

Liquid Supplement in Testosterone Induced Evaluation of Ameliorative Potentials of Cleanshield Benign Prostatic Hyperplastic (BPH) Rat Model

ABSTRACT

Aim: The aim of this study was to evaluate the ameliorative effect of Cleanshield Liquid Supplement in Testosterone Induced Benign Prostatic Hyperplastic (BPH) in male albino rats

Study design: This study is a case controlled experimental study.

Place and Duration of Study: Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria, between December, 2019 and May, 2020.

Methodology: A total of 30 male albino wistar rats were used for this study and divided into 6 groups of 5 rats each. Testosterone propionate (4mg/kg) was used to induce BPH subcutaneously in the rats and then were given (0.5mg/kg) dutasteride, an anti-BPH drug and Cleanshield liquid supplement (0.24ml, 0.48ml and 0.72ml) for 30 days. At the end of the 30 days treatment, the animals were sacrificed. Chloroform was used to anaesthetize the rats and 5ml of whole blood samples were collected through cardiac puncture at the end of 8 hours fast. The blood samples collected were separated and the serum was used to analyze for prostate specific antigen (PSA) using rat specific PSA ELISA kit produced by Shanghai Korain Biotech Co., Ltd, China, while liver enzymes (alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP)) were analyzed using spectrophotometric method. Liver tissues of the rats were excised and used for histological analysis. SPSS version 22.0 was used for statistical analysis and $p < 0.05$ was considered statistically significant.

Results: The results showed that comparison between the Cleanshield treated groups and the Avodart group (group 3) showed no significant difference but there was a non-significant reduction in the mean PSA values of all the clean shield treated group with the group that received the highest amount of Cleanshield having the most reduced mean PSA value. The mean PSA value of the negative control group (NC) compared to dutasteride group and all Cleanshield group showed statistically significant difference. The mean ALT values did not show any significant difference statistically when the groups that received Cleanshield supplement were compared to PC group. In the mean values of AST, only the comparison between anti BPH group and the group that received 0.72ml of Cleanshield produce statistically significant difference ($p = 0.036$). That of the ALP comparison between the mean ALP values of the PC group (grp 2) showed significant difference against group 2 ($p = 0.001$) and against group 5 ($p = 0.003$). Avodart group (group 3) showed a statistical difference when compared to group 4 (0.24ml of Cleanshield) at $p = 0.000$. Group 4 (0.24ml of Cleanshield) showed statistical difference when compared to group 5 (0.48ml of Cleanshield) at $p = 0.001$.

Conclusion: Conclusively, Cleanshield liquid supplement is not toxic to the liver but does not possess significant BPH ameliorative potentials.

Keywords: Ameliorative Potentials Cleanshield Liquid Supplement, Testosterone, Benign Prostatic Hyperplastic (BPH), Rat

1. INTRODUCTION

Benign prostatic hyperplasia (BPH), also known as benign prostatic hypertrophy, is a proliferation of the prostatic stromal cells which results in an enlarged prostate gland [1]. As a result, the prostatic urethra is compressed which restricts the flow of urine from the bladder, [1]. Its histologic diagnosis characterized by proliferation of the cellular elements of the prostate. Cellular accumulation which may result from epithelial and stromal proliferation, impaired preprogrammed cell death (apoptosis), or both [2]. BPH is considered a normal part for the aging process in men and is hormonally dependent on testosterone and

dihydrotestosterone (DHT) production. An estimated 50% of men demonstrate histopathologic BPH by age 60 years. This number increases to 90% by age 85 years generally called elderly people or geriatrics comma [3].

The voiding process dysfunction that arises from the enlarged prostate gland and bladder outlet obstruction (BOO) is known as lower urinary tract symptoms (LUTS). It has also been commonly referred to as prostatism, although this term has decreased in popularity. These entities overlap; not all men with BPH have LUTS, and likewise, not all men with LUTS have BPH. Approximately half of men diagnosed with histopathologic BPH report moderate-to-severe LUTS [2].

