Effects of Balanites aegyptiaca (leaves, seeds, stem bark) extracts on serum level of certain biochemical markers of liver on Nevirapine-induced hepatotoxicity rats

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Abstract- The present study investigated the possible effects of Balanites aegyptiaca (leaves, seeds, stem bark and combination of extracts) extracts on serum level of certain biochemical markers of liver on Nevirapine-induced hepatotoxicity rats. Group I was the normal control, neither administered with Nevirapine nor extract. Groups II to X were orally administered with 6mg/kg Nevirapine for six (6) weeks followed by administration of 100mg/kg leaves, seeds, stem bark and combination of extracts to groups III to VI and 200mg/kg leaves, seeds, stem bark and combination of extracts to groups VII to X respectively for one (1) week. Groups XI to XIV were orally administered concurrently with 6mg/kg Nevirapine and 100mg/kg leaves, seeds, stem bark and combination of extracts and groups XV to XVIII administered concurrently with 6mg/kg Nevirapine and 200mg/kg leaves, seeds, stem bark and combination of extracts respectively for six (6) weeks. Oral administration of 6mg/kg Nevirapine for six (6) weeks significantly increase the activities of liver marker enzymes with the simultaneous reduction in urea but has no effects in the concentration of creatinine, electrolytes and plasma malondialdehyde. Administration of 100mg and 200mg aqueous extracts of Balanite aegyptiaca for 1 week significantly increased the concentration of urea, creatinine and electrolytes (HCO3, K+, Cl- and Na+) and restored all liver marker enzymes to near normal control levels but the concentration of plasma malondialdehyde was not affected. Oral administration of Nevirapine with Balanite aegyptiaca aqueous extracts concurrently for 6 weeks significantly increase the concentration of urea, creatinine and electrolytes (HCO3, K+, Cl- and Na+). The activities of liver enzymes was significantly reduced after the co-administration but has no effect on the concentration of plasma malondialdehyde. The results showed that administration of aqueous extracts after induction of liver damage may have some curative effects as significant decreases in liver enzymes activities were observed. The oral co-administration results showed significant decreases in the liver enzymes activities compared with the test control. This indicates a protective effect against Nevirapine hepatotoxicity. The decreases in the activities of liver enzymes may be due to the Phytochemical constituents of the plant. The increases in renal parameters could be due to dehydration brought about by the administration of the aqueous extracts.

Index term- Balanites aegyptiaca, biochemical markers, hepatotoxicity, liver enzymes, Nevirapine, renal parameters, Phytochemicals.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities [7]. It is estimated that there are between 200,000 and 700,000 species of tropical flowering plants that have medicinal properties [3]. Their actions include: antibacterial, antifungal, antiviral, antihelmintic, anti allergic, anti carcinogenic and larvicidal. These medicinal value lie in some chemical substances they contain. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, are precursors for the synthesis of complex chemical substances. In addition the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies [16]. The use of and search for drug and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists and natural products chemists are combing the earth for phytochemicals that could be developed for treatment of various diseases. In fact, many modern drugs have been derived from plants.

Balanites aegyptiaca is a species of tree, classified either as a member of the *Zygophyllaceae* or the *Balanitaceae*. This tree is native to much of Africa and parts of the Middle East.There are many common names for this plant. In English the fruit has been called desert date, in Arabic it is known as *lalob*, *hidjihi*, and *heglig*. In Hausa it is called *aduwa*, in Swahili *mduguyu*, and in Amharic *bedena* [27]. The *Balanites aegyptiaca* tree reaches 10 m (33 ft) in height with a generally narrow

form. The branches are thorny. The dark green compound leaves are made up of two leaflets which are variable in size and shape. *Balanites aegyptiaca* is one of the most common trees in Senegal [18]. It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay, and climatic moisture levels, from arid to sub humid. It is relatively tolerant of flooding, livestock activity, and wildfire.

The yellow, single-seeded fruit is edible. Many parts of the plant are used as famine foods in Africa; the leaves are eaten raw or cooked, the oily seed is boiled to make it less bitter and eaten mixed with sorghum, and the flowers can be eaten [27]. The tree is considered valuable in arid regions because it produces fruit even in dry times. The fruit can be fermented for alcoholic beverages. The seed contains 30-40% seed oil and contains the sapogenins diosgenin and yamogenin Diosgenin that can be used to produce hormones such as those in combined oral contraceptive pills and corticoids. The oil is used as cooking oil. The seed cake remaining after the oil is extracted is commonly used as animal fodder in Africa.The seeds of the *Balanites aegyptiaca* have molluscicide effect on *Biomphalaria pfeifferi* [5].

Medicinal uses of this plant are many. The fruit is mixed into porridge and eaten by nursing mothers, and the oil is consumed for headache and to improve lactation. Bark extracts and the fruit repel snails and copepods, organisms that host the parasites schistosome and guinea worm, respectively [27].

MATERIALS AND METHOD

MATERIALS

The under listed items were the equipment and apparatus used in the course of this study: Weighing balance (Scout pru spu-402 Ohause corporation, Pine Brook, NJ USA), Water bath (OLS 200 Grant instruments Cambridge LTD), Hot air oven (D 3165 Gallenkamp), spectrophotometer (Jenway, 6051 New life medical instrument England), Centrifugation machine(800D Gulfex Medical and Scientific England).

CHEMICALS AND REAGENTS

Reagents used are of analytical grade and RANDOX Kit reagents from Randox Laboratories UK. The drug used was Nevirapine 200mg tablets manufactured by Aurobindo pharmacy limited, India obtained from Aminu Kano Teaching Hospital Kano.

COLLECTION AND PREPARATION OF PLANT EXTRACT

The leaves, seeds and stem bark of *Balanite aegyptiaca* were collected from the tree at Bayero University, Kano (old Campus). Specimens were authenticated at Botany unit of the Department of Biological Sciences, Bayero Universty, Kano.

