

Effect of *H. sabdariffa* Extract on Crude Oil Linked Biochemical Alterations in the Rabbit (*Oryctolagus cuniculus*)

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This study evaluated the ability of dried calyces of *Hibiscus sabdariffa* aqueous extract (HSE) to change some selected biochemical and gravimetric parameters in crude oil exposed rabbits. A total of 28 rabbits were used for this study. The rabbits in this study were divided into 4 groups of seven rabbits each. Group I rabbits received neither the crude oil fortified growers mash nor the extract. Group II rabbits received the extract only (100 mg extract/kg body weight by gavage). Group III rabbits received the crude oil fortified growers mash only. While group IV rabbits received both the crude oil fortified mash and the extract. Each group was given the treatment once daily for 14 days. The biochemical indices examined were plasma cholesterol levels, glucose and cortisol while the gravimetric parameters evaluated were body weight gain and organ-body weight ratio. Relative to the control (group I), crude oil fed rabbits showed significant ($P < 0.05$) reduction in body weight gain as well as reduction in heart and liver body weight ratios. Plasma total cholesterol, LDL-cholesterol, were significantly ($P < 0.05$) elevated by crude oil fortified mash relative to group I, the control. Crude oil fortified mash only fed rabbits showed statistically significant ($P < 0.05$) increase in plasma cortisol concentration when compared to group I. Prior treatment of rabbits with *Hibiscus sabdariffa* aqueous extract (HSE) before exposure to the crude oil fortified mash, caused a significant ($P < 0.05$) increase in organ body weight ratios and a reduction in body weight gain relative to the control, extract only and crude oil fortified mash fed only groups. This study has demonstrated that crude oil linked decreases in organ body weight ratios as well as its associated increases in plasma total cholesterol, LDL-cholesterol, were reversed by *Hibiscus sabdariffa* aqueous extract.

Key words: Biochemical alterations, Crude oil fortified mash, *H.sabdariffa* extract and Rabbits.

Exposure of offshore drilling workers to hydrocarbons and other chemical hazards is inevitable. Gardner (2003) reported that although base oils have attracted the most interest, workers in off shore drilling operations are potentially exposed to a range of particulates especially during handling of additives like barium sulphate. Stress on the body and its internal organs is

usually the result of such exposure. Stress is also reported to be one of the basic factors in the philosophical investigation of the causes and origin of a number of diseases such as atherosclerosis, coronary heart diseases, diabetes, aging, liver diseases (Selye, 1950) rampant amongst offshore oil and gas workers. ASTDR (1999) reported neurological effect, carcinogenic effect, pulmonary effect as some health effects associated with inhalation exposure of some of these chemical agents. McDougal *et al* (2000), also demonstrated petroleum

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distillates like crude oil and diesel oil associated renal, hepatic, neurological, immunological and pulmonary toxicity. The biochemical feature that are brought into focus in relation to the aetiology and prevention of these stress-induced diseases are serum cholesterol and its factors like low density lipo-protein cholesterol (LDL-C), high density lipo protein cholesterol (HDL-C). Eliot (1974), in his work, showed a good correlation between cholesterol and lipo-protein levels. Increase stress level was also reported to cause an increase in the secretion of cortisol in blood. Cortisol reacts with insulin to cause high fat deposition in visceral tissues, which may be responsible for the increased body weight observed amongst oil and gas workers.

In animal studies, it was found that stress raises serum and tissue cholesterol level of rats on normal diet (Horst *et al*, 1960; 1963.), Berger *et al* (1980), also reported that serum cholesterol level increased after different psychological stress in rats. There are various reports that stress can change the level of certain hormones like insulin, cortisol and epinephrine (Daniel, 1989; River *et al*, 1986; Ghulam *et al*, 2009). All these hormones affect lipid profile of the body to a great extent. Cardiovascular system is more prone to be affected by stress either directly or indirectly (Koolhass *et al*, 1991). Presently, cardiovascular accidents are reported to be the major killer especially in this modern human life that is full of hassles. The use of natural products and secondary metabolites from plants for the treatment of numerous human diseases like cancer, coronary artery disease, diabetes mellitus and infectious diseases have increased over the decades (Omoregie and Okugbo, 2013). These plants natural products and secondary metabolites include alkaloids, tannins, flavonoids phenolic compounds, vitamins and minerals which in their mode of action act as antioxidants. *Hibiscus sabdariffa, l* is one of such plants. The leaf is reported to contain protein, fat, carbohydrate, fibre, ash, calcium, phosphorus, iron, thiamine, B- carotene, riboflavin, niacin and ascorbic acid (Chane, 1949; Duke,1978; Duke,1979; Duke *et al.*,1984; Morton, 1975;Watt and Breyer, 1962; Perry,1980).

