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Research Article

Effect of Liv.52hb, Ganoderma Lucidum, STC30, and Astaxanthin on Serum TNF-α And Liver Function Indices Following CCL4 Induced Hepatocellular Carcinoma in Albino Wistar Rats Model

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Abstract

This study, examines the effect of *Ganoderma lucidum*, Astaxanthin, Liv.52 HB, STC 30, and a combination of the four (4) above-mentioned naturopathic remedies on various biochemical parameters (Serum TNF-α concentration, Liver function indices) of albino Wistar rats induced with Hepatocellular carcinoma using CCl_4. Hepatocellular carcinoma (HCC) or Hepatoma is one of the popularly known types of hepato carcinoma that develops in hepatocytes which are the most common forms of liver cells like most cancers, they occur when the cells avoid apoptosis because of the epigenetic alterations and mutations that affect cellular machinery that result in cells replicating at very high and uncontrollable rates. Thirty-five (35) albino rats of Wistar strain weighing 60–160 g, were put into seven groups of 5 animals each as follows: Group I was given only distilled water as a placebo and served as the normal control, Group II was induced with HCC using CCl_4 but not treated and served as the positive control, Group III was induced with HCC using CCl_4 and animals in this group were treated with *Ganoderma lucidum*, Group IV was also induced with HCC using CCl_4 and treated using Astaxanthin, Group V was induced with HCC using CCl_4 and treated with STC 30, Group VI was induced with HCC and treated with Liv.52 HB and lastly, group VII was as well induced with HCC using CCl_4 and treated using a combination of *Ganoderma lucidum*, Astaxanthin, Liv.52 HB, and STC 30. The treatment lasted for thirty-one (31) days. Sacrifice occurred 24 hours after administration of the last treatment dose and blood samples were collected through cardiac puncture. The biochemical parameters estimated in the blood samples were serum Alanine transaminase, Aspartate transaminase, alkaline phosphatase, Gamma Glutamyl Transferase activities, Total bilirubin, Conjugated bilirubin, and Tumor necrosis factor (TNF) α levels. Results obtained revealed the treatment significantly ($p<0.05$) reduced the concentrations of some biochemical parameters assayed for in the groups which received treatments (groups III–VII) compared with the positive control group (group II) whose biochemical parameter values were significantly ($p<0.05$) elevated compared to the normal control (group I). This reduction in values in the treated groups compared to the untreated group indicates that the treatment had positive or ameliorative effects on the parameters assayed compared with the positive control group, the values of the estimated parameters tended toward normal values of animals in the normal control group (not HCC induced). The estimated parameters that showed significant ($p<0.05$) reduction in the groups that received treatments when compared to the untreated

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group (positive control group) include; AST, ALT, ALP, GGT, CB, TB, CRP, and TNF-α. Some of the observed effects from the administration of these remedies correspond with other research works carried out using some natural remedies. Conclusively, the remedies/treatments were effective in restoring normalcy to the levels of some of the biochemical parameters assayed for in this investigation whose abnormal activities or levels are known to be associated with Hepatocellular carcinoma. The treatments were capable of restoring normal liver physio-biochemical integrity in the treated animals.

Keywords: Hepatocellular carcinoma, TNF-α, *Ganoderma lucidum*, Astaxanthin, Liv.52 HB, STC 30.

Introduction

Hepatocellular carcinoma (HCC) is one of the front runners of deaths linked to cancer globally and is one of the major causes of mortality in patients with liver damage (Serag, 2011). The incidence of Hepatocellular Carcinoma in the United States (US) has risen geometrically and is said to be more than twice its number over the past twenty (20) years and is estimated to continue rising over the next 2 decades, primarily because of the rising number of patients with advanced hepatitis C virus (HCV) and/or nonalcoholic steatohepatitis (NASH). At its current rate, Hepatocellular Carcinoma is estimated to become the third leading cause of cancer-related death in the US by 2030 surpassing breast and colorectal cancer (Rolski, 2015). Currently, only 46% of Hepatocellular Carcinoma cases are detected at an early stage and sadly, most are not therapeutically cured (Ninan, 2015). Epidemiologic and clinical studies have identified potential risk factors for hepatocellular carcinoma and can help identify patients who are at risk and implement prevention measures. Although several advances in hepatocellular carcinoma prevention, early detection, and diagnosis are effective and could reduce the incidences and death of hepatocellular carcinoma patients, efficient dissemination and successful execution are important for these strategies to be effective in medical practice. Some of the challenges faced are difficulty in recognizing at-risk patients, unavailability of well-validated risk stratification measures, and limited or reduced surveillance in high-risk groups.