The leaves, seeds and stem bark of *Balanite aegyptiaca* were allowed to dry under the shade and then ground to powder using mortar and pestle. The extracts of the leaves, seeds, stem bark were prepared by weighing and soaking 150g each of the leaves, seeds, stem bark powders in 250ml of water for 24 hour. The mixture was then filtered using sieve. To separate weighed petri dish, 5ml each of aqueous extract of leaves, seeds, and stem bark were added and evaporate in an oven and the dish weighed again. The weight of the empty petri dish was subtracted from weigh of petrish dish plus plant extracts after drying to obtain the weight of the extract in 5ml.

Experimental animals

Fifty four (54) albino rats were obtained from Biological science department, faculty of science, Bayero University, Kano. The rats were kept for two weeks to acclimatize after which they were weighed, separated into males and females to prevent mating. The rats were fed on commercial diet and water *ad libitum* through out the period of experiment.

Experimental design

Fifty four (54) albino rats were selected at random, weighed between 100g to 120g were used for this research. They were divided into eighteen (18) groups of three rats each.

Group I (Normal control): Neither treated with nevirapine nor extract.

GrpII (Positive control): Nevirapine (252 mg/kg) induce hepatotoxicity rat

Group III: 252 mg/kg Nevirapine + 700mg leaves extract

Grp IV: 252 mg/kg Nevirapine + 700mg seeds extract

Grp V: 252 mg/kg Nevirapine + 700mg stem bark extract

Grp VI: 252 mg/kg Nevirapine + 700mg combination of leaves, seeds and stem bark extract

Grp VII: 252 mg/kg Nevirapine + 1400mg leaves extract

Grp VIII: 252 mg/kg Nevirapine + 1400mg seeds extract

Grp IX: 252 mg/kg Nevirapine + 1400mg stem bark extract

Grp X: 252 mg/kg Nevirapine + 1400mg combination of leaves, seeds and stem bark extract

Grp XI: 252 mg/kg Nevirapine + 4200mg leaves extract

Grp XII: 252 mg/kg Nevirapine + 4200mg seeds extract

Grp XIII: 252 mg/kg Nevirapine + 4200mg stem bark extract

GrpXIV: 252 mg/kg Nevirapine + 4200mg combination of leaves, seeds and stem bark extract

Grp XV: 252 mg/kg Nevirapine + 8400mg leaves extract

Grp XVI: 252 mg/kg Nevirapine + 8400mg seeds extract

Grp XVII: 252 mg/kg Nevirapine + 8400mg stem bark extract

Grp XVIII: 252 mg/kg Nevirapine + 8400mg combination of leaves, seeds and stem bark extract

The rats were sacrificed after 1 week of *Balanite aegyptiaca* extracts administration. Blood sample of each rat was collected into clean test tube, allowed to clot and then centrifuged at 5000 revolution per minute for 5 minutes in a centrifuge. The serum obtained was then used for analysis of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) activities and total bilirubin, direct bilirubin, urea, creatinine and electrolytes (Na^+, K^+, Cl^- and HCO_3^-) and serum malondialdehyde concentrations in order to assess the liver as well as kidney functions.

In the second phase of the experiment (concurrent administration), the rats were sacrificed after six (6) weeks of nevirapine and *Balanite aegyptiaca* (leaves, seeds, stem bark and combination) extracts administration. Blood samples were collected into clean test tubes, allowed to clot and then centrifuged at 5000 revolution per minute for 5 minutes. The serum obtained was then used for analysis of AST, ALT, ALP activities and total bilirubin, direct bilirubin, urea, creatinine, electrolytes(Na^+, K^+, Cl^- and HCO_3^-) and serum malondialdehyde concentrations in order to assess the liver as well as kidney functions.

Statistical analysis

Statistical analysis was carried out using One-way Analysis of Variance (ANOVA) followed by Student-Newman-Keuls Multiple Comparisons Test. The data were expressed as the mean ± Standard deviation. The P value is < 0.0001, considered extremely significant.

RESULTS

Table 1 shows liver function indices Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (T. BIL), Direct Bilirubin (D. BIL) and Serum Malondialdehyde (SMDA) for groups of Nevirapine induced hepatotoxicity rats orally administered with aqueous extracts of *Balinite aegyptiaca* leaves, stem bark, seeds and combination of the extracts for one (1) week. Multiple Comparisons Test of Analysis of variance (ANOVA) showed that AST and ALT in group II is statistically higher ($p<0.001$) compared to normal control group (group I). The result of AST and ALT in groups II showed inducement of liver damage. Values for AST and ALT in groups III to X were significantly lower ($P<0.001$) than that of group II. ALP Values in group II is statistically higher ($P<0.001$) than ALP in group I. Multiple comparison test show that values of ALP in groups III, IV, V, VI, VII, VIII and IX were statistically lower ($P<0.05$) than ALP in group II. On the other hand ALP in group X was not significant ($P>0.05$) when compared with ALP in group II. Values of T. BIL in group II was considered statistically not significant ($P>0.05$) compared to values in group I. Comparison test for values of T. BIL in group III was considered not significant ($P>0.05$) compared with values in group II. The T. BIL of groups; V ($P<0.05$), VI ($P<0.001$) and IV, VII, VIII, IX, X ($P<0.001$) were significantly higher than that of group II. D. BIL values in group II was not significant ($p>0.05$) compared to D. BIL in group I. Values of D. BIL in groups III, IV, V, VI, VII VIII and X were not significant ($p>0.05$) compared to D. BIL in group II. ANOVA showed that values for D. BIL in group IX ($P<0.01$) was significantly higher when compared with D. BIL II. Analysis of Variance (ANOVA) indicated that values of SMDA in group II was not significant ($p>0.05$) compared to SMDA I.

Values SMDA in groups III to X were also not significant (p>0.05) compared to SMDA in group II.