Some clinical manifestation of LUTS include: Frequent urination during the day or night (nocturia) which usually ends up voiding only small amounts of urine with each episode. Urinary urgency (There is a sudden and urgent need to urinate, due to the sensation of imminent loss of urine without control). Hesitancy, (difficulty initiating the urinary stream; interrupted, weak stream), Incomplete bladder emptying (there is persistent feeling of residual urine, even when there is frequency of urination). Straining (the need strain or push (Valsalva maneuver) to initiate and maintain urination in order to evacuate the bladder fully. Decreased force of stream (the subjective loss of force of the urinary stream over time. Dribbling (the loss of small amounts of urine due to a poor urinary stream) [4-5].

The individual's sexual history is important and taken, as epidemiologic studies have identified LUTS as an independent risk factor that causes erectile and ejaculatory dysfunction [6].

The increasing cases of benign prostatic hyperplasia as it stands now require urgent attention for the well-being of the elderly particularly as it affects men in their early forties. From a population-based study done in South-West of Nigeria, the overall prevalence of BPH stands at 23.7% or 237 per 1000 men and the age-adjusted prevalence increases with increasing age. It was stated that very few of the men diagnosed in this study were on medication for BPH and this suggests the need for more public awareness about disease with manifestations that can affect quality of life (QoL) adversely [7]. This organ, the prostate, tends to increase in size as a man ages [8]. Many researchers have carried out studies to determine the primary cause of the disease and to determine its effective management and its related complications. Age, race, lifestyle, both modifiable (example diet, smoking etc) and non-modifiable (example genetics, family history) among others have been implicated in this disease condition, [9].

Cleanshield is a natural organic supplement, and not a conventional drug directed to a particular germ or designed for a particular health condition. Cleanshield is a supplement that is designed to rehabilitate, reinstate and reinforce the body's broken ability to heal and protect against diseases. It takes back the body's immune system to its initial natural position which is actually a shield. This is achieved by the products ability to raise the inner oceans (body) pH to a level that is very harsh and uncomfortable for germs and disease conditions. Any germ present before the pH is raised is automatically, goes away [9].

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of 30 male albino wistar rats weighing between 170 ± 10 g were used in this study. The rats were purchased from the Department of Pharmacology, University of Port Harcourt, Choba, Rivers State, Nigeria. They were randomly divided into 6 groups of 5 rats each. They were housed in the animal house of Department of Applied and Environmental Biology, Rivers State University and were kept in a well-ventilated cage at room temperature under

12hours of dark and light cycle with access to feed (Top Feed Finisher Mash, Sapele, Nigeria) and water *ad libitum*. Humane treatment according to the criteria in the Guide for Care and Use of Laboratory Animals prepared by National Institute of Health [10] were used.

2.2 Drugs Dose Calculations

2.2.1 Avodart (Dutasteride)

Avodart, manufactured by GlaxoSmithKline, UK was purchased from Meridian Pharmacy, Meridian Hospitals, Port Harcourt after explanation of the purpose for procurement. The calculation of the administered dosages was based on guidelines from U.S. Department of Health and Human Services Food and Drug Administration Centre for Drug Evaluation and Research [11].

Human daily dose is 1 capsule of 0.5mg/kg daily

For a rat of 1kg (1000g) = $1000/1000 \times 0.5\text{mg/kg}$

0.5mg \equiv 1kg \equiv 20ml

For a rat of 160g = $160/1000 \times 0.5\text{mg/kg}$

0.08mg \equiv 160g \equiv 3.2ml

Therefore, 160g of rat was given 0.08mg of Avodart.

2.2.2 Testosterone (Testosterone Propionate) and Induction of BPH

Induction of benign prostatic hyperplasia was achieved by intraperitoneal injection of 4mg/kg body weight (b. wt) of testosterone propionate according to [12]. Testosterone propionate (Testost), manufactured by Laborate Pharmaceuticals India Limited) was procured from Sicone Pharmacy and stores, Port Harcourt after comprehensive explanation of the purpose for procurement.

2.2.3 Cleanshield Liquid Supplement

Cleanshield Liquid Supplement was purchased at drug line, Ogbete market in Enugu state and was administered based on extrapolations of manufacturer's recommended clinical dose. Human daily dose is 30ml x 3times /day = 90ml/day for a rat of 160kg = $160/60000 \times 90 = 0.24\text{ml/day}$.