Hepatocellular carcinoma (HCC) which is also the most common type of primary liver cancer in adults is currently the most common cause of death in people with cirrhosis (Forner *et al.,* 2012). Its onset is in the form of chronic liver inflammation and is very closely associated with chronic viral hepatitis infection (hepatitis B or C) or exposure to toxins such as alcohol, aflatoxin, or pyrrolizidine alkaloids. Some diseases, such as hemochromatosis and alpha 1-antitrypsin deficiency, conspicuously increase the risk of developing Hepatocellular Carcinoma. Metabolic syndrome and NASH are also clearly recognized as risk factors for hepatocellular Carcinoma. (Kernn *et al.,* 2015). Like any cancer known, the treatment and prognosis of Hepatocellular Carcinoma vary depending on the specifics of tumor histology, size, the level of cancer spread, and the patient's all round health status. The overwhelming majority of Hepatocellular Carcinoma cases occur in Asia and sub-Saharan Africa, these are continents where hepatitis B infection is endemic and many are infected from birth. The incidence of Hepatocellular Carcinoma in the United States and in developing countries is rising due to an increase in hepatitis C virus infections. For inexplicable reasons, the cases are more in males than females (Klingenberg, 2015).

The occurrence of HCC in the past two decades has increased tremendously and it accounts for about 782,000 new cases and 746,000 deaths worldwide yearly based on 2012 estimates (Ferlay, 2013). Eighty-five percent of cases are in developing countries, and the highest incidence rate is reported in areas where hepatitis B virus (HBV) is endemic. Hepatocellular Carcinoma rarely occurs in patients younger than 40 years old. The peak incidence is approximately 70 years old.

Tumor Necrosis Factor (TNF-α) is a cytokine produced by adipocytes (Freeman et al., 2002; Suganya *et al.,* 2019) and it is important in modulating injuries, tumors, and disease. Tumor necrosis factor (TNF-α) is one of the potent inflammatory cytokines that is involved very dominantly in host defense reactions. However, the solid effect and possible mechanisms of TNF-α on acute liver injury indicate the development and progression of cancer.

Certain naturopathic remedies have been claimed to be effective in combating hepatoma by folklore practitioners,

some of which are; Astaxanthin, *Ganoderma lucidum*, Liv.52 HB, and STC 30.

Astaxanthin (AX) is a pigment that belongs to the family of xanthophylls, the oxygenated derivatives of carotenoids whose synthesis in plants derives from lycopene. Astaxanthin is one of the main pigments contained in crustacean, salmonids, and other farmed fish feeds. Its major role is to ensure that these organisms maintain the desirable reddish-orange color as they do not have access to natural sources of carotenoids. It also has very good antioxidant properties which have been reported to surpass those of β-carotene or even α-tocopherol (Miki, 1991). Because of its excellent antioxidant activity, Astaxanthin has been linked with extraordinary potential for the protection of organisms against a wide range of ailments including cardiovascular problems, various types of cancer, and some diseases of the immunological system. This has generated immense interest in AX and led to numerous research studies concerning its potential benefits to humans and animals. It has been reported that Astaxanthin has been shown to exhibit antioxidant activity, as high as 10 times more than other carotenoids namely zeaxanthin, lutein, canthaxantin, and β-carotene; and 100 times more than α-tocopherol. Due to these facts, Astaxanthin has been named a "super vitamin E" (Miki, 1991).

Ganoderma lucidum or lingzhi as known in China is a well-known medicinal mushroom and has found importance in health and long-life promotion since ancient times. In vitro and in vivo studies have been performed on the anticancer activity of lingzhi, supporting its application for cancer treatment and prevention. The proposed anticancer activity of lingzhi has led to its use by carcinoma patients. It remains a subject of debate if lingzhi is a food supplement used for health maintenance or is a therapeutic agent (drug) for medical purposes. As far as information goes, there is no documented report of the use of lingzhi in human trials as a direct anticancer agent, despite some evidence pointing to the usage of lingzhi as a potential supplement for cancer patients. According to Schraub, (2000), the use of herbs such as lingzhi in cancer curing and preventive treatments remains questionable with unproven methods that have not been proven scientifically. Previous studies on the anticancer activity of lingzhi, from experiments in vitro and animals to humans in vivo, merely supported its applicability for cancer treatment and prevention, and the mechanisms of action have not been fully explored. (Cao and Lin, 2004; Lin and Zhang, 2004).