Table II. shows the concentration of renal function parameters Urea, Creatinine, Bicarbonate (HCO_3^-), Potassium (K^+), Chloride (Cl^-) and Sodium (Na^+) for groups of Nevirapine induced hepatotoxicity rats orally administered with *Balanites aegyptiaca* for 1 week. Analysis of variance (ANOVA) showed that Urea in group II was statistically lower (p>0.001) compared with control (group I). Values for Urea in groups III (p<0.001) and V (p<0.05) were lower than that of group II. Urea values in groups VI and X were higher (p<0.001) than that of group II. Values of Urea in groups IV, VII, VIII and IX were not significant (p>0.05) compared to Urea in group II. Value of Creatinine in group II was statistically not significant (P>0.05) compared with that of group I. Creatinine values in groups III, IV, V, VI, VIII, IX and X were also not significant (P>0.05) compared with Creatinine in group II. While Creatinine value in group VII was significantly higher (P<0.001) comparison with group II. Values of HCO_3^- in group II was not significant (P>0.05) compared with control (group I). HCO_3^- values in groups IV, VII and IX were not significant (P>0.05) when compared with that of group II. Values of HCO_3 in groups III, V, VI, VIII and X were higher (P<0.05) compared with that of group II. Value of K^+ in group II was not significant (P>0.05) compared with control (group I). K^+ values in groups III to VIII were not significant (P>0.05) compared with that in group II. While values of K^+ in groups IX and X were higher (P<0.001) than that in group II. Values for Cl^- in group II was not significant (P>0.05) compared with that of control (group I). Cl^- value in group III was also not significant (P>0.05) compared with that in group II. while Cl^- values in groups V, VII, and IX (P<0.01) and IV, VI, VIII and X (P<0.05) were higher than that group II. Analysis of variance (ANOVA) showed that Na^+ in group II was not significant (P>0.05) compared with that of control (group I). Values of Na^+ in group III was also not significant (P>0.05) compared with that in group II. However Na^+ values in group IV to X (P<0.001) was higher than that in group II.

Table III. shows Liver function indices Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (T. BIL), Direct Bilirubin (D. BIL) and Serum malondialdehyde (SMDA) for groups of rats orally administered concurrently with the Nevirapine and *Balanite aegyptiaca* extracts for 6 weeks. Analysis of variance (ANOVA) showed that AST and ALT in group II was statistically higher (p<0.001) compared with normal control (group I). Values of AST and ALT in groups XI to XVIII were lower (p<0.001) than that in group II. ALP values in group II was higher (p<0.001) compared with normal control (group I). Values of ALP activities in groups XI to XVIII were higher (p<0.001) than that in group II. T. BIL value in group II was not significant (P>0.05) compared with normal control (group I). Values of T. BIL in groups XI to XVIII were significantly higher (p<0.001) than that in group II. D. BIL value in group II was not significant (P>0.05) compared with normal control (group I). Values of D.BIL in groups XII, XIII, XIV, XV and XVI were not significant (P>0.05) compared with group II. D. BIL values in groups XI, XVIII and XVII were significantly higher(p<0.001) than that in group II. Analysis of variance (ANOVA) showed that value of SMDA in group II was not significant (P>0.05) compared with normal control (group I). Values of SMDA in groups XI to XVIII were also not significant (P>0.05) compared with values in group II.

Table IV. shows the concentration of renal function parameters Urea, Creatinine, Bicarbonate (HCO_3^-), Potassium (K^+), Chloride (Cl^-) and Sodium (Na^+) for groups of nevirapine induced hepatotoxicity rats orally administered with concurrently with Nevirapine and *Balanite aegyptiaca* extracts for 6 weeks. Analysis of variance (ANOVA) for Urea showed that groups XI, XIII, XV, XVI, XVII and XVIII were significantly higher (P<0.05) compared with values in group II. Values of Urea in group XII was not significant (P>0.05) compared with values group II. Creatinine values in group II was not significant (P>0.05) compared with normal control (group I). Values of Creatinine in groups XI, XIII, XIV, XV and XVIII were not significant (P>0.05) compared with values in group II, while values for groups XII, XVI and XVII were statistically higher (P<0.05) than that in group II. Analysis of variance (ANOVA) showed values of HCO_3 in group II was not significant (P>0.05) compared with values in group I. Values of HCO_3 in groups XI to XVIII were not significant (P>0.05) compared with values in group II. K^+ values in group II was not significant (P>0.05) compared with values in normal control (group I). Values of K^+ in groups XI, XIII, XIV and XVIII were not significant (P>0.05) compared with values in group II. K^+ values in groups XII, XV and XVI, XVII were significantly lower (P<0.01) than that in group II. Analysis of

variance (ANOVA) showed that Cl^- in group II was not significant (P>0.05) compared with values in normal control (group I). Values of Cl^- in groups XI to XVI were not significant (P>0.05) compared values in group II, while Cl^- values in groups XIV, XVII, XVIII and XV were higher (P<0.001) that in group II. Values of Na^+ in group II was not significant (P>0.05) compared with values in normal control group (group I). Na^+ values in groups XII, XIII and XVI were not significant (P>0.05) compared with values in group II. However values of Na^+ in groups XI, XIV, XV, XVII and XVIII were higher (P<0.001) than that in group II.

