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# Synthesis, Partial Characterization of Chitosan-Iron Complex and its Effects on Alloxan Induced Diabetic Mellitus in Wistar Albino Rats

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**Abstract:**

This present study was undertaken to synthesize and investigate the activity of chitosan-iron complex on alloxan induced diabetic Wistar albino rats. Chitosan was extracted from crab shell and used to form complex with iron salt. Eighteen Wistar Albino rats were acclimatized and divided into three groups of six animals each weighing between 178 and 237g for bioasay: the control; the diabetic and the treated. Alloxan induction caused relative decrease in the weight of the animals in the induced group (153.64g) than control (194.21) and chitosan-iron complex treated (166.19g) groups. Results of blood glucose showed that the group treated with 50mg/kg body weight alloxan possessed higher glucose level (199mg/dL) than the control (57.67mg/dL) and chitosan-iron complex treated (55.33mg/dL) groups. The activity of chitosan-iron complex was evaluated on the plasma, liver and kidney of the rats. The results of the other studied parameters showed that chitosan-iron complex reversed the effects of the alloxan to almost control in the studied organs as the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride, total cholesterol; HDL-Cholesterol, malondialdehyde (MDA), catalase and reduced glutathione were effectively regulated. It can therefore be inferred that chitosan-iron complex can be used in the treatment of diabetes and diabetic related complications.

**Keywords:** Chitosan-Iron Complex, Antioxidant, Diabetic Mellitus, Oxidative Damage, Drug Development

## Introduction:

Diabetes mellitus is a metabolic disorder of the endocrine system that precipitates disturbance in glucose, lipid and protein homoeostasis (Van et al, 2006). People suffering from diabetes cannot produce or properly use insulin and so persistently have high blood glucose. Diabetes is generally characterized by hyperglycemia, glucosuria, polyuria, loss of body weight, disability, coma and even death. The disease is found in all parts of the world and is increasing rapidly worldwide. According to World Health Organization projection, the prevalence of diabetes is likely to increase by 35% by the year 2020. Currently, there are 150 million diabetics worldwide and this is likely to increase to 300 million in the year 2025 (Tende et al, 2011). Currently, an available therapy for diabetes involves treatment with insulin and other oral anti-diabetic agents such as sulfonylurea

and biguanid. Many of these oral anti-diabetic agents have a number of serious adverse effects, thus management of diabetes without side effect is still a challenge. Herbs have become reliable substitutes and have so far played significant role in the management of various disorders and the accompanying oxidative stress. Despite considerable progress in therapies using expensive synthetic drugs, the search for herbal remedies is growing, which can be accounted for its effectiveness, minimal side effects in clinical experience and relatively low cost of the herbal drugs (Wild et al, 2004).

Chitosan is primarily produced from chitin, which is widely distributed in nature, mainly as the structural component of the exoskeletons of arthropods (including crustaceans and insects), in marine diatoms and algae, as well as in some fungal cell walls (Suzuki, 2000). Chitosan has a unique chemical structure as a linear polycation with a high charge density, reactive hydroxyl and amino groups as well as extensive hydrogen bonding. It displays excellent biocompatibility, physical stability and process-ability. The term 'chitosan' describes a heterogenous group of polymers combining a group of physicochemical and biological characteristics, which allow for a wide scope of applications that are both fascinating and as yet uncharted. The increased awareness of the potentials and industrial value of this biopolymer lead to its utilization in many applications of technical interest, and increasingly in the biomedical arena. Although not primarily used as an antimicrobial agent, its utility as an ingredient in both food and pharmaceutical formulations lately gained more interest, when a scientific understanding of at least some of the pharmacological activities of this versatile carbohydrate began to evolve (Dina and Hans-Georg, 2009).

In recent years, several chitosan-based compounds have been developed for various biological utility such as a possible and relatively inexpensive alternative for lowering phosphorus serum levels, CH-FeCL has been suggested as a phosphate binder due to its ability to reduce intestinal absorption of phosphorus in normal and hyperphosphatemic rats and recently, iron-containing biopolymers have been described as possible drug carriers and controlled release beads for various drug models (Zhu *et al.*, 2009).

