Evaluation of the Effect of Acacia Nilotica Extract in Periodontitis Induced Albino Rabbit

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Abstract

Periodontitis is a tooth disease induced by anaerobic gram-negative bacteria especially by Porphyromonus gingivalis and is widespread in the world not only affecting humans but also a number of animal species. Misvak (A.nilotica) is being used by human being to protect teeth from such type of diseases for centuries but scientific data is not available to support Misvak which may be used as reference. Present study was designed to evaluate the effect of A.nilotica extract in periodontitis induced albino rabbits. In this study 5 group of rabbits were used as A, B, C, D and E with 5 rabbits each. For induction of disease experimentally grown P.gingivalus was applied through ligature over B,C,D and E groups on Monday , Wednesday and Friday for 7 weeks while group A was receiving only ligature. After induction of disease, Group A was received only distilled water and used as negative control while group B was used as positive control and Group C , D and E received treatments in suitable dosage 300mg/kg ,500mg/kg of A.nilotica aqueous Extract and 15mg/kg of Amoxillin respectively. The effect of A.nilotica Extract was evaluated by different Morphometric and hematological parameters i.e CBC, ESR, serum creatinine and Liver function tests ALT and AST before and after treatment. After giving A.nilotica extract in measured quantity, it was observed that the disease periodontitis was cured to some extent after receiving mentioned dose orally for 14 consecutive days by morphological and heamatological parameters.

1. Introduction

A number of diseases are present which involve in calculus and cavity formation of teeth. A cavity within the teeth may form by bacteria that live in the mouth in the form of soft coating that covers surface of the teeth and found between teeth and the gum line. Gum disease is an infection of the gums surrounding the teeth and is the main cause of tooth loss in adults. The severe form of a tooth disease is periodontitis which is a result of complex interplay between infection and host response often modified by other factors. A huge adult population have mild to moderate level of periodontitis. Severe forms of periodontitis affect only 5 to 15 percent of the adult population [1]. Bacteria mainly associated with periodontitis are gram negative anaerobic bacteria and may include *Porphyromonus intermedia*, *Porphyromonus gingivalus, B.fersythus, C.rectus, E.nodatum, P.micros, S.intermedius* and *treponema specie* [2]. Considerable research has shown that *P.gingivalus* a gram negative anaerobic bacterium is the major etiologic agent which contributes to chronic periodontitis. This black pigmented bacterium produces a number of virulence factors that cause destruction to periodontal tissues either directly or indirectly by modulating the host inflammatory response. The epithelial cells are infected by bacteria resulting in inflammatory and immune processes which eventually cause destruction of the surrounding tissues of the teeth [3].

The *P.gingivalus* rapidly adheres to the host cells surface followed by internalization through lipid rafts, incorporate in early phagosomes and activates cellular autophagy to provide a replicate niche while suppressing apoptosis. The replicating vacuole contains host proteins delivered by autophagy that use this pathogen to survive and replicate within the host cell [4].

The ability of *P.gingivalus* to adult periodontitis is determined by its virulence factors. Biofilm formation and bacterial dipeptidyl IV(DPPIV) activity contribute to the pathogenic potential of *P.gingivalus*. It contributes to the pathogenesis of aggressive periodontitis by inducing high levels of proinflammatory cytokines such as IL1B and IL-6 by peripheral CD4+ T helper cells. The *P.gingivalus* serotypes K1 and K2 but not others are associated with an increased production of osteoclastogenesis-related factors RANKL. This important information suggests that these serotypes could elicit a greater bone resorption and have a significant role in the periodontitis pathogenesis [5]. The *P.gingivalus* not only effect human teeth but also to some animals i.e. rabbit. Periodontal disease occurs in rabbits when sharp pieces of material become packed into the periodontal tissues. This is more likely to occur when altered tooth eruption and tooth shape provide abnormal spaces between the teeth into which debris can packed [6]. *Acacia nilotica* (kikar) is being used

as chewing stick (miswak) in different regions of the world since ancient times. This plant is found in the tropics and subtropics of Africa, Pakistan, India and Berma and has proved to have antimicrobial property [7]. This has long been used as folk medicine for the treatment of different diseases in different regions of the world.

