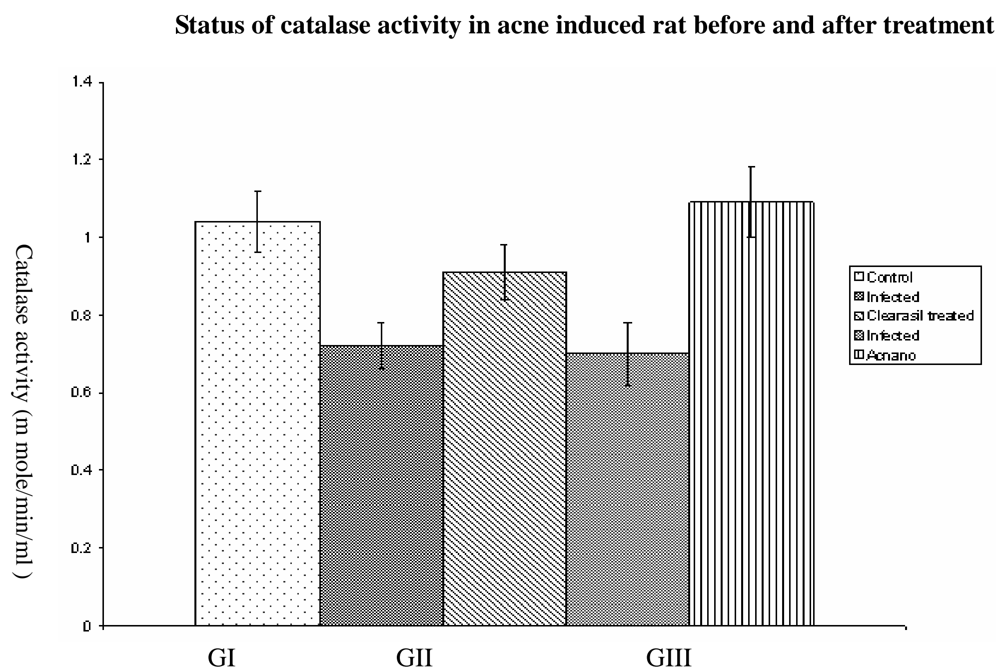

Fig.2. Values are expressed in Mean ± SD. Where G is group. Statistical analysis were determined between control vs infected group and infected vs treated groups. Where $P^{***}<0.001$ (highly significant), $P^{**}<0.01$ (significant), $P^{*}<0.05$(significant) and $P>0.05$(not significant)

Status of GSH (reduced glutathione) level in acne induced rat before and after treatment