Liv.52 HB is an extensively researched product of The Himalaya Drug Company. Numerous clinical studies like open clinical studies, randomized controlled studies, meta-analyses as well as independent investigator-initiated clinical studies have all reported that Liv.52 HB has been shown to have immense benefits in various hepatic conditions. Liv.52 HB contains Nut grass (*Cyperus rotundus*) and Umbrellas Edge (*Cyperus scariosus*) which both contain anti-inflammatory and hepatoprotective properties. Many phytochemicals in Liv.52 HB suppresses hepatitis B surface antigen (HBsAg) and eliminates the hepatitis B virus (HBV) by reverse transcriptase inhibition. It significantly reduces the viral load in chronic hepatitis B infection. The anti-peroxidative activity of Liv.52 HB capsule prevents the loss of functional integrity of hepatic membranes. It also enhances regeneration of the hepatic cells and protects the hepatic parenchyma. Liv.52 HB reverses oxidative damage on hepatocytes and confers an overall hepato-protective action. It also renormalizes liver functions as well as normalizes liver enzyme levels and restores the hepatic glycogen levels.

Superlife Total Care 30 (STC30) is a relatively new health supplement product that has been shown to have lots of uses and benefits and uses. It is very effective in the treatment of several diseases and medical conditions – both mild and chronic, it is the origin of stem cells in the treatment and prevention of diseases. Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to generate healthier stem cells. Stem cells are mother cells that possess the ability to become any type of cell in the body. One of the major characteristics of stem cells is their ability to self-renew or multiply while maintaining the potential to develop into other types of cells. Stem cells can become cells of the heart, kidney, liver, bones, skin, brain, muscle, etc. These basic building blocks of life are rapidly becoming the ultimate repair kit of the future. Stem cells divide limitlessly to replenish other cells thereby serving as a sort of internal repair system, as long as the person or animal is living. Due to the efficiency of the drug, STC 30 has been used as a last-resort medication by physicians when all else fails. It is also being increasingly adopted by many medical personnel as part of treatment regimens for some chronic diseases, one of which is cancer. (SuperLife, 2017).

Hepatocellular carcinoma has been long known to be the most prevalent form of primary liver cancer among adults accounting for more than 700,000 deaths per year. (Torre *et al*., 2012; Marquardt *et al*., 2015). The high incidences of the disease burden create a need for research on treatment regimens for HCC. There is also limited literature on the effects of Astaxanthin, *Ganoderma lucidum*, Liv.52 Hb, and STC 30 on liver function and serum Tissue Necrosis Factor-alpha (TNF-α) level in the liver with oncogenic hepatic cells. There are reports of each of the four natural remedies (*Ganoderma lucidum*, Astaxanthin, Liv.52 Hb, and STC 30) having positive effects on liver function and serum Tissue Necrosis Factor-alpha (TNF-α) levels of individuals with Hepatocellular Carcinoma but all these are without enough scientific data to back up their claims.

This research aimed at studying the individual and combined effects of four known natural remedies, *Ganoderma lucidum*, Astaxanthin, Liv.52 Hb, and STC 30 used currently both by naturopathic and orthodox physicians in the management of Hepatocellular carcinoma on the serum liver functions and Tissue Necrosis Factor-alpha (TNF-α) of rats with HCC.

This study is justified by the fact that, it has made available sufficient scientific data to support or counter those reports on the management of Hepatocellular carcinoma.

Methodology

Experimental Animals

Thirty-five (35) Albino rats of the Wistar strain with a weight range of 60-160g were obtained, housed, and cared for following the standard rules and regulations of the Institute for Laboratory Animal Research (ILAR).

The procured animals were allowed to acclimatize for a period of 7 days at the Federal University Otuoke animal house. They were kept in plastic cages with wire mesh covers to aid ventilation. The animals were under monitored environmental conditions of temperature (28 ± 2°C), relative humidity (50 ± 5%), and a 12-hour light/dark cycle. The animal facility was properly ventilated and the animals were placed on commercial rat pellets as feed and water *ad libitum* throughout the experimental period.