2.3 Experimental Study Design

The rats were randomly selected into 6 groups of five rats each:

Group 1 (Negative Control group- NC).

They were not induced with testosterone propionate. Cleanshield and avodart treatment were not given to them.

Group 2 (Positive Control –PC)

This group was BPH induced through subcutaneous injection of 4mg/kg body weight (b.wt.) of testosterone propionate and were not given treatment (Avodart or Cleanshield) for 30days but allowed to live on rat feed.

Group 3 (BPH + Avt Trt)

This Group was subjected to BPH, induced by subcutaneous injection of 4mg/kg body weight (b.wt.) of testosterone propionate and simultaneous treatment of oral administration of 0.08mg/kg/day of Avodart (Dutasteride) daily for 30 days.

Group 4 (BPH + 0.24mlCS)

This group was subjected to BPH, by subcutaneous injection of 4mg/kg body weight (b.wt.) of testosterone propionate and simultaneous treatment of oral administration of 0.24ml/kg/day of Cleanshield liquid supplement daily for 30 days.

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Comment [A2]: Details of the environmental protocol of the experimental rats, light-dark cycle, humidity. Water consumption: quantity and quality, the latter influences the development of prostate processes in rats.

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Group 5 (BPH + 0.48mlCS)

This group was subjected to BPH induced by subcutaneous injection of 4mg/kg body weight (b.wt.) of testosterone propionate and simultaneous treatment of oral administration of 0.48ml/kg/day of Cleanshield liquid supplement daily for 30 days.

Group 6 (BPH + 0.72mlCS)

This group was subjected to BPH, induced by subcutaneous injection of 4mg/kg body weight (b.wt.) of testosterone propionate and simultaneous treatment of oral administration of 0.72ml/kg/day of Cleanshield liquid supplement daily for 30 days.

2.4 Sample Collection and Preparation

At the end of the 30 days treatment, the animals were sacrificed. Chloroform was used to anaesthetize the rats and 5ml of whole blood samples were collected through cardiac puncture at the end of 18 hours fast. The samples were dispensed into plain container, allowed to stand, retracted, within thirty (30) minutes of obtaining the sample and centrifuged at 12000rpm for 5 minutes to obtain serum for the determination of PSA, AST, ALT and ALP. The serum samples were then stored at -20° C temperature pending the time of determination. For histological analysis, the liver tissues of the rats were harvested, washed in normal saline and preserved in 10% formal saline.

2.5 Laboratory Assay of Biochemical Parameters.

2.5.1 Estimation of Rat Prostate Specific Antigen

Rat PSA was determined using rat specific PSA Enzyme Linked Immunosorbent Assay as demonstrated by [13] and modified by Shanghai Korain Biotech Co., Ltd, China.

2.5.2 Determination of Alanine Aminotransferase (ALT), Aspartate Amino Transferase (AST) and Determination of Alkaline Phosphatase (ALP)

ALT, AST and ALP activities were determined spectrophotometrically as respectively described by [14], [15] and [16].

2.6 Histological Analysis

The liver of the animals in all the groups were harvested for histological analysis, and were fixed in 10% formal saline solution. The tissues were dissected and a good section representative tissue blocks were taken for histological processing and each with identifying label in a tissue cassette. The fixed tissue blocks were subjected to different grades of dehydrating agent, alcohol in ascending order, immersed in xylene for de-alcoholization, infiltrated and embedded in molten paraffin wax. Cutting of sections was done at 3µm using a rotator microtome. The sections were deparaffinized and then satined with the standard Heamatoxylin and Eosin (H & E) techniques of staining and the slides were mounted using DPX. The slides containing the section were then examined and photomicrographs captured using ×40 objective lens of the ScopeTek™ microscope device and software version 1.3.