The present study sought to determine whether CH-Fe complex is capable of ameliorating the effects of diabetics in rats.

## Materials and Methods:

### Materials:

All chemicals and drugs used were obtained commercially and are of analytical grade. All the diagnostic kits were products of Fortress Chemical Ltd. England.

### Methods:

### Experimental Animals:

Live crabs were sourced from Ikorodu in Lagos State of Nigeria, the shells were removed and air dried. Female albino rats weighing between 178 and 237g were also obtained from Animal House of College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria. Before initiation of experiment, the rats were acclimatized for a period of 14 days under standard environmental conditions such as temperature (26-30$^0$C), relative humidity (45-55%) and 12hrs dark/light cycle were maintained in the quarantine. The animals used were grouped with six rats per group and fed with rat pellet diet and water were allowed *ad-libitum* under

strict hygienic conditions

### Induction of Diabetes:

The rats were induced intraperitoneally with 50mg/kg body weight alloxan (2,4,5,6-tetraoxypyrimidine or 2,4,5,6-pyrimidinetetrone) which is an oxygenated pyrimidine derivative.

### Animal Grouping:

The animals were grouped into three groups of six animals each:

GROUP 1: Normal albino rats fed with normal rat feed

GROUP 2: Diabetic induced rats (induced with 50mg/kg body weight alloxan)

GROUP 3: Diabetic induced rats + 50 mg/kg body weight chitosan-iron complex solution

**Extraction of Chitosan:**

Each part of the crab shell was treated with 2.5 M NaOH (10 mL per gram of crab shell at room temperature for 8 hours) and 1.7 M HCl (10 mL per gram of crab shell at room temperature for 8 h). The sample of each part was dehydrated twice with methanol, and once with acetone, transferred to glass tray, and dried after being washed with distilled water to neutral pH. The resulting dried compound which is chitin was kept in a well-stopped polyethylene bottle for the preparation of chitosan.

Chitosan was prepared by a slow addition of the dried chitin powder into a boiling flask containing a solution of 50% (w/v) NaOH to obtain a ratio of solid to alkaline solution of 1:30 (w/v). The temperature of reaction mixture was maintained at 107°C for 2 hours. The chitosan product was washed, dehydrated, dried, weighted and used in preparation of chitosan-iron Complex (modified from No and Meyers, 1995).

**Preparation of Chitosan-Iron Complex:**

Chitosan-iron complex was prepared by coprecipitation method at room temperature. 0.5 g chitosan was dissolved in 30 mL of 1% acetic acid solution for 20 hours, and then filtered to remove insoluble material. 10 mL of $FeSO_4.7H_2O$ aqueous solution, corresponding to a molar ratio, glucosamine:iron, of 1:1, 2:1, 3:1, 4:1, 1:2, 1:3, and 1:4, was added drop wise with stirring for 3 hours. 0.1% $Na_2CO_3$ aqueous solution was used to neutralize and precipitate chitosan-iron complex.

**Dissection and preparations of plasma and organ homogenate:**

The rats in all the groups were anaesthetised individually and quickly dissected to collect blood from the heart into the EDTA bottles and centrifuged at 3000rpm to obtain plasma; while the organs (liver and kidney) were removed and placed into ice-bath. 10% of each organs homogenate were then prepared in 6.7mm potassium phosphate buffer, (pH 7.4) using the electrically top driven homogenizer. The individual organ homogenates were centrifuged at 3,000rpm for 10 minutes at $4^0$C to obtain clear supernatants which were stored at $8^0$C and used for measurement of biochemical parameters.

**Biochemical Assay:**

*Determination of Total Cholesterol.* This experiment was carried out using standard Fortress kit from England. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinonieemine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Sample (10µl) was thoroughly mixed with 1ml of working reagent. The reaction was run at room temperature and incubated for 10 minutes at 20-25°C. The absorbance of the sample against the blank was read at 546nm.