Table 1. **Liver enzyme activities and concentration of T. BIL, D. BIL and SMDA of Nevirapine induced hepatotoxicity rats orally administered with 100mg and 200mg aqueous extracts of *Balanite aegyptiaca* for 1 week.**

Grp	AST IU/l	ALT µ/l	ALP µ/l	T. BIL mg/dl	D. BIL mg/dl	SMDA mmol/l
I 0mg/kg (normal control)	22.6 ± 1.51	35.8 ±1.09	105.5 ±5.817	0.800±0.11	0.382±0.05	1.200±0.35
II 6mg/Kg nevirapine	56.2±2.58[a]	70.4 ±2.51[a]	343.3±17.80[a]	0.606±0.06[d]	0.486±0.11[d]	1.126±0.09[d]
III 100mg/kg Leaves	45 ± 1.87[a]	57.4 ±1.51[a]	294.7 ±8.14[a]	0.752±0.21[d]	0.262±0.03[d]	1.004±0.10[d]
Iv 100mg/kg Seeds	42.2 ±1.09[a]	50.8 ±1.64[a]	146.8 ±14.6[a]	1.448±0.31[a]	0.328±0.04[d]	0.936±0.37[d]
v 100mg/kg S.bark	41.4 ±0.54[a]	50 ± 1.22[a]	192.7 ±2.16[a]	1.006±0.06[c]	0.264±0.02[d]	1.253±0.08[d]
vi 100mg/kg Comb.	39.4 ±2.51[a]	46.8 ±1.09[a]	278.4±27.54[a]	1.076±0.23[b]	0.314±0.02[d]	1.520±0.07[d]
vii 200mg/kg Leaves	48.4 ±2.07[a]	48.4±0.54[a]	131.4±8.13[c]	1.726±0.29[a]	0.334±0.05[d]	1.111±0.22[d]
viii 200mg/kg Seeds	36.8 ±1.09[a]	49 ± 1.22[a]	132.0±7.23[c]	1.880±0.16[a]	0.478±0.03[d]	1.315±1.14[d]
ix 200mg/kg S.bark	38.4 ±1.34[a]	57 ± 1.22[a]	279.3±27.63[a]	1.798±0.17[a]	0.752±0.11[b]	0.887±0.08[d]
x 200mg/kg Comb.	29.8 ±1.09[a]	42 ± 1.22[a]	122.5±4.14[d]	1.974±0.23[a]	0.638±0.06[d]	0.986±0.14[d]

Values in the same column bearing the same letter ([c], [b], [a]) are statistically significant at P<0.05, P<0.01 and P<0.001 respectively and ([d]) are statistically not significant at p>0.05. comparisons were made between group I & II and between group II and the other groups

Table 2. T**he concentration of renal function parameters (Urea, Creatinine, HCO_3, K^+, Cl^- and Na^+) for groups of Nevirapine induced hepatotoxicity rats orally administered with 100mg and 200mg of the aqueous extracts of *Balanite aegyptiaca* for 1 week.**

Grp	Urea mmol/l	Creatinine mg/dl	HCO_3 mmol/l	K^+ mEq/l	Cl^- mmol/l	Na^+ mmol/l

i 0mg/kg (normal control)	0.34±0.02	0.10±0.04	15.9 ± 0.44	1.05±0.45	42.9 ± 12.70	81.3±9.84
ii 6mg/kg nevirapine	0.235±0.01[a]	0.08±0.02[d]	14.4 ±0.05[d]	1.69±0.72[d]	44.8 ± 12.60[d]	77.2±12.42[d]
iii 100mg/kg Leaves	0.17±0.01[a]	0.03±0.01[d]	22.6 ±2.63[a]	1.69±0.57[d]	45.0 ± 2.25[d]	85.3±1.85[d]
iv 100mg/kg Seeds	0.25±0.03[d]	0.04±0.02[d]	15.4 ±0.25[d]	1.57±0.83[d]	80.5 ± 24.0[c]	102.4±8.00[b]
v 100mg/kg S.bark	0.20±0.01[c]	0.09±0.04[d]	23.5 ±0.16[a]	1.99±0.90[d]	88.0 ± 37.20[b]	107.8±9.82[a]
vi 100mg/kg Comb.	0.29±0.01[a]	0.16±0.07[d]	20.9 ±0.50[a]	1.54±0.62[d]	77.5 ± 0.73[c]	116.8±2.18[a]
vii 200mg/kg Leaves	0.25±0.02[d]	0.46±0.090[a]	14.8 ±0.40[d]	1.39±0.05[d]	94.8 ± 13.50[b]	128.2±13.40[a]
viii 200mg/kg Seeds	0.22±0.01[d]	0.12±0.01[d]	17.7 ±0.15[a]	1.64±0.12[d]	85.8 ± 20.20[c]	119.3±12.58[a]
ix 200mg/kg S.bark	0.24±0.01[d]	0.20±0.19[d]	14.9 ±0.38[d]	3.21±0.54[b]	89.6 ± 20.01[b]	109.4±4.70[a]
x 200mg/kg Comb.	0.28±0.01[a]	0.20±0.12[d]	23.0 ±0.40[a]	4.15±0.54[a]	78.5 ± 4.00[c]	117.1±3.57[a]

Values in the same column bearing the same letter ([c], [b], [a]) are statistically significant at P<0.05, P<0.01 and P<0.001 respectively and ([d]) are statistically not significant at p>0.05. comparisons were made between group I & II and between group II and the other groups

Table 3. **Serum liver enzymes activities and concentration of T. BIL, D. BIL and SMDA for groups of rats orally administered concurrently with Nevirapine (6mg/kg), 100mg and 200mg of aqueous extracts of *Balanite aegyptiaca* for 6 weeks.**