The gum, bark, leaves and fruits of *A.nilotica* have been used medicinally for colds, bronchitis, pneumonia, ophthalmia, diarrhea and hemorrhage [8]. The fruit juice and the stem bark are used as a hemostatic. The fruit and the stem bark are regarded as tonic and astringent and are used internally to treat diarrhea and dysentery [9]. A decoction of the fruits is used as a remedy for sore throat, chest complaints, dysentery and leprosy. The water extract is used externally to treat syphilitic lesions and other venereal diseases [8]. In the Sudanese traditional medicine an infusion of about 5 g of the fruits of A.nilotica in 200 ml cold water overnight is used to treat sore throat and common cold. The decoction of the stem bark and root, which is Correspondence to: Emil C. Reisinger, Medizinische Winik Karl-Franzens-Universitat, Auenbruggerpiatz 15, A-8036 Graz, Austria. taken by the Massai to acquire courage, has been claimed to have an intoxicating effect as a nerve stimulant [8]. Here we report on the in vivo antibacterial effect of A.nilotica fresh sticks water extracts against a specific specie P.gingivalus. This is first attempt to evaluate Porphyrmonos gungivolus induced periodontitis in animal model. Further work is required to assess same potential of this plant species in Humans.

2. Material and Method

Total of 25 rabbits divided in five groups each containing 5 rabbits, were used in this experimentation. All animals were purchased from the market with minor age differences and were weighed. They received water ad-libitum and were feed on Vitapol Karma (complete feed for rabbits) for at least 5 days before the experiment. The animals were cared for by experienced laboratory technicians at the JIPS animal house.

2.1. Preparation of Plant extract

To prepare *A.nilotica* extract, 100g of newly growing sticks were ground into moderately fine powder and mixed in 1000ml of distilled water. Mixture was kept for 24 hours and filtered through whatmann filter paper no. 4. The filtrate was freeze dried and was mixed with distilled water containing 0.25% carboxymethyl cellulose (CMC) prior to use [10].

2.2. Preparation of P.gingivalis strain

Vials of bacterial cell were purchased and cultured on agar plates containing trypticase soya agar supplemented with 0.5% (wt/vol) yeast extract, 5 micro gram of hemin per ml and 1.0 micro gram of vitamin K per ml. Plates were incubated for 3 days at 37C in jars that were anaerobically maintained through palladium catalyzed hydrogen carbon dioxide envelops. Colonies were randomly selected and anaerobically cultured over night at 37C in Schaedlers Broth supplemented with vitamin K and hemin.

2.3. Inducement of Periodontitis in Experimental Animals

Ligature were placed under general anesthesia using 40 mg ketamine/kg of body. A 3.0 silk suture placed around the second premolar of both mandibular quadrants. Group A rabbits were given only ligatures, while groups B,C,D and E received *P.gingivalus* in addition to ligature placement for the first 7 weeks of the experiment,109 C.F.U of the *P.gingivalus* were placed topically around the suture on Monday ,Wednesday and Friday with the rabbits under anesthesia. At these times suture was also checked and lost and lose sutures were replaced. The control group was anesthetized using the same protocol and was given carboxy methyl cellulose slurry only without *P.gingivalus*. Weekly measurements of body weight and standard hematological parameters including clinical chemistry (CBC and CRP) were recorded for all animals. After 14 weeks one rabbit from group A and one from group C was studied in detail considering the disease state by ESR, CRP and Liver Function Test ALT and AST, which was used to compare the groups after given different ingredients orally.

2.4. Experimental Design

Inducing periodontitis in 4 out of 5 groups, following treatment was given to these groups:

- Group A was used as negative control and was given only distilled water.
- Group B was used as positive control. Group C,D and E were given treatments as.
- Group C was given 300mg/kg of body weight, the extract of *A.nilotica* for 14 consecutive days after periodontitis.
- Group D was given 500mg/kg of body weight, the extract of *A.nilotica* for 14 days after the periodontitis.