This study was therefore conducted to evaluate the effect of *Hibiscus sabdariffa* calyx

extract on crude oil associated biochemical changes in the blood and organs of the rabbit (*Oryctolagus cuniculus*).

MATERIALS AND METHOD

Plant materials

The dried flowers (calyces) of *H.sabdariffa, l.* locally known as Zobo in Nigeria was bought from Hausa quarters in Igbudu market, Warri, Delta State, Nigeria and was authenticated in the Department of Plant Biology and Biotechnology, Faculty of life science, University of Benin, Benin city, Nigeria.

Animals

A total of 28 rabbits weighing between 700g and 800g bred in Ajuwawa market, Benin city, were used for this study. The rabbits were housed in a wooden frame steel mesh cage under standard environmental conditions. The rabbits were given standard rabbit food (pelleted growers mash) and tap water *ad libitum* and left to acclimatize to the laboratory conditions for 7days before the commencement of the study.

Crude oil sampling

Sample of crude oil from a land rig of KCA-Deutag Oil Company located in Rivers state, Nigeria, was used for this study.

Preparation of extract

100g of dried calyces of *Hibiscus sabdariffa, L.*, was soaked in 600ml ice cold de-ionized water for 4hours and filtered, a solution of the red pigment obtained and the residue discarded. The filtrate was made up to 1000ml with ice cold de-ionised water.

Determination of solid residue of *H.sabdariffa* Extract

The solid residue of the filtrate was determined by drying 1ml of the filtrate at 200°C in a preweighed watch glass. The solid content was found to be 52.0mg/ml. A 5% (v/V) solution of the extract was prepared by making 5ml of the filtrate up to 100ml with deionised water.

Preparation of crude oil fortified diet

Growers' mash bought from PTI road was air dried at room temperature until completely dried. It was sieved using a 2mm mesh. The sieved growers' mash was supplemented or fortified with 2% crude oil/growers' mash w/W. the mixture was homogenized sieved again and air dried at room

temperature. The fortified diet was packed in a celophene and stored in a refrigerator until required for feeding animals. The CFD feeding protocol was once weekly for 2 weeks.

EXPERIMENTAL

Twenty eight (28) rabbits were used for this study. The rabbits were divided into 4 groups of seven rabbits each. The rabbits were weighed and left to acclimatize for seven days. They were housed in wooden framed cages with top and side metal mesh. They were given water and pelleted growers mash *ad libitum*. At the end of the acclimatization period, they were weighed again just before the commencement of the study.

Group I Rabbits

Were given neither crude oil fortified diet nor given the extract (Minus CFD minus HSE).

Group II Rabbits

Were not given CFD but received the extract (100mg kg⁻¹ bd.wt). (Minus CFD Plus HSE).

Group III Rabbits

Were given CFD (2% w/W) once weekly for 2 weeks, but not given the extract. (Minus HSE Plus CFD)

Group IV Rabbits received

The extract (100 mg kg⁻¹ bd wt) and also received the crude oil fortified diet once weekly for 2 weeks (2% w/W) (Plus HSE Plus CFD).

Blood Sample Collection

Blood samples were collected from the pinna of each rabbit just before commencement of study and analyzed for glucose using the glucometer. At the end of the study duration (day 14), the external ear of each rabbit was pricked again for glucose assay using the glucometer. The rabbits were later sacrificed and blood samples collected into heparinized tubes for plasma cortisol, total cholesterol via cardiac puncture. The blood samples were centrifuged thereafter at 3000 rpm for 5 minutes and the plasma was stored at -20°C until required for the analyses.