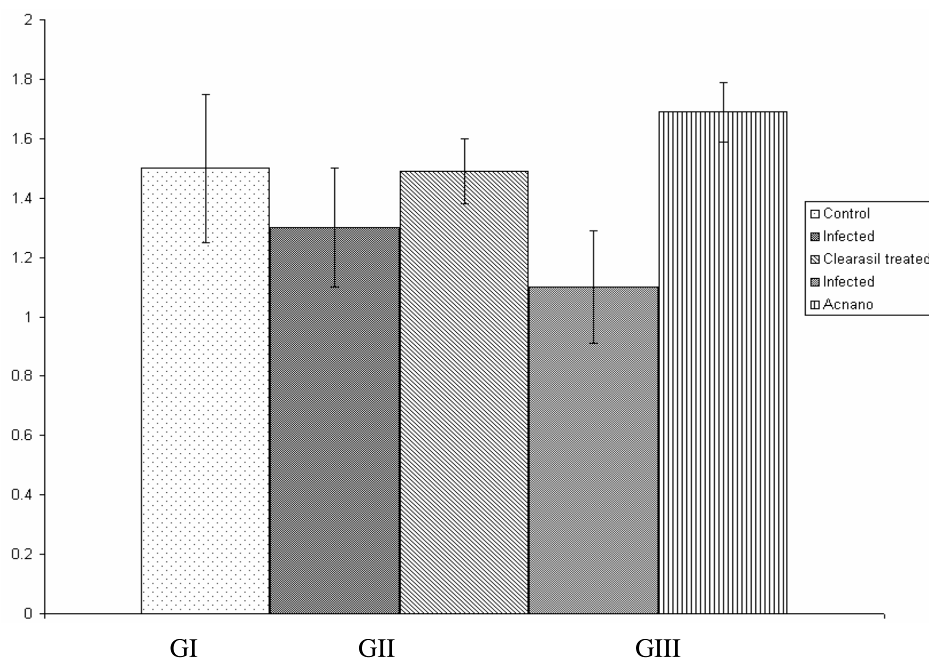

Fig.3. Values are expressed in Mean ± SD. Where G is group. Statistical analysis were determined between control vs infected group and infected vs treated groups. Where $P^{***}<0.001$ (highly significant), $P^{**}<0.01$ (significant), $P^{*}<0.05$(significant) and $P>0.05$(not significant)

Table 1: Status of myloperoxidase activity and MDA level in acne induced rat before and after treatment

S.No	Parameters	Control	Infected	Clearasil treated	Infected	Acnano treated
1	MPO (mmole/min/ml)	0.46 ± 0.03	0.89 ± 0.021[a]	0.27 ± 0.05[a]	0.53± 0.018[a]	0.25± 0.04[a]
2	MDA (nmole/ml)	0.026 ± 0.003	0.084 ± 0.011[a]	0.042 ± 0.008[a]	0.107± 0.024[a]	0.0606 ± 0.010[a]

Values are expressed in Mean ± SD. Where G is group.
Statistical analysis was determined between control vs infected group and infected vs treated groups as well as both treated group . Where a; $P^{***}<0.001$ (highly significant), b; $P^{**}<0.01$ (significant), c; $P^{*}<0.05$(significant) and d; $P>0.05$(not significant)

Table 2: Status of hepatic and renal enzymes in acne before and after treatment

S.No	Parameters	Control	Infected	Clearasil treated	Infected	Acnano treated
1	SGOT (mg/dL)	31.83 ± 2.11	42.51 ± 2.14[a]	41.17± 1.18[d]	35.6± 5.20[c]	31.9± 2.09[a]
2	SGPT(mg/dL)	31.34 ± 4.31	35.12± 2.27[d]	31.36± 4.36[d]	43.56± 3.09[a]	33.16± 1.89[a]
3	Creatinine (mg/dL)	0.98 ± 0.07	1.41± 0.23[a]	1.11± 0.06[a]	1.20± 0.10[b]	0.83± 0.12[a]
4	Uric acid(mg/dL)	1.88± 0.12	2.13± 0.08[a]	1.92± 0.13[a]	2.23± 0.12[a]	1.87± 0.06[a]

Values are present in Mean ± SD. Statistical values were analyzed between control vs both infected group and infected vs both treated group.T
Where a; $P^{***}<0.001$ (highly significant), b; $P^{**}<0.01$ (significant), c; $P^{*}<0.05$ (significant) d; $P>0.05$ (not significant).

Conclusions

On the basis our findings, it was concluded that the results of acnano were found to be comparable to the standard drug clearasil because of their ability to reduced oxidative stress along with increase in antioxidant enzymes activity. It also improve the liver and kidney function tests due to hormonal imbalance during administration of anti-androgenic drug .

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References

1) *Bergfeld WF. The pathophysiology of acne vulgaris in children and adolescents, Part 1. Cutis 2004; **74**: 92-97.*
2) *Gollnick H. Current concepts of the pathogenesis of acne: implications for drug treatment. Drugs 2003; **63** :1579–1596.*
3) *Burkhart CN. Clinical assessment of acne pathogenesis with treatment implications. Int Pediatr 2003; **18**:14–19.*
4) *Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What's new? J Eur Acad Dermatol Venereol 2003; **17**: 663–669.*
5) *Webster GF. Inflammation in acne vulgaris. J Am Acad Dermatol 1995; **33** : 247–253.*
6) *Basak PY, Gultekin F, Kilinc I. The role of the antioxidative defense system in papulopustular acne. J Dermatol 2001; **28**:123–127.*
7) *Kurutas EB, Arican O, Sasmaz S. Superoxide dismutase and myeloperoxidase activities in polymorphonuclear leukocytes in acne vulgaris.*