- Group E was given Amoxillin 15mg/kg of body weight for 14 consecutive days (Hematological Analysis and Biochemical Analysis).

3. Results

Group A - Physical parameters were found at baseline in Group-A which was used as negative control by pricking and strong visual observation. The results are presented in Tabulated (Table 1). Almost all the rabbits show healthy teeth and gums with no bleeding on pricking and not presence of any calculus OR pathological pockets.

Table 1. Group A periodontal condition

Animal No	**Bleeding on Pricking**	**Calculus formation**	**Pathological pockets formation**	**Code**
1	No	No	No	0
2	No	No	No	0
3	No	No	No	0
4	No	No	No	0
5	No	No	No	0

Group B - Physical parameters were found at baseline in Group-B by pricking and strong visual observation. The results are presented in Tabulated (see Table 2). Almost all the rabbits show diseased state with bleeding on pricking and presence of calculus and pathological pockets, respectively.

Table 2. Group B periodontal condition

Animal No	**Bleeding on Pricking**	**Calculus formation**	**Pathological pockets formation**	**Code**
1	Yes	Yes	Yes mild	3
2	Yes	Yes	No	2
3	Yes	Yes	Yes mild	3
4	Yes	Yes	Yes mild	3
5	Yes	Yes	Yes severe	4

Group C - Physical parameters were found in Group-C by pricking and strong visual observation. The results are presented in Tabulated (see Table 3). The rabbits show some betterment in bleeding on pricking and presence of calculus and pathological pockets as represented in the mentioned table.

Table 3. Group C periodontal condition

Animal No	Bleeding on Pricking	Calculus formation	Pathological pockets formation	Code
1	Yes	Yes	NO	2
2	Yes	Yes	NO	2
3	Yes	Yes	NO	2
4	NO	NO	Yes	1
5	Yes	Yes	Yes mild	3

Group D - Physical parameters were found in Group-D by pricking and strong visual observation. The results are presented in Tabulated (see Table 4). The rabbits show betterment in bleeding on pricking and presence of calculus and pathological pockets as represented in the mentioned table.

Table 4. Group D periodontal condition

Animal No	**Bleeding on Pricking**	**Calculus formation**	**Pathological pockets formation**	**Code**
1	Yes	Yes	NO	2
2	Yes	NO	Yes	2
3	Yes	NO	NO	1
4	Yes	Yes	NO	2
5	Yes	Yes	NO	3

Group E - Physical parameters were found in Group-E by pricking and strong visual observation. The results are presented in Tabulated (see Table 5). The rabbits show betterment in bleeding on pricking and presence of calculus and pathological pockets as represented in the mentioned table.

Animal No	**Bleeding on Pricking**	**Calculus formation**	**Pathological pockets formation**	**Code**
1	No	Yes	No	2
2	No	Yes	No	2
3	No	No	No	1
4	No	No	No	2
5	No	Yes	No	3

Table 5. Group E periodontal condition

4. Hematological Analysis

The RBC count were found at baseline in Group-A, Group-B, Group-C, Group-D and Group-E as 5.16 ± 0.27, 5.12 ± 0.512,5.06 ± 0.344,5.18 ± 0.239 and 5.10 ± 0.596 respectively. The results are presented in both Tabulated (Table 6) and graphical form (Figure 1). One-way analysis of variance showed non-significance differences among means of groups as compared to control group. The non-significance variance shows that disease does not affect RBC count.