Grp	AST µ/l	ALT µ/l	ALP µ/l	T. BIL mg/dl	D. BIL mg/dl	SMDA mmol/l
I 0mg/kg (normal control)	22.6±1.51	35.8 ±1.09	105.5±5.81	0.800±0.11	0.38±0.05	1.20±0.35
Ii 6mg/kg nevirapine(NVP)	56.2±2.58[a]	70.4±2.51[a]	343.3±17.0[a]	0.60±0.06[d]	0.28±0.10[d]	1.12±0.09[d]
xi 100mg/kg Leaves+NVP	27.8 ±1.09[a]	43.2 ±1.09[a]	216.9±0.43[a]	1.91±0.43[a]	1.33±0.56[a]	1.263±0.727[d]
xii 100mg/kg Seeds+NVP	27.4 ±0.54[a]	39.8 ±3.03[a]	144.5±8.00[a]	2.55±0.120[a]	0.54±0.09[d]	0.99±0.87[d]
Xiii 100mg/kg S.bark+NVP	28.4 ±1.51[a]	42.8 ±1.09[a]	161.2±15.69[a]	2.62±0.05[a]	0.69±0.07[d]	1.48±0.24[d]
Xiv 100mg/kg Comb+NVP	26.4 ±0.54[a]	40.4 ±1.51[a]	133.7±4.24[a]	2.48±0.22[a]	0.81±0.09[d]	1.21±0.50[d]
Xv 200mg/kg Leaves+NVP	27.6 ±2.51[a]	42.6 ±2.51[a]	178.5±4.79[a]	2.40±0.05[a]	0.76±0.12[d]	0.58±0.38[d]
xvi 200mg/kg Seeds+NVP	30.4 ±0.54[a]	40.4 ±0.54[a]	178.2±5.99[a]	2.32±0.06[a]	0.73±0.14[d]	0.28±0.18[d]
Xvii 200mg/kg S.bark+NVP	28.8 ±1.09[a]	41.4 ±1.51[a]	201.7±2.74[a]	2.77±0.33[a]	1.05±0.21[b]	0.29±0.24[d]
Xviii 200mg/kg Comb+NVP	24.4 ±1.51[a]	40.8 ±1.09[a]	161.4±6.10[a]	2.33±0.31[a]	1.46±0.57[a]	1.33±0.73[d]

Values in the same column bearing the same letter ([c], [b], [a]) are statistically significant at P<0.05, P<0.01 and P<0.001 respectively and ([d]) are statistically not significant at p>0.05. comparisons were made between group I & II and between group II and the other groups

Table 4. **The concentration of renal function parameters (Urea, Creatinine, HCO3, K^+, Cl^- and Na^+) for groups of Nevirapine induced hepatotoxicity rats orally administered concurrently with Nevirapine (6mg/kg), 100mg and 200mg of aqueous extracts of *Balanite aegyptiaca* for 6 weeks.**

ISSN 2229-5518

Grp	Urea mmol/l	Creatinine mg/dl	HCO3 mmol/l	K^+ mEq/l	Cl^- mmol/l	Na^+ mmol/l
I 0mg/kg (normal control)	0.34±0.03	0.09±0.05	16.12±0.55	1.05±0.28	42.92±12.17	81.3±9.84
ii 6mg/kg nevirapine(NVP)	0.23±0.01^{c}	0.08±0.02^{d}	14.40±0.05^{d}	1.69±0.71^{d}	44.82±12.6^{d}	77.28±12.42^{d}
xi 100mg/kg Leave+NVP	0.32±0.13^{c}	0.21±0.10^{d}	21.80 ± 6.41^{b}	1.69±0.38^{d}	75.70 ± 8.38^{d}	116.3 ± 8.72^{c}
Xii 100mg/kg Seeds+NVP	0.22±0.02^{d}	0.30±0.07^{b}	19.21 ± 3.55^{d}	0.60±0.38^{b}	70.40 ± 10.9^{d}	105.1 ± 7.84^{d}
Xiii 100mg/kg S.bark+NVP	0.38±0.01^{b}	0.05±0.01^{d}	19.31 ± 2.20^{d}	1.29±0.18^{d}	62.51 ± 5.35^{d}	104.4 ± 7.51^{d}
Xiv 100mg/kg Comb+NVP	0.42±0.03^{a}	0.04±0.02^{d}	20.13 ± 1.65^{d}	1.42±0.31^{d}	124.30 ±28.71^{a}	176.9 ± 22.1^{a}
Xv 200 mg/kg Leave+NVP	0.37±0.01^{b}	0.22±0.22^{d}	19.21 ± 1.92^{d}	0.62±0.28^{b}	99.51 ± 59.50^{c}	171.8 ±45.4^{a}
Xvi 200mg/kg Seeds+NVP	0.37±0.01^{b}	0.36±0.08^{a}	17.40 ± 3.21^{d}	0.50±0.18^{a}	62.0 ± 2.80^{d}	98.6 ± 2.62^{d}
Xvii 200mg/kg S.bark+NVP	0.44±0.09^{a}	0.27±0.04^{c}	17.60 ± 1.10^{d}	0.40±0.09^{a}	138.2 ±37.10^{a}	198.7 ± 8.95^{a}
Xviii 200mg/kg Comb+NVP	0.40±0.06^{a}	0.08±0.01^{d}	19.51 ± 1.01^{d}	1.10±0.60^{d}	149.0 ±6.90^{a}	183.9 ± 6.59^{a}

Values in the same column bearing the same letter (^{c}, ^{b}, ^{a}) are statistically significant at P<0.05, P<0.01 and P<0.001 respectively and (^{d}) are statistically not significant at p>0.05. comparisons were made between group I & II and between group II and the other groups

DISCUSSION

This study demonstrated that Nevirapine causes liver injury in rats (table 1). There was various evidence of hepatotoxicity, including elevation of serum activities of AST, ALT, and ALP. It was found that the serum levels of both AST,ALT and ALP were elevated more than twofold in the Nevirapine administered group (group 2) in comparison with the normal control (group I). Similarly, different studies have reported that hepatocytes are targeted by Nevirapine [8].