*Determination of Triglyceride.* This experiment was carried out using standard Fortress kit from England. The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of perioxidase. Sample (10µl) was thoroughly mixed with 1ml of working reagent. The reaction was run at room temperature and incubated for 10 minutes at 20-25°C. Within 60 minutes the absorbance of the sample against the blank was read at 500nm.

*Determination of HDL-Cholesterol.* This experiment was carried out using standard Fortress kit from England. Low density lipoprotein (LDL and VLDL) and chylomychron frakctions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (High density lipoprotein) fraction, which remains in the supernatant, is determined. Sample (5µl) was added to450µl of buffer solution (R1). This mixture was mixed well and incubated at 37°C in water bath for 5 minutes. Abs 1 was measured at 600 nm. 150µl of enzyme reagent (R2) was later added to the mixture,

incubated for 5 minutes at 37ºC. Abs 2 of the sample against the blank was read at 600nm.

*Determination of LDL-Cholesterol.* This experiment was carried out using standard Fortress kit from England. Sample (5µl) was added to 450µl of buffer solution. This mixture was mixed well and incubated at 37ºC in water bath for 5 minutes. Abs 1 was measured at 600 nm. 150µl of enzyme reagent was later added to the mixture, incubated for 5 minutes at 37ºC. Abs 2 of the sample against the blank was read at 600nm.

*Determination of Alkaline Phosphatase.* This experiment was carried out using standard Fortress kit from England. The alkaline phosphatase acts on the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured at 590nm. Substrate (500 µl) was equilibrated at 37ºC for 3 minutes. 50 µl of sample was added to the equilibrated substrate and incubated for 10 minutes at 37ºC. 2.5ml of colour reagent (mixture of NaOH, 0.09M and $Na_2CO_3$, 0.1M) was added. This mixture was thoroughly mixed. Absorbance of the sample against the blank was read at 590nm.

*Determination of Aspartate Transaminase.* This experiment was carried out using standard Fortress kit from England. AST is measured by the monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. Sample (100 µl) was added to 500 µl of AST buffer. Mixed well and incubated for 30 minutes at 37ºC. 0.5ml of dye reagent (2,4 Dinitrophenyl Hydrazine, 2.0 mmol/L) was added. This mixture was thoroughly mixed and allowed to stand for 20 minutes at 20ºC. 5.0 ml of diluted NaOH was then added and thoroughly mixed. Absorbance of the sample against the blank was read at 546 nm.

*Determination of Catalase Activities.* 3.0mL of assay mixture for the catalase activity comprised: 2.5 ml of 50 mmol phosphate buffer (pH 7.0), 0.4 ml of 5.9 mmol H2O2 and 0.1 mL of enzyme extract. Decrease in absorbance of the reaction solution at 240 nm was recorded after every 20sec. An absorbance change of 0.01 units $min^{-1}$ was defined as one unit of CAT activity. Enzyme activities were expressed on the units/mg protein basis (Sinha, 1972).

*Estimation of Lipid Peroxidation Assay (TBARS).* The assay for lipid peroxidation was carried out with modified method of Iqbal et al.(1996). The reaction mixture in a total volume of 1.0 ml contained: 0.58 ml phosphate buffer (0.1 mol; pH 7.4), 0.2 ml homogenate sample, 0.2 ml ascorbic acid (100 mmol), and 0.02 ml ferric chloride (100 mmol). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by addition of 1.0 ml 10% trichloroacetic acid.

Following addition of 1.0 ml 0.67% thiobarbituric acid, all the tubes were placed in boiling water bath for 20 min and then shifted to crushed ice-bath before centrifuging at 2500 × g for 10 min. The amount of TBARS formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank.. The results were expressed as nmol of TBARS/min/mg tissue protein at $37^0$C using molar extinction coefficient of $1.56x10^5M^{-1}$ $cm^{-1}$.

was computed with a molar extinction coefficient of 1.56 x 105 M-1Cm-1.