Table 6. RBC count with their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Group E
1	5.2	5.1	5.2	5.2	5.2
2	5.5	5.3	5.4	5.5	5.9
3	5.3	5.2	5.3	5.3	5.3
4	4.8	5.7	4.8	4.9	4.8
5	5	4.3	4.6	5	4.3
Mean	5.16	5.12	5.06	5.18	5.10
SD	0.270	0.512	0.344	0.239	0.596

RBC Count (Million/cmm)

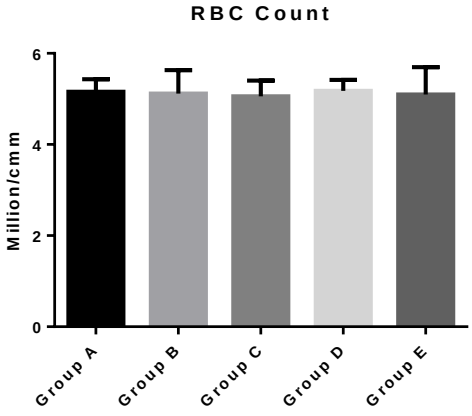

Figure 1. Graphical representation of RBC count

WBCs Count were found at baseline in Group-A, Group-B, Group-C, Group-D and Group-E were 6130 ± 366.7, 7780 ± 130.4, 6920 ± 238.7,6800 ± 316.2 and 6200 ± 158.1 respectively. The results are presented in both Tabulated (Table 7) and graphical form (Figure 2). One-way analysis of variance showed significance differences among means of different groups. Significant Increase (P>0.001) in WBC count was observed in Group 2 and group 4 as compared to control and similarly WBC count in group 4 was also increased significantly (P>0.01) as compared to control. However, group 5 showed non-significant increase in WBC as compared to control group.

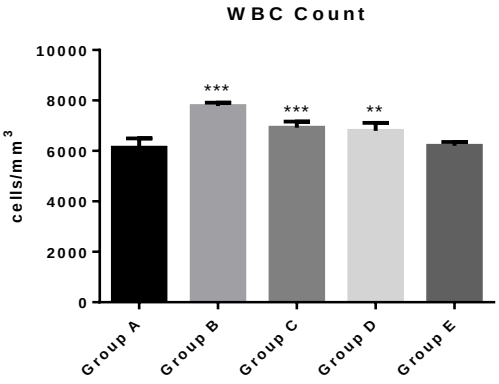

Figure 2. Graphical representation of WBC count

Table 7. WBCs count in their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Grou p E
1	5900	7700	7100	6700	6100
2	6600	7600	7200	6900	6200
3	6450	7900	6900	7300	6000
4	5800	7800	6800	6600	6400
5	5900	7900	6600	6500	6300
Mean	6130	7780	6920	6800	6200
SD	366.7	130.4	238.7	316.2	158.1

WBCs Count(cells/mm^3)

ESR level was found in Group-A, Group-B, Group-C, Group-D and Group-E as 3.8 ± 0.836, 6.8 ± 0.8367,6.4 ± 1.14,5.8 ± 0.836 and 5.4 ± 1.14 respectively. The results are presented in Figure 3 and Table 8. One-way analysis of variance showed significance differences among means of different groups. As in positive Group B (Positive control group) the value is much high showing diseased state of the group.

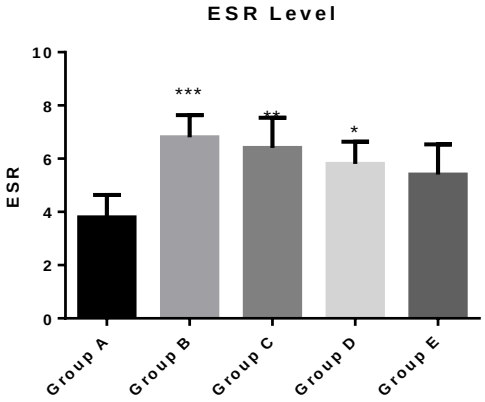

Figure 3. Graphical representation of ESR values

Table 9. Platelets count in their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Group E
1	718000	618000	618000	618000	698000
2	554000	604000	594000	654000	564000
3	621000	621000	621000	621000	621000
4	612000	611000	612000	612000	613000
5	549000	591000	549000	589000	559000
Mean	610800	609000	598800	618800	611000
SD	68269	12021	29744	23339	56094

Platelets count (cells/mm^3)