The activities of AST and ALT in group II administered with Nevirapine alone were statistically higher (P<0.001) than control (group I). This indicated the inducement of liver toxicity in group II by Nevirapine. The raise in the activity of ALT is due to hepatocellular damage and is usually accompanied by raise in AST [21]. Nevirapine-containing regimens have been associated with a risk of significant elevations of liver transaminase levels [24]. Administration of aqueous extracts of B. *aegyptiaca* resulted in significant reduction (P<0.001) in the activities of AST and ALT of groups III to X (P<0.001) administered with 100mg and 200mg of leaves, seeds, stem bark and combination extracts respectively compared with groupII. This showed that the effectiveness of the plant may ameliorate hepatotoxicity induced by Nevirapine, due to the Phytochemical constituents of the plant. The plant leaves are used in curing anthrax, for antihelminthic a ctivities and to clean malignant wound [10].Different studies ha ve found that the plant acts as antioxidant against adriamycin in duced cardiotoxicity inexperimental mice [14]. More recently, 12 hydroxy nevirapine has been proposed as a factor in Nevirapin e hepatocarcinogenicity [2]as well as skin rash [4]. The effect of lyophilized extracts of *B. aegyptiaca* (1g/kg) and Silymarin (0.1g/kg), a standard heptoprotective agent, given for 5 consecutive days, was tested on liver damage induced by paracetamol (0.6 g/kg) in the mice. *B. aegyptiaca* had a relatively modest hepatoprotective activity [1]. Administration of met hanol extract and aqueous extract of *P. marsupium*stem bark sh owed significant hepatoprotective activity, whichwas compara ble with the standard drug silymarin [12]. The level of ALP activity in group II significantly increase (P<0.001) compared with control (group I). Severe and potentially life-threatening skin reactions and hepatotoxicity have occurred among HIV-infectedpersons taking nevirapine [22]. The activity of ALP signi ficantly decrease (P<0.001) in groups III to X administered with 100mg, 200mg leaves, seeds, stem bark and combination respectively when compared with group II.

The extracts of leaf, stem bark and root of *B. aegyptiaca* were screened for hepatoprotective activity in Wistar albino rats. The extracts of the plant showed significant ($P < 0.05$) hepatoprotective effects as revealed by a decrease in the activity of serum transaminase and alkaline phosphatase enzymes as compared to control rats [20]. Administration of the aqueous extract of *Balanite aegyptiaca* to biliary duct-ligated rats showed a dose independent significant decrease in serum level of liver marker enzymes [15]. Various evidence have indicated that the ethanolic extract of*B. Aegyptiaca* exhibited more significant activity in the treatment of pain and inflammation [9]. This study has found that the Nevirapine administration followed by aqueous extracts of *Balanite aegyptiaca* (leaves, seeds, stem bark and combination) showed reduced level of the liver enzyme activities compared with the Nevirapine administration alone (group II). This indicated the effectiveness of the aqueous extracts of *Balanite aegyptiaca* (leaves, seeds, stem bark and combination) in the treatment of Nevirapine induced hepatotoxicity.

Bilirubin is a useful tool in the assessment of haemolytic anaemia. In this study the concentration of T. BIL and D. BIL of Nevirapine administered group (group II) were statistically not significant (P>0.05) when compared with control (group I). The concentration of T. BIL and D. BIL in group III administered with 100mg leaves extract were also statistically not significant (P>0.05) when compared with group II. However the concentration of T. BIL in group IV significantly increase (P<0.001) when administered with 100mg seeds extract compared with group II. D. BIL in group IV was found to be statistically not significant (P>0.05) when administered with 100mg seeds extract compared with group II. Concentration of T.BIL significantly increase in group V (P<0.05) and VI (P<0.01) administered with 100mg of stem bark and combination of extracts respectively compared with group II. No significant differences (P>0.05) were observed in the concentration of D. BIL of groups V and VI administered with 100mg stem bark and combination respectively compared with group II. When the dose was increased, the concentration of T. BIL in groups VII, VIII, IX and X were found to be significantly elevated (P<0.001) after administration of 200mg leaves, seeds, stem bark and combination of aqeueos extracts respectively compared with group II. This elevation may be attributed to haemolytic anaemia caused as a result of extracts used which may have some toxic components. Concentration of D. BIL in groups VII, VIII and X were found to be not significant (P>0.05) while group IX was significantly higher (P<0.01) compared with group II.

Serum MDA level is an important indicator of lipid peroxidation. This study showed that there were no significant difference (P>0.05) observed in serum MDA level in group II compared with control (group I). The concentration of serum MDA of all the Nevirapine administered groups III to X was also statistically not significant in comparison with group II. This indicated that both Nevirapine and aqueous extracts of *Balanite aegyptiaca* have no potential of causing lipid peroxidation.

The level of Urea, Creatinine and electrolytes are the outcome of fine regulatory mechanism of ionic charges and the osmotic balance. This homeostasis is achieved by an interplay involving the kidney, the lungs and endocrine system [23] . Creatinine is a waste product formed from creatine in muscles and is renally excreted allowing it to serve as a measure of renal function. Urea is rised in dehydration, dietary creatinine intake in body building, and in renal failure. A low creatinine is a sign of low or falling muscle mass and is seen in the very old and malnourished patients. Urea is a waste product formed from condensation of two ammonia molecules and one molecule of carbon dioxide and serves to allow excretion of excessive nitrogen from the body. As a waste product it has a minimal concentration of zero that is never seen clinically. It is rised due to increased synthesis from a high protein meal, upper gastrointestinal bleed and renal failure. A high urea with a normal creatinine often points to dehydration. Symptomatic uraemia in the presence of anuria is an indication to renal replacement therapy if there is no treatable urinary obstruction [6],[23].

Sodium is the major cation of the extracellular fluid where it regulates acid-base equilibrium and protects the body against excessive fluid loss. Potassium is the major intracellular cation with similar role to those of sodium. Hyperkalaemia is usually encountered frequently in renal failure, improper use of K^+ sparing diuretics, hypoaldosteronism, insulin deficiency associated hyperglycaemia, Addison's disease and massive tissue destruction[6],[23], [26],[13]. Plasma bicarbonate ion concentration is increased in respiratory acidosis and metabolic alkalosis but decreased in respiratory alkalosis and metabolic acidosis[6],[23].