*Determination of Glutathione.* The method of Jollow et al. (1974) was followed in estimating the level of reduced glutathione (GSH). (0.2ml) was added to 1.8mL of distilled water and 3mL of the precipitating solution was mixed with sample. The mixture was then allowed to stand for approximately 10 minutes and then centrifuged at 3000g for 5 minutes. 0.5mL of the supernatant was added to 4mL of 0.1M phosphate buffer. Finally, 0.5mL of the Ellman's reagent was added. The absorbance of the reaction mixture was read within 30 minutes of colour development at 412nm against a reagent blank.

*Determination of Superoxide Dismutase (SOD).* The level of SOD activity was determined by the

method of Misra and Fridovich (1972). Sample (1ml) was diluted in 9mL of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

*Determination of Alanine Transferase.* This experiment was carried out using standard Fortress kit from England. 100 µl of sample was added to 500 µl of ALT buffer. Mixed well and incubated for 30 minutes at 37oC in water bath. 0.5 ml of dye reagent (2,4 Dinitrophenyl Hydrazine, 2.0 mmol/l) was added. This mixture was thoroughly mixed and allowed to stand for 20 minutes at 20oC. 5.0 ml of diluted NaOH was then added and thoroughly mixed. Absorbance of the sample against the blank was read at 546 nm.

*Determination of Total Protein.* This experiment was carried out using standard Fortress kit from England. Copper ions react in alkaline solution with protein peptide bonds to give a purple coloured biuret complex. The amount of complex formed is directly proporyional to the amount of protein in the specimen. 20µl of sample was thoroughly mixed with 1ml of biuret reagent. The reaction was run at room temperature, thoroughly mixed and incubated for 10 minutes at 20-25oC. The absorbance of the sample against the blank was read at 546nm.

**Results and Discussion:**

The results of the analyses in Tables 1 to 2 were grouped and represented as follows:

**GROUP** 1: CONTROL = Normal albino rats fed with rat feed

**GROUP** 2: INDUCED = Diabetic induced rats (induced with 50mg/kg body weight alloxan)

**GROUP** 3: TREATED = Diabetic induced rats + standard drug

**GROUP** 4: TREATED = Diabetic induced rats + 50 mg/kg body weight chitosan-Fe complex solution

**Table 1: Average weights of rats in the groups (g)**

| Group | Weight Before Induction | Weight After Induction | Weight After Treatment |
|---|---|---|---|
| CONTROL (UNINDUCED) | 178.45 | 185.67 | 195.35 |
| INDUCED (UNTREATED) | 201.34 | 187.45 | 194.68 |
| METFORMIN TREATED | 237.27 | 203.45 | 226.46 |
| CHITOSAN-FE COMPLEX TREATED | 194.21 | 153.64 | 166.19 |

The results of the weights of the animals during the experiment show that induction with

alloxan caused a slight decrease in the weights of the animals except the control group that was not induced while treatment with the chitosan-Fe complex and standard drug caused slight increase as obtained in Table 1. The observations in this work were however in agreement with some earlier works which showed that diabetics caused a decrease in the weight of induced animals as reported by Adaramoye (2012); Prince *et al.* (2011); Punithavathi *et al.* (2011a); Latha and Daisy (2011) and Punithavathi *et al.* (2011b) in their various studies.

**Table 2: Blood Glucose concentration of rats in the groups (mg/dL)**

| Group | Glucose Level Before Induction | Glucose Level After Induction | Glucose Level After Treatment |
|---|---|---|---|
| CONTROL (UNINDUCED) | 76.37 | 85.34 | 93.34 |
| INDUCED (UNTREATED) | 83.56 | 378.48 | 306.23 |
| METFORMIN TREATED | 73.87 | 356.54 | 97.98 |
| CHITOSAN-FE COMPLEX TREATED | 57.67 | 199.33 | 55.33 |