Experimental Design and Treatment of Animals

Administration of treatment was done twice daily for a period of thirty-one (31) days via orogastric intubation. The experimental design employed comprised 35 Wistar rats of albino strain divided into 7 groups of 5 animals each. Group I was the normal control group and the animals in this group received only distilled water as a placebo. Group II was the positive control group, it was induced with HCC (along with groups 3 to 7) using CCl_4 and the animals in this group received distilled water as a placebo. Animals in groups III, IV, V, VI, and VII which were also induced with HCC received various doses of *Ganoderma lucidum*, Liv.52 HB, Astaxanthin, STC 30, and a combination of the four aforementioned remedies respectively.

Collection of Blood Samples for Analysis

The animals were sacrificed 12 hours after the administration of the last treatment regimen; whole blood was collected from the heart via cardiac puncture using a sterile syringe and needle. The blood samples were put into plain tubes. The blood samples in the plain tubes were allowed to clot by standing for 2 hours at room temperature and later centrifuged at 4000rpm for 10 minutes to separate from the serum red blood cells. Sera from each centrifuged plain tube were collected into another plain tube labeled accordingly using Pasteur pipettes. The separated sera were then kept frozen in a freezer until when needed for various biochemical assays.

Biochemical Assay

All biochemical assays were carried out using Cusabio diagnostic kits and the required biochemical analyzers.

The biochemical parameters assays include; Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Bilirubin (total and conjugated), Gamma Glutamyl transferase (GGT). The serum electrolyte levels (Sodium, Potassium, Chloride, and Bicarbonate), Urea, Creatinine, and Tumour necrosis factor α (TNF α) in the serum samples of the animals were also assayed.

The estimation of Aspartate Aminotransferase (AST) and Alanine aminotransferase (ALT) activities was done using randox kits based on the method of Reitman and Frankel (1957), while catalase and glutathione peroxidase activities was determined by the method of Johnanson, *et al.*, (1988). Total Protein and albumin concentration was determined using randox kits based on the method of Tietz (1995) and Grant *et al.,* (1987) respectively. Serum creatinine level was determined using the method of Henry (1974) while serum urea concentration was estimated by the method

of Weatherburn (1967). However, the method of Tietz, (1976) as modified was used to estimate the concentration of sodium, potassium, chloride, magnesium, bicarbonate in the serum. Tissue Necrosis Factor alpha assay was also conducted using ELISA.

Statistical Analysis

Data obtained will be expressed as Mean ± SEM and analysis will be done using the Analysis of Variance (ANOVA; f-ratio) (Welkowitz, *et al.,* 2006) and Statistical Package for Social Scientists (SPSS version 23.0). Values at P<0.05 will be considered significant in comparison with appropriate controls.

Table 1. Experimental grouping and treatment regimen

Groups	Number of rats	Treatment regimen
1 (Normal Control)	5	Distill water intra-gastrically twice daily for 3 weeks
2 (Positive Control)	5	Distill water intra-gastrically twice daily for 3 weeks
3 (Treated)	5	*Ganoderma lucidum* twice daily for 3 weeks
4 (Treated)	5	Liv.52 HB (1.78mg/kg BW)twice daily for 3 weeks
5 (Treated)	5	Astaxanthin (10.29 mg/kg bw) for 2 weeks
6 (Treated)	5	STC 30 twice daily for 3 weeks
7 (Treated)	5	Combination (*Ganoderma lucidum*, Astaxanthin, Liv.52 HB, STC 30) twice daily for 3 weeks

Results

The Effects of the various treatments on different serum biochemical parameters were evaluated and the results are presented in table 2 and 3 below.

AST

Results from analysis carried out on animal serum samples showed that the AST values of animals in groups II or PC (208.20±2.27), III (176.60±2.50), IV (211.40±2.73), V (190.60±2.29), VI (180.40±3.65), and VII (161.40±0.93) were significantly (p<0.05) higher than that of the normal control group/group I (145.80±3.40). Aspartate transaminase levels of animals in groups III (176.60±2.50), V (190.60±2.29), VI (180.40±3.65), and VII (161.40±0.93) were significantly (p<0.05) lower compared to the positive control group or group II (208.20±2.27). There was no significant (p>0.05) difference when the AST value of group IV (211.40±2.73) was compared to that of the positive control group (208.20±2.27).

ALT

Obtained results from the rats' serum sample analysis revealed that ALT values of animals in group II or positive control (123.60±2.42) and group IV (134.20±1.71) were significantly (p<0.05) higher than that of the normal control group or group I (108.20±1.80). Also, ALT values of animals in groups III (92.20±1.39), V (64.60±1.57), VI (61.00±1.22), and VII (81.40±0.93) were found to be significantly (p<0.05) lower than that of the normal control group (108.20±1.80). Compared to the positive control group/ group II (123.60±2.42), ALT values of groups III (92.20±1.39), V (64.60±1.57), VI (61.00±1.22), and VII (81.40±0.93) were significantly (p<0.05) lower. The ALT values of animals in group IV (134.20±1.71) were significantly (p<0.05) higher than that of the positive control group (123.60±2.42).