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In this study (table 2), there was a significant reduction (P<0.001) in the concentration of Urea in rats administered with Nevirapine alone (group II) compared with control (group I). Significant decreases were observed in groups III (P<0.001) and V (P<0.01) administered with 100mg leaves and stem bark

aqeueos extracts in comparison with group II. Groups VI and X was significantly higher (P<0.001) administered with 100mg and 200mg combination of aqeueos extracts respectively compared with group II. Groups IV,VII, VIII and IX administered with 100mg seeds, 200mg leaves, seeds and stem bark aqeueos extracts were statistically not significant (P>0.05) compared with group II. Administration of Nevirapine-alone in group II revealed no significant difference (P>0.05) in the concentration of Creatinine in comparison with control (group I). This showed that oral administration of Nevirapine at a daily dose of 6mg/kg for 6 weeks could not lead to renal dysfunction. No significant difference (P>0.05) in the concentration of Creatinine was also observed in groups III, IV, V and VI treated with 100mg leaves, seeds, stem bark and combination aqeueos extracts respectively, when compared with group II. There were also no significant differences (P>0.05) in the concentrations of Creatinine observed in groups VIII, IX, and X administered with 200mg seeds, stem bark and combination respectively in comparison with group II, While a significant difference (P<0.001) was observed in Creatinine concentration of group VII treated with 200mg leaves extract compared with group II. A high blood level of creatinine indicates that the kidneys may not be working properly. Creatinine concentration is raised in dehydration, dietary creatinine intake in body building, and in renal failure, the increase in group VII could be due to dehydration as the drug was not able to induce renal damage. There was no significant difference (P>0.05) in the concentration of HCO_3 in group II administered with Nevirapine-alone in comparison with control (group I). No significant difference (P>0.05) was also observed in the concentration of HCO_3 in group IV administered with 100mg seeds in comparison with group II, while groups III, V and VI administered with 100mg leaves, stem bark and combination extracts produced significant increases (P<0.001) in the concentration of HCO_3 when compared with group II. When the dose was increased, significant increases were observed in groups VIII (P<0.01) and X (P<0.001) treated with 200mg seeds and combination when compared with group II. High blood bicarbonate can be as a result of chronic lung condition due to the poor exchange of gases [25]. There were statistically not significant difference (P>0.05) in groups VII and IX administered with 200mg leaves and stem bark extracts in comparison with group II. Administration of nevirapine to group II resulted in statistically not significant difference (P>0.05) in the concentration of K^+ compared to control (group I). There were no significant difference (P>0.05) in the level of K^+ in groups III, IV, V and VI administered with 100mg leaves, seeds, stem bark and combination and also in groups VII and VIII administered with 200mg leaves and stem bark when compared with group II. The concentration of K^+ significantly increases (P<0.001) in groups IX and X administered with 200mg stem bark and combination extracts compared with group II. There were no significant difference (P>0.05) in the concentration of Cl^- in group II (Nevirapine-alone) compared with control (group I). The concentration of Cl^- in group III was not significant (P>0.05) when compared with group II. Administration of aqueous extracts of *Balanite aegyptiaca* produced a statistically significant increase in the concentration of Cl^- in groups IV, VI (P<0.05) and in group V (P<0.01) administered with 100mg seeds, combination and stem bark extracts respectively when compared with group II. When the dose was increased the concentration of Cl^- were also higher in groups VII, IX (P<0.01) and groups VIII and X (P<0.05) administered with 200mg leaves, seeds and stem bark, combination of the aqeueos extracts respectively in comparison with group II. High level of chloride in the blood is an indication of electrolyte inbalance a condition known as hyperchloremia. Increase in serum chloride is also seen in dehydration, renal tubular acidosis, acute renal failure, [11]. There were no significant difference (P>0.05) in Na^+ concentration in group II administered with nevirapine alone in comparison with control (group I). The concentration of Na^+ was also not significant in group III (P>0.05) administered with 100mg leaves compared with group II. A significant increase (P<0.001) in the concentration of Na^+ was seen in groups IV to X administered with 100mg, 200mg of seeds, stem bark and combination extracts respectively when compared with group II. A high serum concentration of sodium is associated with condition hypernatraemia [19]

The statistically not significant difference observed in the concentration of renal function parameters (creatinine, HCO_3, K^+, Cl^-, and Na^+, excluding Urea in which there was significant reduction (P<0.001)) of rats administered with Nevirapine at a daily dose of 6mg/kg for 6 weeks was an indication that nevirapine has no effect on the kidney.

In the concurrent administration (table 3) the activities of AST and ALT in group II were elevated (P<0.001) in comparison with the control (group I). Statistically significant decreases (P<0.001) were observed in groups XI to XVIII administered with 100mg , 200mg leaves, seeds, stem bark and combination of the aqueo s extracts in comparison with group II. This may be due to the Phytochemical constituents of the plant. The concentration of T. BIL in group II (Nevirapine-alone) was statistically not significant (P>0.05) when compared with control (group I). A significant increase (P<0.001) was observed in groups XI to XVIII administered concurrently with 100mg, 200mg leaves,

seeds, stem bark and combination of aqeueos extracts compared with group II. The elevation may be attributed to haemolytic anaemia caused as a result of extracts used which may have some toxic component. Statistically no significant difference (P>0.05) was observed in concentration of D. BIL in groupII administered with Nevirapine alone in comparison with the control (group I). Statistically not signifi cant differences (P>0.05) were observed in groups XII to XVI administered with 100mg, 200mg seeds, s. bark and combination respectivey when compared wi th group II. However there were significant increases in the concentrations of D. BIL in groups XI, XVIII (P<0.001) and group XVII (P<0.01) in comparison with group II. There was no significant difference observed in the concentration of SMDA in group II in comparison with control (group I). The concentration of SMDA was also not significant (P>0.05) in groups XI to XVIII administered with aqueous extracts of *Balanite aegyptiaca* when compared with group II. This indicated that both Nevirapine and aqueous extracts of *Balanite aegyptiaca* have no potential of causing lipid peroxidation

Reduction in the activities of AST and ALT towards the respective normal value is an indication of stabilization of plasma membrane permeability as well as repair of hepatic tissue damages caused by nevirapine. Suppression of increased ALP activity also suggests the stability of biliary dysfunction in rat liver during chronic hepatic injury with Nevrapine [17]. The reduction in the activities of liver transaminases towards the respective normal value may be due the Phytochemical constituents of the plant.