The liver helps to maintain normal blood glucose concentration in the fasting and postprandial states. Loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose production. Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues such as the liver are an early manifestation of conditions characterized by insulin resistance and are detectable earlier than fasting hyperglycemia (Harris, 2005). As it was observed From Table 2, that the average glucose level in the diabetic induced rats group was 199.33 which was drastically reduced after treatment with the Chitosan-iron complex to 55.33 mg/dL that produced about 72% reduction in the blood glucose level which can be compared with metformin which is standard drug used in the treatment of diabetic which also brought about 72% reduction in the blood glucose level after treatment. It was also observed that the untreated induced group remained almost unabated for the period of the experiment. Our observation in this work also corroborates the findings of many researchers o the alloxan induction and treatments using various extracts as reported by Owa et al., 2016; Asgary et al., 2012; Okwesili *et al.*, 2016; Bako *et al.*, 2014 and Oseni 2017

The results of the analyses in Tables 3 to 11 were grouped and represented as follows:

**GROUP** 1: CONTROL = Normal albino rats fed with rat feed

**GROUP** 2: INDUCED = Diabetic induced rats (induced with 50mg/kg body weight alloxan)

**GROUP** 3: TREATED = Diabetic induced rats + 50 mg/kg body weight chitosan-Fe complex solution

Values with different superscripts along the columns in Tables 3-11 are significantly different at $p$-value $\leq 0.05$.

**Table 3: Effect of Chitosan-iron complex on Triglyceride in alloxan induced diabetic rats (mg/dL)**

| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| 1 | 66.01±3.35$^{a}$ | 161.08±5.22$^{a}$ | 120.81±1.62$^{a}$ |
| 2 | 193.60±4.07$^{b}$ | 81.28±1.95$^{b}$ | 26.14±1.30$^{b}$ |
| 3 | 107.88±1.76$^{c}$ | 105.91±3.48$^{c}$ | 40.14±1.36$^{c}$ |

**Table 4: Effect of Chitosan-iron complex on Total Cholesterol in alloxan induced diabetic rats (mg/dL)**

| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| 1 | 119.05 ±1.75$^{a}$ | 459.52±2.34$^{a}$ | 423.81±1.62$^{a}$ |
| 2 | 311.90 ±3.62$^{b}$ | 195.24±2.94$^{b}$ | 107.14±3.30$^{b}$ |
| 3 | 195.24 ±2.03$^{c}$ | 259.52±3.98$^{c}$ | 157.14±1.36$^{c}$ |

Tables 3 and 4 showed the results of the triglyceride and cholesterol concentrations in the normal, induced and treated groups of the animals. The observed variations in the two cases after induction were however corrected during the treatments as the results were reversed towards the normal control groups. The alloxan induction however produced a significant increase in both triglyceride and total cholesterol concentrations in the plasma while significant reductions were observed in the studied organs when compared with the control, these parameters were also significantly corrected and reversed towards the normal when treated with chitosan-iron complex in both cases. These observations however corroborated the earlier works of Okwesili *et al.*, 2016; Bako *et al.*, 2014 and Oseni 2017 in their various works on phytochemical, antihyperglycaemic and Lipid profile effects of methanol extract fraction of *Ricinus comminus* seeds in alloxan induced diabetic male Wistar albino rats; lipid profile of alloxan-induced diabetic Wistar rats treated with methanolic extract of *Adansonia digitata* fruit pulp and lipid profile and protective effects of aqueous extracts of some leguminous plant sprouts against doxorubicin-induced organs impairment in female Wistar rats respectively

**Table 5: Effect of Chitosan-iron complex on HDL-cholesterol in alloxan induced diabetic rats (mg/dL)**

| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| 1 | 653.85±3.51$^{a}$ | 700.34±3.75$^{a}$ | 588.46±1.98$^{a}$ |
| 2 | 215.38±5.27$^{b}$ | 515.38±2.09$^{b}$ | 280.77±5.83$^{b}$ |
| 3 | 238.46±4.39$^{b}$ | 600.21±1.66$^{b}$ | 292.31±5.10$^{b}$ |