ALP

Alkaline phosphatase levels of animals in groups III (202.20±2.63), V (211.00±4.01), VI (210.60±3.71), and

VII (188.40±1.08) were significantly ($p<0.05$) lower than that of the normal control group (290.00±6.73). More so ALP values of animals in the positive control group (336.60±2.42) were significantly ($p<0.05$) higher than that of the normal control (290.00±6.73). There was no significant ($p>0.05$) difference when group IV (294.40±3.93) ALP values were compared to that of the normal control group (290.00±6.73). Alkaline phosphatase levels of animals in groups III (202.20±2.63), IV (294.40±3.93), V (211.00±4.01), VI (210.60±3.71), and VII (188.40±1.08) were significantly ($p<0.05$) lower than that of the positive control group (336.60±2.42).

Table 2. *Effects of the various treatments on different serum biochemical parameters*

	AST	ALT	ALP	TB	CB	GGT	TNF- α	CRP
NC	145.80±3.40	108.20±1.80	290.00±6.73	18.00±0.17	6.76±0.14	7.40±0.68	42.80±1.98	21.44±1.35
PC	208.20±2.27*	123.60±2.42*	336.60±2.42*	23.58±0.69*	14.66±1.61*	14.40±0.51*	495.20±26.95*	584.20±21.08
Gl	176.60±2.50*[a]	92.20±1.39*[a]	202.20±2.63*[a]	16.84±0.44[a]	6.68±0.20[a]	11.60±0.40*[a]	348.80±7.28*[a]	485.130±14.67
Liv. 52 HB	180.40±3.65*[acd]	61.00±1.22*[abc]	210.60±3.71*[ac]	22.12±1.21*[bc]	9.62±0.58*[abcd]	12.40±0.51*[acd]	360.40±12.72*[a]	491.00±15.63
ASX	211.40±2.73*[b]	134.20±1.71*[ab]	294.40±3.93[ab]	28.98±0.90*[ab]	12.08±0.77*[ab]	15.00±0.84*[b]	391.40±2.14*[ab]	410.21±12.64
STC	190.60±2.29*[abc]	64.60±1.57*[abc]	211.00±4.01*[ac]	22.06±1.22*[bc]	14.54±0.27*[bc]	16.60±0.51*[abc]	345.80±2.08*[ac]	381.22±18.10
GASL	161.40±0.93*[abcde]	81.40±0.93*[abcde]	188.40±1.08*[abcde]	12.78±0.87*[abcde]	6.74±0.31[acde]	9.80±0.37*[abcde]	299.00±3.81*[abcde]	278.320±21

NC- Normal Control, PC- Positive Control, Gl- *Ganoderma lucidum*, AST- Astaxanthin, STC- STC 30, GASL- Combination of *Ganoderma lucidum*, AST- Astaxanthin, Liv. 52 HB STC- STC 30, CRP- Capsule Reactive protein.

TB

Results showed that total bilirubin levels of animals in groups II or PC (23.58±0.69), IV (28.98±0.90), V (22.06±1.22), and VI (22.12±1.21) were significantly ($p<0.05$) higher than that of the normal control group (18.00±0.17) while TB levels of animals in group VII (12.78±0.87) was significantly ($p<0.05$) lower than that of the normal control group (18.00±0.17). There was no significant ($p>0.05$) difference when the total bilirubin levels of animals in group III (16.84±0.44) were compared to that of the normal control group/group I (18.00±0.17). Total bilirubin levels of animals in group III (16.84±0.44), and VII (12.78±0.87) were significantly ($p<0.05$) lower when compared to that of the positive control group (23.58±0.69) but total bilirubin levels of animals in group IV (28.98±0.90) was significantly ($p<0.05$) higher when compared to the positive control group (23.58±0.69). Group V (22.06±1.22) and group VI (22.12±1.21) TB levels showed no significant ($p>0.05$) difference when compared with the positive control group (23.58±0.69).