2.6.1 Staining Procedures

Two changes of xylene were used to clear the paraffin. The slide was then immersed in alcohol (absolute) for 30 seconds and hydrated in alcohol of descending grades in this order, 90%, 70%, for 30 seconds each. Rinsed in tap water for 1 minute and stained with Erlich's heamatoxylin for 30 minutes and rinsed in running tap water for 5 minutes until the colour turns blue. A counterstain was done with 1% aqueous eosin for 5 minutes and rinsed in tap water for 30 seconds. Then the slide was dehydrated now in ascending grades of alcohol 70% and 90% for 30 seconds each and immersed in absolute alcohol twice for 30 seconds

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Was this protocol under which this technique was carried out described by some other author? Under what bibliographic criteria was it made?

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It is important not to leave the data to the imagination and describe the technique in detail, for example the approximate amount in grams of each liver sample collected.

each. It was then cleared in xylene finally for 1 minute. It was mounted using DPX and microscope for focal examination.

2.7 Statistical Analysis

SPSS version 22.0 of windows statistical package was used for data analysis from the generated data. Statistical tools such as mean & SD were used. One-way Analysis of Variance (ANOVA) with Turkey's multiple comparison test was also done. Then using the values obtained, statistical decisions and inferential evaluation were made. The probability (p) value less than 0.05 was used and considered statistically significant. Results were expressed as mean \pm standard deviation. GraphPad Prism version 8.0.2 was used to plot the bar charts.

3. RESULTS AND DISCUSSION

In this study, there was statistically significant increase when mean PSA for negative control was compared with other groups ($p < 0.0001$), which showed that the induction worked. However, when group 2 (Positive control) was compared with treatment groups, the PSA values in the treatment groups were observed to record lower values but not statistically significant on treatment of a period of 30 days of daily administration of dutasteride, and Cleanshield liquid supplement. Again, non-significant higher values were seen in rats treated with Cleanshield compared against rats treated Avodart alone, (Table 1)

The non-significant reduction may be because of the buffering ability of the Cleanshield liquid supplement imposed by sodium carbonate (0.49%), sodium phosphorus (0.514%) and stabilizers such as Guar and locust bean gum. These substances make the body more alkaline and reduce the effects of increased acidic body fluids due to some disease. When the body organs are not functioning properly, under the influence of acidifying factors, acid production becomes excessive and waste products are bioaccumulated in connective tissue in order to alter the normal blood pH value. This acidification process can lead to chronic tissue acidosis, which accelerates the ageing process and creates an environment conducive to the development of a number of diseases and also, experimental and epidemiological data support the notion that alkalinising foods have a beneficial effect on bone [17-18]. Sodium phosphorus/phosphate works by allowing the phosphate to combine with calcium to strengthen bones and by this promote more formation or lead to increased plasma levels of 1,25-(OH)₂D, the active metabolite of vitamin D and reduce the risk of ageing men to develop prostatic diseases, both BPH and/or carcinoma of Prostate [19].

Table 1: ANOVA Table for PSA Levels for all Groups

Groups	PSA (pg/ml)
GRP 1(NC)	311.26 \pm 9.14
GRP 2(PC)	585.38 \pm 92.33 ¹
GRP 3(BPH +Avt Trt)	490.34 \pm 24.57 ¹
GRP 4 (BPH + 0.24mICS)	576.10 \pm 66.60 ¹
GRP 5 (BPH + 0.48mICS)	558.42 \pm 124.33 ¹
GRP 6 (BPH + 0.72mICS)	515.3 \pm 80.17 ¹
F-value	8.816
P-value	<0.0001
Remark	S

S- Significant p-value, 1- significant compared with Grp 1 (post hoc test), Avt Trt- Avodart Treated, CS – Cleanshield

It was observed that when the dose of Cleanshield was increased, the PSA value further decreased, though not statistically significant. This could be attributed to the increased buffering ability of the Cleanshield liquid supplement. The highest percentage reduction (88.7%) was observed in the group treated with 0.72ml CS. Although there are no work or research to support this presently but the reduction in PSA value may be associated with the reduced hyperplasia as a direct consequence of 5 α -reductase inhibition or anti-inflammatory/antiproliferative action coming from the anti BPH dutasteride and the 5 α -reductase inhibitors that block the conversion of testosterone to DHT and thus reduce the growth effects of androgens on the prostate and in turn stops the BPH development [20].