Table 5 shows the HDL cholesterol concentrations in both plasma and the two organs in study which was grossly reduced after diabetic induction but slightly increased after treatment with chitosan-iron complex. The observed results showed that the chitosan metal complex slightly reversed the effect of the alloxan in both plasma and the organs in study. It has been earlier reported that elevated ALT leads to higher triglycerides and lower HDL cholesterol (Saligram *et al.*, 2012). Low levels of HDL (high-density lipoprotein) cholesterol are consistently associated with increased risk of type 2 diabetes in epidemiological studies. Low levels of HDL cholesterol and high levels of triglycerides are part of the diabetic dyslipidemia, and high levels of triglycerides have recently been shown to be a marker of type 2 diabetes rather than playing a causal role. Experimental evidence suggests that levels of HDL cholesterol may contribute to the pathophysiology of type 2 diabetes through direct effects on plasma glucose levels. Indeed, HDL cholesterol stimulates pancreatic β-cell insulin secretion and modulates glucose uptake in skeletal muscle in different experimental and human settings (Haase *et al.*, 2015).

**Table 6: Effect of Chitosan-iron complex on MDA in alloxan induced diabetic rats (nmolTBARS/min/mg protein)**

| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| 1 | 0.0029±0.00$^{a}$ | 0.0051±0.00$^{a}$ | 0.0064±0.00$^{a}$ |
| 2 | 0.0063±0.00$^{b}$ | 0.0083±0.00$^{b}$ | 0.0126±0.00$^{b}$ |
| 3 | 0.0035±0.00$^{a}$ | 0.0065±0.00$^{a}$ | 0.0057±0.00$^{a}$ |

The observations in this work showed that the alloxan induction produced a significant increase in the concentration of malondialdehyde when compared to the normal control in both plasma and the studied organs. It has however been reported that diabetic animals show higher MDA plasma levels compared (Noberasco *et al.*, 1991). However, treatment of the diabetic with the chitosan-iron complex produced significant reversal in MDA concentration towards the control in both plasma and the studied organs. Similar trends as observed in this work have been reported in other studies by Oseni *et al.*, (2016) and Adeleye *et al.*, (2018)

**Table 7: Effect of Chitosan-iron complex on Reduced Glutathione (GSH) in alloxan induced diabetic rats (µg/mL)**

| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| 1 | 1.13±0.01$^{a}$ | 1.30±1.36$^{a}$ | 0.93±0.54$^{a}$ |
| 2 | 0.13±0.16$^{b}$ | 0.33±0.00$^{b}$ | 0.29±0.08$^{b}$ |
| 3 | 1.58±1.75$^{c}$ | 0.92±0.18$^{c}$ | 0.65±0.30$^{c}$ |

The results of alloxan induction obtained in this study showed that GSH concentration was grossly reduced while treatment with the chitosan-iron complex significantly reversed this effect towards the control. It has been earlier reported that patients with uncontrolled type 2 diabetes have severely deficient synthesis of glutathione attributed to limited precursor availability because sustained hyperglycaemia is associated with low cellular levels of the antioxidant glutathione (GSH) (Sekhar *et al.*, 2011). However, the observed trends in this study for GSH has also been reported by Oseni *et al.*, 2015

**Table 8: Effect of Chitosan-Iron Complex on Catalase in alloxan induced diabetic rats (units/mg of protein)**

| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| 1 | 7.05x10$^{-03a}$ | 9.03x10$^{-03a}$ | 1.71x10$^{-03a}$ |
| 2 | 5.97x10$^{-05b}$ | 9.37x10$^{-05b}$ | 6.86x10$^{-05b}$ |
| 3 | 6.32x10$^{-03a}$ | 9.09x10$^{-03a}$ | 1.01x10$^{-02c}$ |

The results of catalase activities in Table 8 showed a significant reduction when induced with alloxan when compared with the control with corresponding increase after treatment with the chitosan-iron

complex in both plasma and the studied organs. Similar trend were however reported for catalase in various research works by Abul *et al.*, 2013; Adeleye *et al.*, 2018 and Oseni *et al.*, 2015

**Table 9: Effect of Chitosan-iron complex on Aspartate Transaminase (AST) in alloxan induced diabetic rats (U/L)**

| Group | Plasma | Liver |
|---|---|---|
| 1 | 165.60±5.57[a] | 106.00±1.11[a] |
| 2 | 259.00±2.30[b] | 130.60±3.66[b] |
| 3 | 151.18±7.71[a] | 113.00±1.25[a] |