The concentration of Urea (table 4) increased significantly in groups XI (P<0.05), XIII (P<0.01) and XIV (P<0.001) administered with 100mg leave, stem bark and combination extracts compared with group II. No statistically significant difference (P>0.05) was observed in the concentration of Urea in group XII administered with 100mg seeds extracts compared with group II. When the dose was increased, there was significant increase observed in the concentration of Urea in groups XV, XVI (P<0.05) and in groups XVII and XVIII (P<0.001) administered with 200mg leaves, seeds, stem bark and combination respectively when compared with group II. Administration of Nevirapine to group II revealed no significant difference (P>0.05) in the concentration of Creatinine compared with control (group I). There was also no statistically significant difference (P>0.05) in the concentration of Creatinine in groups XI, XIII and XIV administered with 100mg leaves, stem bark and combination respectively compared with group II. Administration with 100mg seeds produced a significant increase (P<0.001) in the concentration of Creatinine in group XII compared with group II. When the dose was increased, there was significant elevation observed in the concentration of Creatinine in groups XVI (P<0.001) and XVII (P<0.05) administered with 200mg seeds and stem bark aqeueos extracts respectively in comparison with group II. A raise in the level of creatinine is associated with dehydration, dietary creatinine intake in body building, and in renal failure. The concentration of HCO_3 in groups XII to XVIII were found to be statistically not significant (P>0.05) in comparison with group II. Concentration of HCO_3 in group XI was statistically higher (P<0.01) compared with group II. No significant difference (P>0.05) was observed in the concentration of K^+ in groups XI, XIII, XIV and XVIII administered with 100mg leaves, combination and 200mg combination of aqeueos extracts respectively in comparison with group II. The concentration of K^+ significantly reduced (P<0.01) in groups XII and XV administered with 100mg seeds and 200mg leaves extracts respectively in comparison with group II. The concentration of K^+ also significantly reduced (P<0.001) in groups XVI and XVII administered with 200mg seeds and stem bark extracts respectively in comparison with group II. The concentration of Cl^- in group II (Nevirapine-alone) was found to be statistically not significant (P>0.05) in comparison with control (group I). There was also not significant difference (P>0.05) in the concentration of Cl^- in groups XI, XII and XIII administered with 100mg leaves, seeds and stem bark respectively compared with group II. The concentration of Cl^- in group XIV administered with 100mg combination of extracts was found to be significantly higher (P<0.001) than group II. However, increasing the dose, significantly increase the concentration of Cl^- in groups XV(P<0.05), XVII and XVIII (P<0.001 administered with 200mg leaves, stem bark and combination extracts respectively in comparison with group II. Increase in serum chloride is also seen in dehydration, renal tubular acidosis, acute renal failure, diabetes insipidus, prolonged diarrhea (Haouzi, *et al.*, 2000). Increased in the levels of HCO_3 and Cl^- in this study may suggest that the extract induced some kind of metabolic alkalosis. No significant difference (P>0.05) in Cl^- concentration was observed in group XVI administered with 200mg seeds extract compared with group II. There was no significant difference (P>0.05) in the concentration of Na^+ in group II (treated with nevirapine alone) when compared with control (group I). No significant difference (P>0.05) was also seen in groups XII and XIII administered with 100mg seeds and stem

bark extracts respectively when compared with group II. Administration of 100mg leave and combination extracts to groups XI ($P<0.05$) and XIV ($P<0.001$) respectively produced significant increase in the concentration of Na^+ when compared with group II. When the dose was increased, there was statistically significant increase ($P<0.001$) in Na^+ concentration in groups XV, XVII and XVIII administered with 200mg leaves, stem bark and combination extracts respectively compared with group II. A high serum concentration of sodium is associated with condition hypernatraemia [19]. No significant difference ($P>0.05$) was observed in group XVI administered with 200mg seeds compared with group II.

The statistically significant increase observed in the concentration of renal function parameters (creatinine, HCO_3, K^+, Cl^-, and Na^+) in the co-administration or concurrent administration experiment may be because aqueous extracts of *Balanite aegyptiaca* at high doses could lead to dehydration.

There was no difference in the concentrations of renal function parameters between Nevirapine administration alone followed by aqeueos extracts and concurrent administration. There was also no regular pattern of treatment by aqueous extracts of *Balanites aegyptiaca* because increasing the dose to 200mg/kg from 100mg/kg does not change the effect of extracts on the experimental rat (dose-independent) and also the pattern of treatment by individual extracts (leaves, seeds, stem bark and combination) was not regular.

CONCLUSION

The results showed that administration of aqueous extracts after induction of liver damage may have some curative effects as significant decreases in liver enzymes activities were observed. The oral co-administration results showed significant decreases in the liver enzymes activities compared with the test control. This indicates a protective effect against Nevirapine hepatotoxicity. The decreases in the activities of liver enzymes may be due to the Phytochemical constituents of the plant. The increases in renal parameters could be due to dehydration brought about by the administration of the aqueous extracts.

From this study it can be concluded that extracts of *Balanites aegyptiaca* protects liver from oxidative damage and could be used as an effective protector in Nevirapine induced damage,but further experimental and clinical studies are required to confirm these findings.

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