Conjugated Bilirubin

Results of the labouratory assay showed the level of conjugated bilirubin in animals of groups II (14.66±1.61), IV (12.08±0.77), V (14.54±0.27), and VI (9.62±0.58) were significantly ($p<0.05$) higher than that of the normal control group or group I (6.76±0.14) but CB levels of animals in groups III (6.68±0.20), and VII (6.74±0.31) showed no significant ($p>0.05$) difference when compared with the normal control group (6.76±0.14). Conjugated bilirubin levels of animals in groups III (6.68±0.20), IV (12.08±0.77), VI (9.62±0.58), and VII (6.74±0.31) were significantly ($p<0.05$) lower compared to that of the positive control group (14.66±1.61). There was no significant ($p>0.05$) difference when CB values of animals in group V (14.54±0.27) were compared to that of the positive control group (14.66±1.61).

GGT

Results obtained showed that GGT levels of animals in groups II or PC (14.40±0.51), III (11.60±0.40), IV (15.00±0.84), V (16.60±0.51), VI (12.40±0.51), and VII (9.80±0.37) were significantly ($p<0.05$) higher than that of the normal control group or group I (7.40±0.68). Gamma-glutamyl transferase levels of animals in groups III (11.60±0.40), VI (12.40±0.51), and VII (9.80±0.37) were significantly ($p<0.05$) lower compared to the positive control group (14.40±0.51) while GGT levels of animals in group V (16.60±0.51) was significantly ($p<0.05$) higher than that of the positive control group (14.40±0.51). There was no significant ($p>0.05$) difference when GGT levels for animals in group IV (15.00±0.84) were compared to that of the positive control group (14.40±0.51).

Tissue Necrosis Factor- α

Levels of TNF-α in groups II or PC (495.20±26.95), III (348.80±7.28), IV (391.40±2.14), V (345.80±2.08), VI (360.40±12.72), and VII (299.00±3.81) were found to be significantly ($p<0.05$) higher than that of the normal control group or group I (42.80±1.98). Results also showed that TNF α levels of animals in groups III (348.80±7.28), IV (391.40±2.14), V (345.80±2.08), VI (360.40±12.72), and VII (299.00±3.81) were significantly ($p<0.05$) lower compared to that of the positive control group (495.20±26.95).

Discussion

The liver is the primary site for detoxification or biotransformation (this is a process by which a toxic compound has been transformed to a less harmful form to reduce toxicity (Hodgson, 2004). It is also an organ where intense metabolism takes place and its therefore susceptible to several disorders because of exposure to both the intrinsic and extrinsic forms of toxins. The liver is at the forefront when it comes to metabolism and this ensures the energy level of the structural stability of the body is maintained (Guyton *et al*., 2002).

The liver catabolizes and filters out harmful substances in the blood. It also produces proteins, enzymes, and hormones which are used by the body to fend off infections (Abdel-Misih *et al*., 2010). It also converts vitamins, nutrients, and medicines into substances that the bodies can utilise. The liver also purifies the blood, leading to the production of bile for digestion and storage of glycogen for energy (Zakim *et al*., 2002). It is a specialised organ in terms of its metabolic synthetic and detoxifying functions, it is a vulnerable organ as various noxious agents can be assimilated by the intestine and transported into and through the liver, inducing inflammation, necrosis, fibrosis (Grattagliano *et al*., 2000) and eventually hepatocellular carcinoma (HCC). Over 80% of HCC occurs in patients with hepatic fibrosis and thus develops in a setting of hepatocellular injury,

regenerating, infiltration of inflammatory cells, and an abundance of activated myofibroblast (Bohan *et al*.,2003), unfortunately, at present the exact mechanisms of liver injury are still being studied and also the treatment for liver injury is still a big challenge.

The serum activity of aminotransferases is a measure of the concentration of intracellular hepatic enzymes leaked into the circulation, thus serving as a marker of hepatocyte injury (Shelli, *et al*., 2013). The liver contains aspartate amino transaminases (AST) and alanine amino transaminases (ALT) as hepatobiliary enzymes in high concentrations which are released into the blood circulation following hepatic damage and necrosis. This results in raised serum concentration of these enzymes as a result of cellular breakage and loss of functional integrity of cell membranes in hepatic tissues, making both enzymes indicators of possible liver damage/toxicity (Attiah *et al*., 2013).