**Table 10: Effect of Chitosan-iron complex on Alanine Transferase (ALT) in alloxan induced diabetic rats (U/L)**

| Groups | Plasma | Liver |
|---|---|---|
| 1 | 3.42±0.59[a] | 5.42±2.24[a] |
| 2 | 2.67±0.71[b] | 10.83±8.72[b] |
| 3 | 2.58±0.11[b] | 8.17±1.41[c] |

Tables 9 and 10 showed respectively the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) results for the experimental rats. It was observed that both transferases levels were significantly increased in both plasma and liver after induction with alloxan while treatment with Chitosan-iron complex brought downward trends in both cases. Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury (Harris, 2005).

An association exists between diabetes and liver injury. Liver pathology among diabetics is similar to that of alcoholic liver disease, including fatty liver (steatosis), steatohepatitis, fibrosis, and cirrhosis. Elevated serum activity of the two aminotransferases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), is the most frequently measured indicator of liver disease and occurs in diabetics more frequently than in the general population (Meltzer and Everhart, 1997).

Individuals with type 2 diabetes have a higher incidence of liver function test abnormalities than individuals who do not have diabetes. Mild chronic elevations of transaminases often reflect underlying insulin resistance. Elevation of transaminases within three times the upper limits of normal is not a contraindication for starting oral antidiabetic or lipid modifying therapy. In contrast, antidiabetic agents have generally been shown to decrease alanine aminotransferase levels as tighter blood glucose levels are achieved (Harris, 2005).

**Table 11: Effect of Chitosan-iron complex on Total Protein in alloxan induced diabetic rats (g/dL)**

| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| 1 | 53.42±6.20[a] | 75.95±1.97[a] | 46.25±25.37[a] |
| 2 | 34.88±8.74[b] | 83.32±8.46[b] | 46.45±26.78[a] |
| 3 | 46.44±15.50[c] | 56.21±3.95[c] | 53.82±4.51[a] |

The total protein contents of the plasma, liver and kidney homogenates were shown as as obtained in Table 11. Though changes were obtained in the concentrations of the total protein after induction with alloxan and treatments with the chitosan-metal treatments

**Fourier Transform Infra-Red Spectra:**

**Table 12: Fourier Transform Infra-Red (FTIR) *Spectra of* synthesized Chitosan and Chitosan-iron complex**

| IR-Region | Chitosan | Chitosan-Fe Complex |
|---|---|---|
| 400-800 | 678 | 650,760 |
| 800-1000 | 872 | - |
| 1000-1200 | 1056,1100,1152 | - |
| 1200-1400 | 1360,1326,1254 | - |
| 1400-1600 | 1587 | 1561 |
| 1600-1800 | 1647 | 1680 |
| 1800-2000 | 1841,1872 | 1960 |
| 2000-2200 | - | 2050,2150 |
| 2200-2400 | 2301,2380 | - |
| 2400-2600 | 2415 | - |
| 2600-3000 | 2928,2882 | - |
| 3000-3200 | 3001 | - |
| 3200-3400 | 3381 | 3400 |
| 3400-3600 | - | - |
| 3600-3800 | 3652,3724 | 3750 |
| 3800-4000 | 3829 | 3830 |

Table 12 describes the FTIR spectra of the Chitosan and the Chitosan-Iron complex. The comparative results have shown that Iron coordinates through $NH_2$ group with strong band between 1587-1647$cm^{-1}$ in the ligand (Chitosan) with a shift to 1680$cm^{-1}$ in the complex. Furthermore, a prominent band around 3381$cm^{-1}$ in the Chitosan has shifted to 3400, 3750$cm^{-1}$ bands in the metal complex, suggesting coordination of metals to the Chitosan is through OH group. The strong absorptions or bands displayed at 650$cm^{-1}$ and 760$cm^{-1}$ by the Chitosan-iron complex and nothing found around the regions in the Chitosan confirmed the formation of Chitosan-iron complex and presence of inner water molecules in the complex respectively.