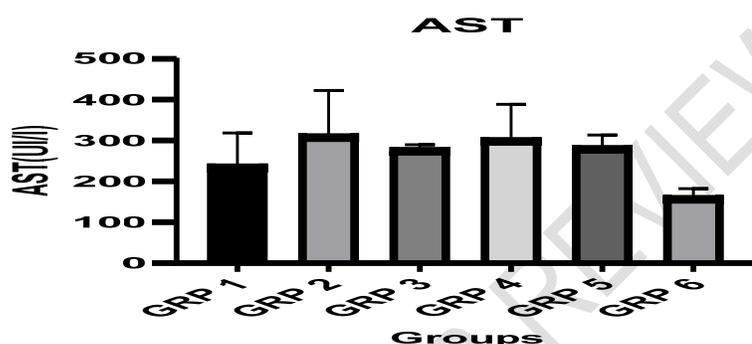


Fig. 1: Graphical comparison of the effect of Cleanshield treatment on AST across the groups

Also, this agrees with work that ethanol extract of five traditional plants exhibited high antiproliferative potential against the tested cancer cell lines with some significant differences [21]. Again, according to [12], increased combination of higher doses of ethanolic extract of pomegranate with dutasteride had more BPH reducing effect than dutasteride administered alone. Although this work did not combine treatment drugs but is in line with the increased dose of the administered Cleanshield that produced more anti-BPH effect. However, Avodart recorded a more reduced PSA value compared to the increased dose of Cleanshield. Some clinical observations have supported the importance of DHT in causing a nodular hyperplasia, thereby leading to the administration of 5 α -reductase, inhibitor to men with BPH which in turn reduces the DHT content of the prostate and as well reduces the size or volume and the symptoms associated to BPH [22]. This agrees with the recent work done with the Cleanshield liquid supplement.

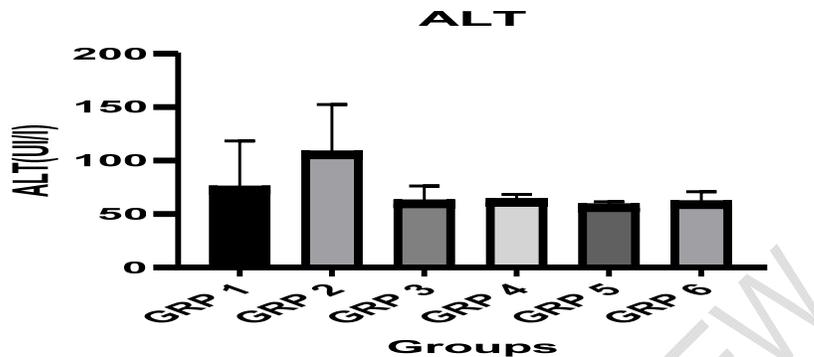


Fig. 2: Graphical comparison of the effect of Cleanshield treatment on ALT across the groups

ALT levels showed no significant difference across the groups while AST and ALP were significantly reduced difference across the group using one-way ANOVA. When group 1 (negative control) was compared to group 2 (positive control), there was no significant difference in ALT, AST and ALP (Figs. 1-3) even though there was an increase in enzyme levels. This could be attributed to the induced BPH which may have caused an increase in the liver cell activities or that may have caused some level of inflammation to the liver and being the power house of drug and toxin metabolism. However, in group 3, there was a non-significant reduced difference in ALT, AST while ALP recorded significant reduced difference ($p=0.001$) (Fig. 3). This may be as a result of anti-proliferative or ameliorative activities of dutasteride which may have extended its effects on the stroma/muscle cells of the prostate organ. Also, the group 2 was compared to group 5 for ALT, AST and ALP and there was a reduced non-significant difference for ALT and AST which was reduced more than that of the values seen in group 4. This could be as a result of the increased dosage of the Cleanshield liquid supplement and consequently, increased buffering Cleanshield activities. However, for ALP there was a reduced significant value ($p=0.0003$), (Fig. 3). These reduced enzyme values could be as a result of increased buffering activities of the Cleanshield supplement or due to loss of parenchymal materials. Comparing group 6 liver enzymes to that of group 2, there was a non-significant reduced difference for ALT and ALP, but AST gave a significant reduced difference ($p<0.036$). Group 6 received the highest dose (0.72ml) of Cleanshield liquid supplement and this could have been the reason as seen in AST values. The non-significant differences observed within the groups treated with Cleanshield as seen in ALT using one way ANOVA, showed that it was not significant ($p=0.06$) across the treated groups when compared to group 1 and 2. Also the significant decrease in ALP and AST in the treated groups using one way ANOVA could be attributed to the amelioration of the toxic effects caused to the liver cells by the induced BPH on the prostate muscle since AST is not only found in the liver but also in various other tissues [23] and since ALP is expressed in abundance in other tissues like skeletal muscles, renal tissues and probably prostate glands. [23]. Hence, significant ALP levels may not be of hepatic origin also the non-significant reduction in these enzymes could as well and most importantly be a loss of parenchymal materials in the liver with the group 5 having the highest reduced enzyme value. [24] reported that an increase in dose of ethanolic extract of *Zingiber Officinale* rhizome caused a higher toxic effect on liver.