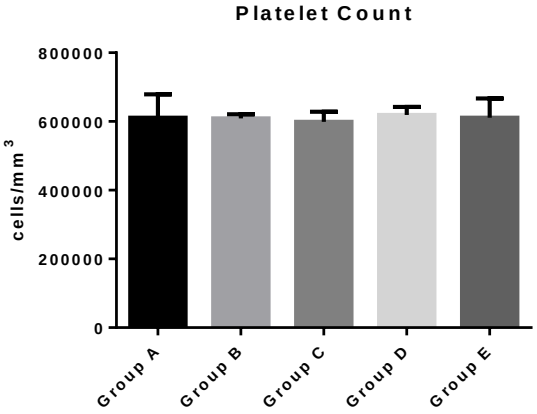

Figure 4. Graphical representation of Platelets count

MCH was found in Group-A, Group-B, Group-C, Group-D and Group-E as 29 ± 5.148, 31.2 ± 1.483,30.8 ± 2.387,30.2 ± 3.114 and 29.4 ± 4.278 respectively. The results are presented in Table 10 and Figure 5. One-way analysis of variance showed non-significance differences among means of different groups.

Table 10. MCH level with their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Group E
1	30	31	29	30	30
2	20	29	28	25	22
3	31	31	31	31	31
4	33	33	32	32	33
5	31	32	34	33	31
Mean	29	31.2	30.8	30.2	29.4
SD	5.148	1.483	2.387	3.114	4.278

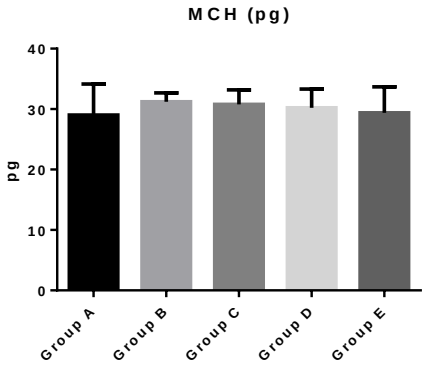

Figure 5. Graphical representation of MCH values

MCV (fl) was found in Group-A, Group-B, Group-C, Group-D and Group-E as 69.8 +- 1.924, 72.2 +- 3.834,71.8 +- 3.033,71 +- 3.937 and 70.6 +- 2.191 respectively. The results are presented in Table 11 and Figure 6. One-way analysis of variance showed non-significance differences among means of different groups.

Table 11. MCV level with their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Grou p E
1	70	75	75	71	71
2	67	68	69	67	68
3	72	72	71	72	70
4	71	77	75	77	74
5	69	69	69	68	70
Mean	69.8	72.2	71.8	71	70.6
SD	1.924	3.834	3.033	3.937	2.191

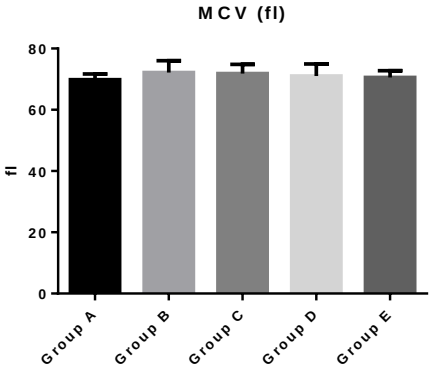

Figure 6. Graphical representation of MCV values

5. Biochemical Analysis

Serum creatinine was found in Group-A, Group-B, Group-C, Group-D and Group-E as 1.2 +- 0.0707,1.48 +- 0.08367,1.36 +- 0.114,1.32 +- 0.083 and 1.3 +- 0.0707 respectively. The results are presented in both Tabulated (Table 12) and graphical form (Figure 7). One-way analysis of variance showed significance differences among means of different groups.