Methods of analysis

Body and organ weights were obtained using a Mettler electronic balance as outlined by Emmanuel *et al.*, (2013). Total cholesterol and HDL-cholesterol were estimated by the

cholesterol oxidase method (Richmond, 1973). LDL- cholesterol was estimated using Freidewald's formulae. Glucose was estimated based on the glucose oxidase method by means of digital glucometer. Cortisol was analyzed by the Enzyme linked immuno-sorbent assay (ELISA) method outlined by Ramnik (2006).

Statistical analysis

Results are expressed as mean \pm SEM. Analysis of variance (Anova) was used to test for differences between treatments effects while Turkey multiple comparison test was used to test for significant differences between treatment means. Values were considered significant at $P < 0.05$.

RESULTS

Gravimetry

The effects of crude oil fortified diet and *H.sabdariffa* extract (HSE) on the body weight gain and organ body weight ratios of the experimental rabbits is presented in tables 3.1 and 3.2.

Administration of the HSE caused significant ($P < 0.05$) reduction in body weight gain of rabbits relative to the control (group I) (Table 3.1.). CFD also caused significant ($P < 0.05$) reduction in body weight gain relative to the control (Table 3.1.). When animals were given the extract and subsequently CFD, there was a significant ($P < 0.05$) reduction in body weight gain relative to the CFD only group (group III) (Table 3.1.)

Administration of extract caused significant increase ($P < 0.05$) in heart body weight ratio relative to group I (Table 3.2). CFD also caused a significant ($P < 0.05$) decrease in kidney and heart / body weight ratios relative to their respective group II. In this study, liver body weight was not significantly ($P > 0.05$) affected by any one of the treatments.

Cholesterol status

Administration of the extract caused a slight decrease in plasma total cholesterol status (Table 3.3) but it was not statistically significant ($P > 0.05$). the extract also caused a significant increase in HDL-Cholesterol status ($P < 0.05$), significant decrease in LDL-Cholesterol and Total Cholesterol/HDL ratio ($P < 0.05$) relative to their

corresponding group I. CFD caused a significant (P<0.05) increase in total cholesterol levels, LDL-Cholesterol status, total cholesterol/HDL ratio and a significant (P<0.05) decrease in HDL-Cholesterol status relative to group II.

Prior administration of the extract to

rabbits before exposure to CFD caused a significant decrease in total cholesterol status (P<0.05), a significant (P<0.05) decrease in LDL-cholesterol and a significant (P<0.05) decrease in total cholesterol/HDL ratio relative to their respective CFD only group (group III).

Table 1. Effects of CFD and aqueous extract of *H.sabdariffa* on body weight gain of rabbits

| Group no. | Treatment | Body wt. gain(g)mean±SEM (n=7) |
|------------|--|--------------------------------|
| <i>I</i> | Minus Hibiscus Sabdariffa,I extract (HSE)* | |
| | Minus crudeoil feed | 15.57±0.22 |
| <i>II</i> | Plus HSE(100mg/kg bd.wt) Minus crude oil feed | 12.57±0.22 ^a |
| <i>III</i> | Minus HSEPlus crude oil feed (CFD)* | 10.86±0.27 ^{ab} |
| <i>IV</i> | Plus HSE (100mg/kg bd.wt)Plus crude oil feed | 9.14±0.024 ^{abc} |

^a value significantly different from group I (P<0.05)
^b value significantly different from group II (P<0.05)
^c value significantly different from group III (P<0.05)

Table 2. Effect of *H. sabdariffa* aqueous extract on organ/body weight ratio of crude oil-exposed rabbits

| Groupno | Treatment | Organ/body wt ratio (n=7) | | Mean ± SEM)×10 ⁻³ |
|------------|--------------------|---------------------------|--------------------------|------------------------------|
| | | Kidney/bd .wt | Heart/bd. wt | |
| <i>I</i> | Minus CFDMinus HSE | 13.45±0.76 | 37.32±0.80 | 47.01±1.22 |
| <i>II</i> | Minus CFDPlus HSE | 11.25±0.11 ^a | 37.09±0.15 ^b | 47.18±0.25 ^a |
| <i>III</i> | Plus CFD*Minus HSE | 9.01±0.55 ^{bc} | 25.87±0.47 ^{ac} | 44.67±0.09 ^{ad} |
| <i>IV</i> | Plus CFDPlus HSE* | 13.60±0.88 ^{ade} | 35.20±1.32 ^{de} | 45.60±0.97 ^{adf} |