**Conclusion and Recommendation:**

**Conclusion:**

In this study, Wistar albino rats were induced with diabetes, Chitosan was produced from crab shell and complexed with Iron (II) Sulphate salt. The resulting complex solution was used on the diabetic rats to test for its antidiabetic properties. Anti-diabetes evaluation of the complex revealed that it was active in controlling high glucose level characteristics of diabetes.

**Recommendation:**

Indeed, several works have been carried out on the control of diabetes using various drugs, however further studies should be carried out on the usage of Chitosan-metal complexes in the treatment of diabetics.

**Conflicts Of Interest:** The Authors declare that no conflict of interest in relation to the publication of this manuscript

**Appendix:**

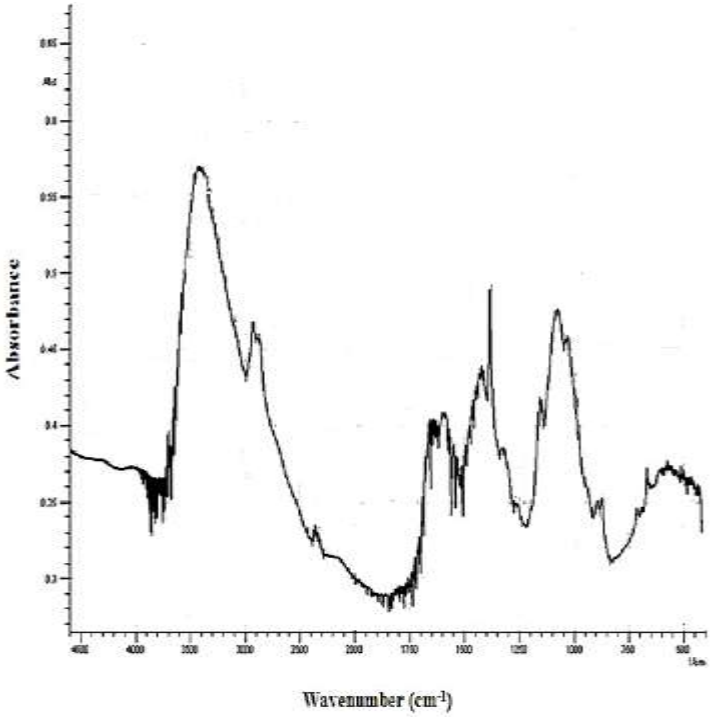


**Figure 1 FTIR Spectrum of Chitosan from Crab Shell**

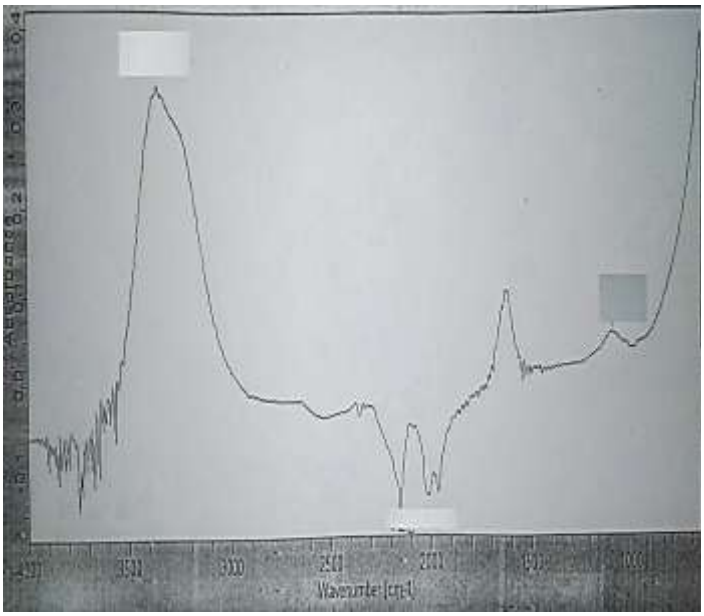

**Figure 2 FTIR Spectrum of Chitosan-Fe Complex**

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