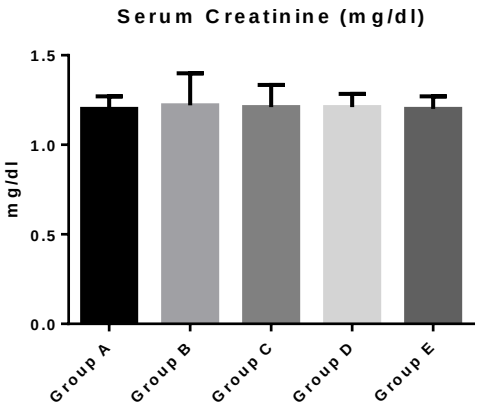

Figure 7. Graphical representation of serum creatinine values

Table 12. Serum creatinine level with their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Group E
1	1.1	1	1.25	1.2	1.2
2	1.2	1.4	1.2	1.1	1.2
3	1.2	1.4	1.3	1.25	1.3
4	1.3	1.1	1	1.3	1.1
5	1.2	1.2	1.3	1.2	1.2
Mean	1.2	1.22	1.21	1.21	1.2
SEM	0.071	0.179	0.125	0.074	0.071

Serum Creatinine mg/dl

ALT(U/L) was found in Group-A, Group-B, Group-C, Group-D and Group-E as 61.8 +- 4.087,66 +- 3.162,65 +- 1.581,64 +- 4.183 and 63.2 +- 3.701 respectively. The results are presented in Table 13 and Figure 8. One-way analysis of variance showed non-significance differences among means of different groups.

Table 13. ALT with their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Group E
1	57	67	67	60	61
2	66	69	66	67	66
3	65	65	65	68	68
4	58	61	64	59	59
5	63	68	63	66	62
Mean	61.8	66	65	64	63.2
SD	4.087	3.162	1.581	4.183	3.701

ALT (U/L)

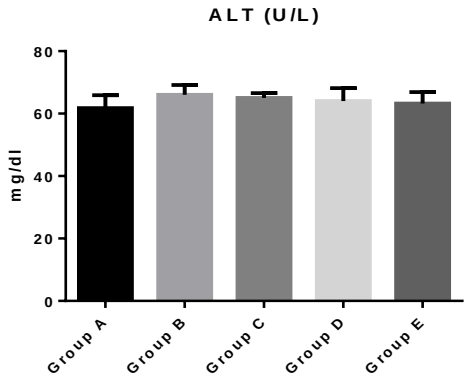

Figure 8. Graphical representation of ALT values

AST(U/L) was found in Group-A, Group-B, Group-C, Group-D and Group-E as 63.4 +- 3.647,78.8 +- 12.91,65.2 +- 1.304,65 +- 4.583 and 63.8 +- 3.421 respectively. The results are presented Table 14 and Figure 9. One-way analysis of variance showed non-significance differences among means of different groups.

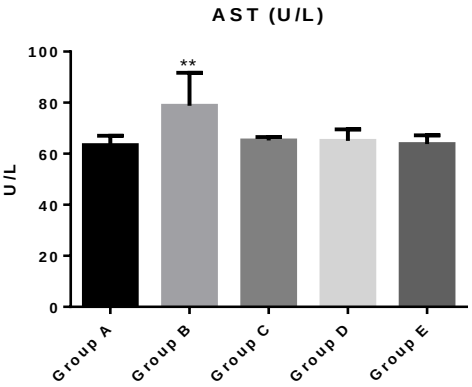

Figure 9. Graphical representation of AST values

Table 14. AST with their mean and SD values

Animal No	Group A	Group B	Group C	Group D	Group E
1	63	84	67	64	65
2	61	65	66	65	62
3	59	77	65	58	59
4	66	98	64	68	68
5	68	70	64	70	65
Mean	63.4	78.8	65.2	65	63.8
SD	3.647	12.91	1.304	4.583	3.421

AST (U/L)

6. Discussion

The present study shows treated group values near the negative control group A indicating that *A.nilotica* has positive effect in the removal of *p.gingivalus* induced periodontitis. This was a first in vivo evaluation of *A.nilotica* extract in the raw form against a gram negative an-aerobic bacteria.