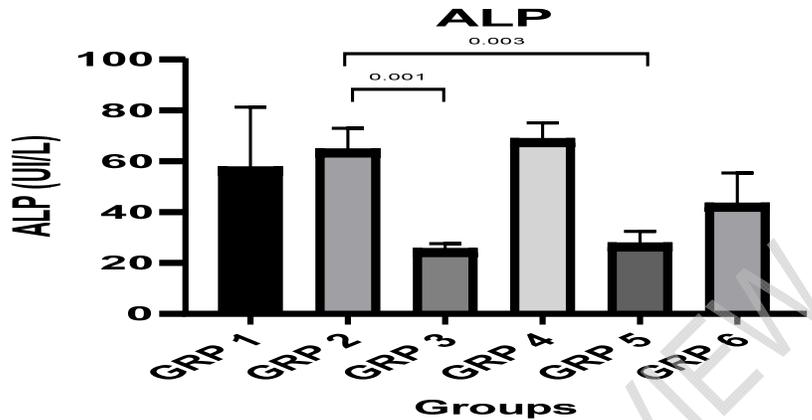
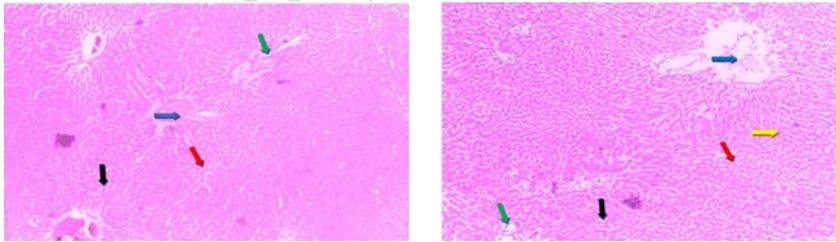


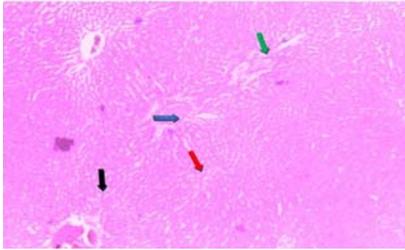
Fig. 3: Graphical comparison of the effect of Cleanshield treatment on ALP across the groups

This does not agree with recent work where an increase in dose of the supplements caused non-significant reduction in the enzyme (liver) ALP, AST and ALT activities. Although there are fears that liver disease induced by herbal drugs or supplements consumption will increase or is on the increase globally. However, assumptions that these herbal drugs and supplements are safe since they occur naturally abounds or still exists in hearts of people. The American association for the study of liver diseases (AASLD) hepatotoxicity special interest groups (SIG) presented a research where it noted that a wide range of over the counter products including vitamins, minerals, dietary elements, herbal preparations and synthetic compounds and their increasing consumptions that is leading to HDS-related hepatotoxicity [25].