^avalue not significantly different from corresponding group I value(P>0.05)
^bvalue significantly different from corresponding group I value(P<0.05)
^cvalue significantly different from corresponding group II value(P<0.05)
^dvalue not significantly different from corresponding group II value (P>0.05)
^evalue significantly different from corresponding group III value (P<0.05)

Table 3. Effect of CFD and *H. Sabdariffa* calyx aqueous extract on plasma cholesterol status

| Group | Treatment | Plasma cholesterol status(mg/dl)(mean±SEM)×10 ⁻³ n=7 | | | |
|------------|--------------------|---|---------------------------|---------------------------|--------------------------|
| | | Total chol | HDL-C | LDL-C | Tchol/HDLratio |
| <i>I</i> | Minus HSEMinus CFD | 104.05±3.81 | 22.53±1.12 | 42.51±2.43 | 4.67±0.30 |
| <i>II</i> | Plus HSEMinus CFD | 98.19±3.58 ^b | 28.28±2.99 ^a | 33.10±2.02 ^a | 3.58±0.41 ^a |
| <i>III</i> | Minus HSEPlus CFD | 133.43±4.43 ^{ac} | 23.07±1.35 ^{bc} | 68.90±5.43 ^{ac} | 5.57±0.29 ^{bc} |
| <i>V</i> | Plus HSEPlus CFD | 104.01±3.38 ^{bde} | 26.54±1.97 ^{ade} | 38.61±2.62 ^{bde} | 3.72±0.50 ^{bde} |

^a value significantly (p<0.05) different from corresponding group I value (P<0.05)
^b value not significantly different from corresponding group I value (P>0.05)
^c value significantly different from corresponding group II value (P<0.05)
^d value not significantly different from corresponding group II value (P>0.05)
^e value significantly different from corresponding group III value (P<0.05)

*see table 3.1 for interpretation of abbreviation and CFD protocol

Crude oil contamination and associated biochemical changes

Blood glucose

The effects of CFD and whole aqueous extract of *H.sabdariffa*, L on blood glucose of rabbits are represented in Table 3.4. The effects of CFD and aqueous extract of *H.sabdariffa*, L calyx on blood glucose

concentration of rabbit are presented in Table 3.4. CFD caused a significant (P<0.05) increase in blood glucose concentration of rabbits relative to group II, post treatment (Table 3.4.). Treatment of the rabbits with HSE prior to exposure to CFD caused significant (P<0.05) decrease in blood glucose concentration relative to group III value, post treatment (Table 3.4.)

Table 4. Effects of CFD and whole aqueous extract of *H.sabdariffa*, l calyx on blood glucose concentration of rabbit

| Group | Treatment | Blood glucose (mg/dl) mean± SEM (n=7) | |
|-------|---------------------|---------------------------------------|------------------------------|
| | | Pre- treatment | Post-treatment |
| I | Minus CFD | 117.20±5.94** | 138.60±12.50** |
| II | Minus CFD plus HSE | 112.75±3.52 ^a | 129.75±4.77 ^a |
| III | Minus HSE Plus CFD* | 123.80±8.45 ^{ac**} | 148.60±18.45 ^{ab**} |
| IV | Plus HSE *Plus CFD | 114.00±4.14 ^{acc} | 121.75±3.33 ^{acd} |

^avalue (s) not significantly different from corresponding group I value (P>0.05)
^bvalue(s) significantly different from corresponding group II (P<0.05).
^cvalue(s) not significantly different from corresponding group II (P>0.05)
^dvalue(s) significantly different from corresponding group III (P<0.05).
^evalue(s) not significantly different from corresponding group III (P>0.05)
 *see table 3.1 for interpretation of abbreviation and CFD protocol
 values on the same row having **superscript are statistically significant