The physical parameters show a clear-cut periodontitis in acute form in all the rabbits of group B positive control. After this the disease indications gradually decrease due to continuous use of *A.nilotica* extract up to 14 days, in different doses. In group A all the teeth of rabbits were quite healthy, having similar physical parameters. In group B, the disease was induced in all the animals, but Animal no.2 showed least disease induction which may be due to strong immune system. In the same way Animal no.5 of this group showed severe disease induction which perhaps is due to weak pathogen fighting power of the animal.

The Group C shows positive effect of *A.nilotica* dose. While considering the detail of this group, Animal no. 4 shows most effective result while group 5 has least effect due to some unknown reasons which needs further investigation. In group D when the dose of A.nilotica extract was increased, cure intensity also increased this increase in the cure intensity clear cut shows the there is some certain mechanism by which removal OR decrease of Porphyromonus bacteria takes place. Perhaps there is some certain compound in the raw form of *A.nilotica* which affect the growth of bacteria in the body. Group E shows values near to the negative control group as it was given standard amoxicillin dose available in the market.

While considering hematological parameters, indicators of inflammation and infection, WBCs and ESR show increased value in positive control group B while in group A these are least as this group was only receiving ligature during disease induction. When mentioned dosages were given to the groups C and D, WBCs and ESR decreases gradually showing positive results of *A.nilotica* effect in controlling the disease. RBCs count do not show enormous change in all the groups showing not any particular effect of infection OR infection control.

Similarly, MCH (Mean corpuscular heamoglobin) do not show too much increase OR decrease indicating that *P.gingivalus* do not affect RBCs production, movement OR structure and *A.nilotica* extract given in particular dosage also do not have any bad effect over the RBCs. Group B positive control shows a slight increase which may be due to increase inflammatory mediaters OR some other such type of reason which needs further investigation.

MCV (Mean corpuscular volume of erythrocytes) in group B shows a slight increase which may be due to increased inflammatory mediaters as already discussed in MCH.

Biochemical parameters i.e. serum creatinine, ALT and AST do not show much variation in groups indicating *A.nilotica* Extract in the given amount do not harms kidney and liver function instead in AST case after using *A.niltica extract* the value moved towards normal.

Serum creatinine shows slight increase in positive control group B, indicating increased inflammation and infection may affect glomerular filtration of creatinine to a small extent. Perhaps it would be due to some certain changes in the glomerulus tubules which needs further investigation and research. In group C and D the value moves towards normal showing positive effect of *A.nilotica* extract.

ALT (Alanine amino transferase) is an enzyme indicating liver function. In Group B its value show slight increase which may be due to infection OR inflammation. In the remaining groups Group C,D and E it is slightly decreased. The scientific reason of this, needs further investigation. As a whole there is not any huge change in any of the given groups. The AST (Aspartate amino transferase) show increased value in group B due to some unknown reasons. In group C and D, it gradually decreases. In group E when amoxil was given its value moves towards the value of Group A which was used as negative control.

The *A.nilotica* aquous extract is effective against oral pathogens such as *p.gingivalus* which is responsible of tooth decay and periodontitis. It is demonstrated that this antibacterial activity may be related to the presence of hydrolysable tannins and polyphenolics in the *A.nilotica* extract specifically. It means that the antimicrobial effect of tannins is related to its toxicity and molecular structure. Tannins may act on the cell wall and across the cell membrane because they can precipitate proteins. Hence, the antibacterial activity of *A.nilotica* be related to polyphenol structures because polyphenols may affect the bacterial cell wall, inhibit enzymes by oxidized agents, interact with proteins and disturb co-aggregation of microorganisms [11].

7. Conclusions

In the present study, the extract of *A.nilotica* freshly growing sticks have an effect on *P. gingivalus* in different dosages. This is an important finding for future use as antibiotics even for gram negative bacterial species. Furthermore, the extract has effect on the growth of *P.gingivalus* and the in vivo method for the antimicrobial evaluation in Albino Rabbits. However, the MIC method (Minimum Inhibitory Concentration) applied along with disk diffusion may be recommended in future studies.

8. References

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