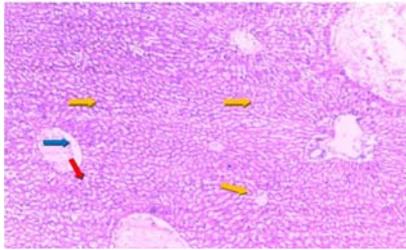


A
B
 Key: Green – portal tract, Black – hepatocytes, Blue – central vein, Red – hepatic sinusoids, Yellow– lymphocytes

Plate 1: Photomicrograph of the liver of two (2) Normal Control group labeled A and B of the experimental animals Using Hematoxylin and Eosin (H&E) Staining Technique with 200x Magnification



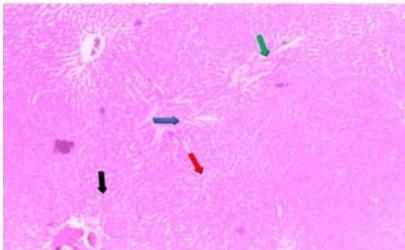
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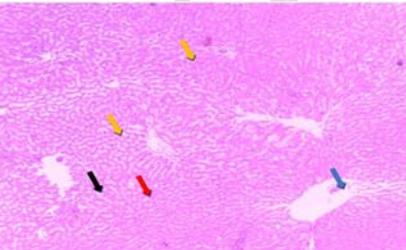
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Key: Green – portal tract, Black – hepatocytes, Blue – central vein, Red – hepatic sinusoids, Yellow– lymphocytes

Plate 2: Photomicrograph of the liver of group 1 rats, normal control labeled plate A and group 2 rats, induced control, labeled B, showing Central veins and Hepatocytes, sinusoids and Portal tract. Histology sections shows normal histologic features for plate A. Mild intraparenchymal inflammation and vacuolar change for plate B.



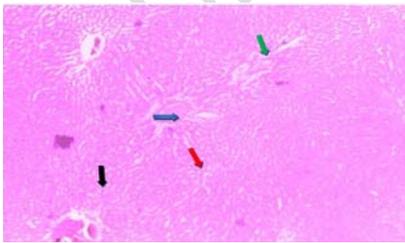
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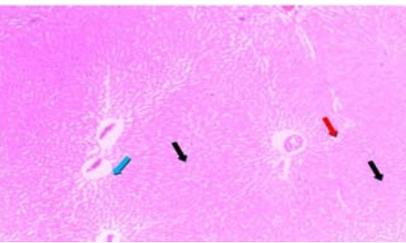
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Key: Green – portal tract, Black – hepatocytes, Blue – central vein, Red – hepatic sinusoids, Yellow– lymphocytes

Plate 3: Photomicrograph of the liver of the group 1 rats, normal control labeled plate A and group 3 rats, avodart treated, labeled plate B showing, Central veins and Hepatocytes, sinusoids and Portal tract. Histology sections show normal histologic features for plate A and mild intraparenchymal inflammation for plate B.



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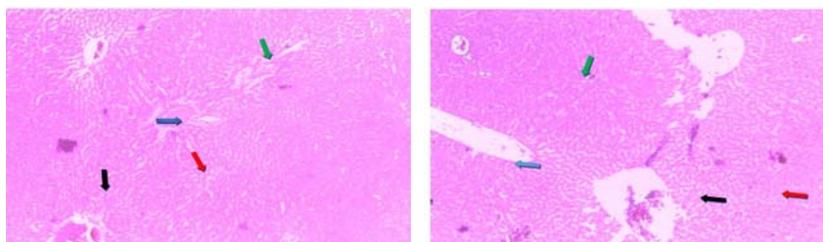


B

Key: Green – portal tract, Black – hepatocytes, Blue – central vein, Red – hepatic sinusoids, Yellow– lymphocytes

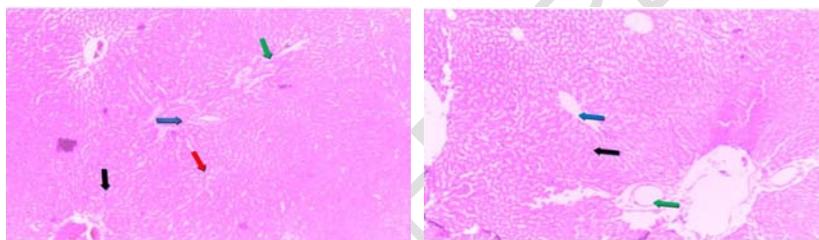
Plate 4: Photomicrograph of the liver of the group 1, normal control, labeled plate A and group 4 rats, cleanshield treated, labeled plate B showing, Central veins and

Hepatocytes, sinusoids and Portal tract. Histology sections show normal histologic features for plate A and mild inflammation for plate B.