Table 5. Effects of stress and aqueous extract of *H.sabdariffa*, L. calyx on plasma cortisol status of rabbit

| Group | Treatment | plasma cortisol (n=7) (Mean ±SEM)Mg/dl |
|-------|---------------------|---|
| I | Minus HSE | 41.33±3.57 |
| II | Plus HSE | 42.36±3.33 ^a |
| III | Minus HSE Plus CFD* | 66.07±2.18 ^{bc} |
| IV | Plus HSE* Plus CFD | 61.99±3.08 ^{bcd} |

^avalue not significantly different from group I (P>0.05)
^bvalue significantly different from group I (P<0.05)
^cvalue significantly different from group II (P<0.05)
^dvalue not significantly different from group III (P>0.05)
 *see table 3.1 for interpretation of abbreviation and CFD protocol

Blood cortisol status

The effects of stress and aqueous extract of *H.sabdariffa*, l calyx on plasma cortisol status are presented in (Table 3.5).

HSE caused a slight increase in plasma cortisol status relative to group I but it was not statistically significant. CFD caused a significant

(P<0.05) increase in cortisol status relative to group I and II.

Prior treatment of rabbits with HSE before exposure to CFD caused a significant (P<0.05) increase in plasma cortisol status relative to group I and II but group III remained unaffected.

DISCUSSION

This study examined the effects of *H.sabdariffa*, L calyx aqueous extract (HSE) on crude fortified diet linked biochemical changes in rabbit (*Oryctolagus cuniculus*).

Alterations in body weight gain are usually seen as toxicity indices (Horiguchi *et al.*, 1996; Timbrell, 1991). The significant ($P < 0.05$) reduction in body weight gain (Table 3.1) and organ body weight ratio (Table 3.2) of rabbits in the crude fortified diet groups (III and IV) relative to the body weight gain of their respective non-crude diet groups (I and II) observed in this study, reveals the toxic nature of crude oil. This result aligns with the works of Timbrell, 1991; Horiguchi *et al.*, 1996; and the later works of (Asagba, *et al.*, 2004; Asagba, *et al.*, 2007,) who demonstrated how Cd, a chemical stressor caused a significant reduction in male reproductive organ/body weight ratios in rats and HSE completely reversed the effect of Cd. In the present study, prior administration of the extract before exposure of rabbits to the crude oil fortified diet resulted in further reduction in body weight gain instead of a reversal as observed by (Asagba *et al.*, 2004, Asagba *et al.*, 2007. This could be due to the weight reducing property of the extract. This aligns with the work of Emmanuel *et al.*, 2013). The result obtained in table 3.1 is not surprising, this is because on examination of Table 3.4, plasma cortisol (Table 3.4) concentration was also reduced in group (IV) rabbits, and this may also be responsible for the observed weight loss. This is because under stressful situation (whether physical, chemical, biological or psychological stress), cortisol floods the blood with glucose supplying immediate energy, this leads to cell starvation and the consequence may be over-eating in the crude oil fortified diet fed rabbits (group III). The unused glucose can later be stored as body fats which could lead to increase body weight gain. Examination of Table 3.4 revealed a reduction in cortisol concentration of group IV rabbits, although, it was not statistically significant ($P > 0.05$). The reduced cortisol level of group IV rabbits (Table 3.4) could also explain the reduction in body weight gain observed by group IV rabbits (Table 3.1) in this study. An

investigation of Table 3.2 showed that the extract caused a significant ($P < 0.05$) increase in kidney and heart body weight ratio of extract treated CFD exposed rabbits (group IV) when compared to CFD only group, (group III). HSE is seen to be repairing the kidney and heart organs in this study. This restoration potential of the extract on the organs in this study could possibly be due to some of the bioactive principles of HSE. Phytochemical screening of HSE revealed the presence of flavonoids and phenolic compounds like anthocyanins, glycosides and hydroxycitric acid. This agrees with the works of Ali (2003), Tseng (1996) and Obi *et al* (2005) who demonstrated the protective effect of the extract and its anthocyanin component on paracetamol, tertbutyl hydroperoxide and carbon tetrachloride - induced cell and organs toxicity in rats respectively.