The finding of this study aligns with the report of Attiah *et al*., (2013) which shows that there is a significant ($p \leq 0.05$) decrease in TB, CB serum concentration, and AST, ALT, ALP, GGT activities as observed following the administration of Liv.52 Hb when compared with the PC, NC, and the treated group and also confirmed by the work of Ekpo and Johnson (2021) who reported that following the administration of the above-listed remedies in their studies, there was a significant ($p \leq 0.05$) reduction in the serum concentration of CRP (an inflammatory marker) and a marked improvement of GFR indices. This may have been due to a plethora of complex biochemical changes occasioned by many antioxidants domicile in the natural products used in this study. Ekpo and Johnson (2021)

Nevertheless, the activities of ALT outside the liver cell are very low and therefore ALT is considered more specific for hepatocellular damage than other enzymes (Emeka *et al*., 2009). A significant ($p<0.05$) increase in AST may have been extrahepatic as this enzyme is also found in other tissues such as the kidney, brain, pancreas, lungs, and cardiac and skeletal muscles (Kasper *et al*., 2005). There was a significant ($p<0.05$) decrease with the administration of Liv.52HB observed in all the biochemical parameters in the treated group as compared with the positive control and normal control group.

Administration of astaxanthin to animals induced with HCC resulted in a decrease in serum ALP activity and conjugated bilirubin levels ($P<0.05$) when compared to the positive control. Aspartate Aminotransferase and GGT activities did not differ significantly ($p>0.05$) from the positive control.

A significant rise in serum activities of the liver enzymes accompanied by a rise in both total and conjugated bilirubin concentrations is often an indication of not only damage to the hepatic cells but also biliary obstruction (cholestasis). According to Giannini *et al.,* (2005), injury to the liver, whether acute or chronic, eventually increases serum concentrations of aminotransferases. Serum ALP, GGT, and bilirubin concentrations are of significance in cholestasis (obstruction of bile flow). Cholestasis enhances the synthesis and release of ALP and may be accompanied by hyperbilirubinemia. Changes in ALP activity due to common bile duct obstruction are preceded by a peak in aminotransferase levels, and conjugated hyperbilirubinemia; abnormal ALP levels may be a sign of metastatic cancer of the liver. GGT is a sensitive marker for liver disease and is useful for identifying causes of altered ALP levels, or elevated levels, together with other biochemical abnormalities. An increase in GGT levels is associated with bile duct damage and fibrosis in chronic liver disease. In normal healthy individuals, conjugated bilirubin is often absent from serum and only increases when the liver has lost at least half of its excretory capacity. Its presence in the serum indicates liver disorder. A cholestatic picture demonstrated by conjugated hyperbilirubinemia, an increase in ALP and GGT levels, and a significant increase in aminotransferase levels, is often attributed to chemical-induced hepatobiliary cholestasis. In this present study, a case of anti-cholestatic mechanisms has been observed following administration of astaxanthin post-HCC induction, demonstrated by a significant decrease in serum ALP activity and conjugated bilirubin levels and insignificant changes in serum AST and GGT activities. This finding agrees with earlier findings by Islam *et al*., (2017) who reported that astaxanthin exhibited a significant reduction of ALT, AST, and ALP activities in CCl4-administered rats and showed a protective effect against CCl4-induced hepatocellular toxicity. The authors also reported an ameliorating effect of astaxanthin on CCl4-induced hepatocellular damage. Astaxanthin is a potent antioxidant compound; it exhibits a protective effect against CCL4 toxicity possibly by scavenging free radicals generated from the biotransformation of CCl4 by

so doing inhibiting lipid peroxidation of the hepatic cell membranes as supported by Islam *et al*., (2017). Chen and Kotani, (2016) documented that astaxanthin has been shown to inhibit the level of lipid peroxidation, as measured by thiobarbituric acid reactive substances, and increase the level of cellular antioxidants, as measured by glutathione and superoxide dismutase, in rat liver tissues treated with carbon tetrachloride. Li *et al*., (2020) also reported that astaxanthin significantly reduced liver injury induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and significantly increased the activity of inhibited antioxidant enzymes in cell and animal models. Tripathi and Jena, (2010) earlier confirmed that astaxanthin reduced the number and area of liver cancer lesions in rats in early cyclophosphamide-induced liver tumors, and played an important role in preventing the development of liver cancer.