A B
Key: Green – portal tract, Black – hepatocytes, Blue – central vein, Red – hepatic sinusoids, Yellow– lymphocytes

Plate 5: Photomicrograph of the liver of the group 1 rats, normal control, labeled plate A and group 5 rats, Cleanshield treated, labeled plate B showing, Central veins and Hepatocytes, sinusoids and Portal tract. Histology sections show normal histologic features for plate A and B.



A B
Key: Green – portal tract, Black – hepatocytes, Blue – central vein, Red – hepatic sinusoids, Yellow– lymphocytes

Plate 6: Photomicrograph of the liver of the group 1 rats, normal control labeled A and group 6 rats, Cleanshield treated, labeled B, showing, Central veins and Hepatocytes, sinusoids and Portal tract. Histology sections show normal histologic features for plates A and B.

Plate 1 shows a section of the liver of the negative control group, plate A of the rats with the portal tract, hepatocyte, central vein hepatic sinusoids and lymphocytes with all being intact and no necrotic and histologic change seen. But when this was compared to plate B, the positive control, there was a histologic change as seen in Plate 2. The section showed dilated sinusoids, congested central vein and lymphocytes. The sinusoids and congested central vein could be as a result of the induction of the BPH in the plate B rats and is an indication of liver impairment. These changes follow an intraparenchymal inflammation and vacuolar change as reported in the histologic section and could be as a result of the induction. These findings correspond to the biochemical findings of the work. However, the inflammation was mild maybe as a result of the time frame of induction and may be severe if the induction was extended.

The plate B of Plate 3 that is the group induced and treated with Avodart (dutasteride). The section of the liver of the animals in this group showed mild intraparenchymal inflammation probably due to the induction. Hence, the parenchymal cells adjust to the effect of the drugs.

This shows that the dutasteride has mild, little or no significant effect to the liver. Again, it is possible that the effect of the dutasteride was not significant due to the time frame of the study.

In Plate 4, the histology section of the liver in group 4 labeled as plate B was compared to that of group 1, labeled plate A, it showed that the group 4 histology section with congested central vein, dilated sinusoids has almost normal histologic features with little inflammation probably from the induction. This depicts that the Cleanshield liquid supplement caused no toxic effect to the liver.

In Plate 5, the histologic section of group 5 labeled plate B was compared to that of group 1, the normal control group labeled plate B and it was observed that from the section of the liver of the rats in plate B has normal histologic features. This may be attributed to the buffering activities of Cleanshield and owing that the inflammation caused here may be mild and did not show any liver toxicity. Thus, depicting that the Cleanshield liquid supplement does not give any significant toxic effect to the liver.

Lastly, in Plate 6, the histologic section of the liver of group 6 rats, labeled plate B was compared to that of group 1 rats labeled A and the section showed that there were no significant histology change in the plates A and B sections and all have normal histologic features which may also be as a result of the buffering activities of Cleanshield as stated above and denotes that the Cleanshield liquid supplement at the level of 0.72ml taken by the rats did cause any significant toxicity effect and could have repaired the mild inflammation as seen in the other groups.

4. CONCLUSION

In conclusion, oral administration of Cleanshield liquid supplement after induction of BPH, did not produce significant ameliorative effect on BPH induced rats. Although there were reduced PSA levels in the treatment groups compared with the induced rat group. This study also depicted that treating the rats with different doses of the supplement did not produce any significant toxic effect on the liver biochemically and histologically.

ETHICAL APPROVAL

Authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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Comment [A7]: In order to allow continuity in the study of such an interesting product, perhaps it is prudent to leave a gap that studies molecular tests, that follow the same line of research

4. Mobley, D., Feibus, A. & Baum, N. Benign prostatic hyperplasia and urinary symptoms: evaluation and treatment. *Postg Med J*, 2015; 127:301-307.
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