Acta Dermatovenerol Alp Panonica Adriat 2005; ***14*** *: 39–42.*

8) *Neumann F, Elger W. The effect of a new antiandrogenic steroid, 6-chloro-17-hydroxy-1□,2□-methylenepregena-4,6-diene-3,20-dione acetate (cyproterone acetate) on the sebaceous glands of mice. J Invest Dermatol 1966;* ***46*** *:561–572.*

9) *Misra HP, Fridovich I . The role of superoxide anion in the auto-oxidation of epinephrine and a sample assay for Super-oxide dismutase. J Biol Chem 1972;* ***247****: 3170-3175.*

10) *Luck Catalase. In: Bergmeyer HU, editor. Method in Enzymatic analysis. NY: Academic Press. 1965; 885-894.*

11) *Worthing Enzyme Manual Worthington Biochemical Corporation Freehold, New Jersey, USA, 1972.*

12) *Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 1976;* ***74****: 214–26.*

13) *Ohkawa H, Ohishi N, Yagi K.Assay of lipid per-oxidation in animal tissue by thio barbutric acid reaction. Anal Biochem 1979;* ***95****: 351-358.*

14) *Lowery OH, Roseborogugh MJ, Farr AL et al. Protein measurement in Folin phenol reagent. J Biol Chem 1951;* ***193 :*** *265-275.*

15) *Burkhart CG, Burkhart CN, Lehmann PL. Acne: a review of immunologic and microbiologic factors. Postgrad Med J 1999;****75****: 328-31.*

16) *Kaiser E and Gruner. H.S. Liver structure and function during long-term treatment with cyproterone acetate. Arch Gynecol Obstetric 1987; 240: 217-223.*

17) *Kaymak Y. Creatine phosphokinase values during isotretinoin treatment for acne. Int J Dermatol 2008;* ***47*** *: 398-441.*

18) *Oztas MO, Balk M, Ogus E et al. The role of free oxygen radicals in the aetiopathogenesis of rosacea. Clin Exp Dermatol 2003;****28*** *:188–192.*

19) *Akamatsu H, Niwa Y, Matsunaga K. Effect of palmitic acid on neutrophil functions in vitro. Int J Dermatol 2001;* ***40****: 640–3.*

20) *Akamatsu H, Horio T, Hattori K. Increased hydrogen peroxide generation by neutrophils from patients with acne inflammation. Int J Dermatol 2003;* ***42****: 366–369.*

21) *Brown SK, Shalita AR. Acne vulgaris. Lancet 1998;* ***351****:1871–1876.*

22) *Ozer Arican, Ergul Belge Kurutas, and Sezai. Oxidative Stress in Patients With Acne Vulgaris. Mediators Inflamm 2005;* ***14****: 380–384.*

23) *Mates JM, Perez-Gomez C, Nunez de Castro I. Antioxidant enzymes and human diseases. Clin Biochem 1999;* ***32*** *: 595–603.*

24) *Pugliese PT. The skin's antioxidant systems. Dermatol Nurs 1998;* ***10*** *:401–416.*

25) *Maccarrone M, Catani MV, Iraci S. et al. A survey of reactive oxygen species and their role in dermatology. J Eur Acad Dermatol Venereol. 1997;* ***8****:185–202.*

26) *DeChatelet LR, McCall CE, McPhail LC. et al. Superoxide dismutase activity in leukocytes. J Clin Invest 1974;* ***53****: 1197–201.*

27) *Borrello S, Seccia A, Galeotti T. et al. Protective enzymes in human epidermal carcinomas and psoriasis. Arch Dermatol Res 1984;* ***276****: 338–40.*

28) *Carson C F, Hammer K A, and. Riley TV. Melaleuca alternifolia (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. Clin Microbiol Rev 2006;* ***19*** *: 50-62.*

29) *Bozin B, Mimica-Dukic N, Samojlik I et al. Anti-microbial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. J Agricultural Food Chem. 2007;* ***55****: 7879-7885.*

30) *Mimica-Djukic N and Božin B. Mentha L. Species (Lamiaceae) as Promising Sources of Bioactive Secondary Metabolites. Curr Pharma Design 2008;* ***29*** *: 3141-3150.*

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