From the results of the labouratory assessment obtained, it was observed that administration of the treatment mixture caused a significant reduction in activities of AST, ALT, ALP, and GGT compared with the positive control group which wasn't administered any treatment. In the case of ALT, ALP, and GGT, the reduction in activities of the enzymes was also significant in comparison with the normal control group. Increased ALT, ALP AST, and GGT activity has been known as an indicator of liver damage of which cancer may be one of the factors and this rise in activity can be observed in group 2 which had CCl_4-induced HCC. A significant reduction in Total Bilirubin and Conjugated Bilirubin levels compared to the positive control group was also observed in group VII and it may be as a result of the properties of some of the natural products in the treatment mixture. The results of this study align with the findings of Oluwafemi *et al*., (2020) which showed that *Ganoderma lucidum* (a component of the treatment mixture) caused a reduction in AST, ALT, ALP, and Total bilirubin levels in comparison with the untreated group that had induced liver damage with CCl4. Research by Yang *et al*., (2014) is in line with the findings of this study in that Astaxanthin which is a part of the treatment mixture, caused a lowering of AST and ALT levels compared to the positive control. Shao *et al*., (2016) demonstrated that Astaxanthin inhibited cell proliferation and promoted cell apoptosis in vitro and in vivo and that Astaxanthin mainly locked the cell cycle in the G2 phase thus preventing the growth of cancerous cells. Liv.52 HB which is also part of the treatment mixture has been shown by the work of Girish *et al*., (2009) to be capable of reducing AST, ALT, and ALP activity to normal levels in animal models with induced liver damage. Some of the components of STC 30 have been shown by various publications to possess hepatoprotective abilities; *Malus domestica* which is one of the compositions was shown by the research of Ismail and El-Gawad (2020) to be capable of lowering AST and ALT levels. *Vitis vinifera* according to Gan *et al*., (2015) caused a decrease in serum levels of AST, ALT, ALP, and Total bilirubin in comparison with the control.

The findings of this research and the above-mentioned works may indicate that the treatment mixture which was a combination of natural products is capable of preventing the progression of Hepatocellular carcinoma and stimulating healing and reversal of liver damage due to Hepatocellular carcinoma.

Tumour Necrosis Factor (TNF-α) is a cytokine and it is important in modulating injuries, tumors, and disease. Tumor necrosis factor (TNF-α) is one of the potent inflammatory cytokines that play a dominant role in host defense reaction. However, the concrete effect and possible mechanisms of TNF-α on acute liver injury indicate the development and progression of cancer.

The results of the experiment showed that the administration of Liv.52 HB caused a significant decrease in TNF alpha levels of the test group in comparison with the positive control group after the TNF alpha levels increased significantly in the positive control group compared to the normal control group. The effects observed in the treated group after administration of Liv.52 were similarly observed in the work of Roy *et al.,* (1994), where Liv.52 HB was used as a treatment on rats with hepatotoxicity and was in line with an earlier study by Ekpo and Johnson, (2021) who revealed that LIV 52 HB lowers serum CRP levels in CCl4-induced animals which is one of the major inflammatory makers and Adias *et al.,* (2013) who report declined in oxidatively stressed indices occasioned by declined in certain haematological indices following administration of *Telfairia occidentalis* which have some phytochemical constituents in common with some of our remedies. This shows that the treatments may be capable of reversing damage caused by HCC.

Astaxanthin (AX) is a pigment that belongs to the family of Xanthophylls, which has antioxidant properties that

help in the protection of cells against different types of damage. The reduction in TNF-α level in the treated group compared to the positive group may be attributed to Astaxanthin administration as Astaxanthin was shown to have the same TNF-α and other inflammatory indices level-reducing effect in comparison with the positive control group in a study by Sutji *et al*, (2020); Ekpo and Johnson (2021).

Conclusion

This study observed that administration of *Ganoderma lucidum*, Astaxanthin, Liv. 52 HB and STC 30 and the treatment mixture caused a general decrease in serum activities of Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), and Gamma Glutamyl Transferase (GGT), and levels of CRP and TNF α at varying degree in comparison with the non-treated group or positive control group. This shows that the treatments may have had hepatic rejuvenating or restoring effects on the hepatocytes which would have led to the regeneration, restored hepatic cells membrane integrity, and subsequently led to renormalization of liver metabolism by the decrease in serum parameters tested and stated earlier in comparison with appropriate control groups. It may then be concluded that the combination of *Ganoderma lucidum*, Astaxanthin, Liv.52 HB, and STC 30 is effective and may be suitable for the management of Hepatocellular carcinoma.

Compliance with ethical standards

This study adhered to all standard ethical practices as applied to this research.

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Disclosure of conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement Of Ethical Approval

Ethical approval for this study was provided by the Research and Quality Control Unit of Federal University Otuoke in line with guidelines of the European Convention for the Protection of Vertebrate animals used for experimental and other Scientific Purposes ETS-123

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