Also in this study, a decrease in total cholesterol and LDL-cholesterol concentration occurred in the extract treated CFD exposed rabbit group, (group IV). These are all component of total lipid. It is reasonable therefore to suggest that the lipid reducing effect of the extract observed in this study could be as a result of the reduction of other components of plasma total lipid besides cholesterol. This result aligns with the work of (Ajay *et al* 2007; Faraji *et al.*, 1999; Onyenekwe *et al.*, 1997 ; Odigwe *et al.*, 2003; Herrera-arellamo *et al.*, 2004; who demonstrated that HSE extract exhibits some anti hypertensive and cardio protective effects in rats.

The hormone responsible for stimulating glucose uptake also regulates blood lipid concentration as well. The decrease in plasma glucose concentration of extract treated -crude fortified diet exposed rabbit group (group IV) relative to CFD only group agrees with the finding. The findings from this present study is in agreement with the works of El Saadany *et al* (1991), Chau *et al* (2004), Carajal- Zarrabel *et al* (2005) , Hirunpanich *et al* (2006).

Reduction in total cholesterol status and an increase in HDL-Cholesterol is usually an indication of low risk of coronary artery diseases. In this study (Table 3.3), there was a significant ($P < 0.05$) decrease in total cholesterol LDL-Cholesterol status and Cholesterol/HDL ratio. There was also a significant ($P < 0.05$) increase in HDL-cholesterol in extract only treated rabbit

group (group II). The decrease in plasma cholesterol observed in the extract treated CFD group (group IV) could be as a result of increase HDL-C which may have caused the increase removal of cholesterol from tissues for metabolism by the liver. This report aligns with the work of Prince *et al*, 1999. The decreases in plasma total cholesterol status and LDL-C of rabbits treated with the extract before giving CFD observed in this study could also be due to the inhibitory effect of HSE on LDL-C oxidation. This could also be as a result of the glycoside delphinidine-3- glucoside, content of HSE. This confirms the works of Chau *et al* (2004); Obi *et al*, 2013, where the authors demonstrated the inhibitory effects of HSE extract on low density lipoprotein oxidation; the hypocholesterolemic; the antioxidants effects of the extract.

Stress has been reported to cause glycogen immobilization Inoguchi and his co authors in a previous work demonstrated the direct relationship between high palmitate level and increase level of reactive oxygen species in patients. In the present study, crude oil fortified diet increased post treatment whole blood glucose concentration while prior administration of the extract caused a reduction in whole blood glucose concentration. This glucose reducing effect of the extract could be due to either hormonal stimulation of blood glucose or its ability to reduce plasma total lipid. Prior administration of extract (HSE) before exposure to the crude oil fortified diet (CFD) caused a decrease in blood glucose concentration relative to the extract only group (group II) although, the value was not statistically significant ($P>0.05$). This finding agrees with the previous works of Irondell *et al*, 1977, Kim *et al.*, 1966 and Merson *et al.*, 1978. Who demonstrated how stress leads to the immobilization of glycogen and enhance production of pyruvate.

Cortisol is a glucocorticoid hormone synthesized from cholesterol. Cortisol aids the synthesis of triacylglycerol to sustain energy during stress. Cortisol acts by binding to cortisol binding globulin (CBG). Stress increases cortisol level in blood and increase cortisol level enhances the synthesis of cortisol binding globulin (CBG) allowing for more cortisol- CBG interaction and drastically reducing the presence of free cortisol

thereby preventing the negative effect of prolong cortisol in circulation during prolong stress. But in this study, the chemical stressor (crude oil fortified diet) elevated cortisol level but test animals who received HSE before exposure to the CFD showed an insignificant ($P>0.05$) reduction in cortisol concentration. This seemingly cortisol increasing potency of the extract (HSE) may be due to the presence of mavidin and delphinidine anthocyanins reported from the previous work done by (Ologundudu *et al* 2009), who demonstrated the structural resembles of the structure of collapsed cortisol and delphinidine. This may have been responsible for the observed increased cortisol concentration observed in this study. The mechanism could be that since HSE structurally resembles collapsed cortisol, there is competition for cortisol binding site on CBG and hence the apparently increased pool of cortisol observed in this study.

In conclusion, this study advocates the regimented and regular intake of H.sabdariffa aqueous extract amongst offshore workers for organ protection. However, further studies are needed to determine the exact remedial concentration.

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