Functional Oil from Black Seed Differentially Inhibits Aldose-reductase and Ectonucleotidase Activities by Up-regulating Cellular Energy in Haloperidol-induced Hepatic Toxicity in Rat Liver

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Abstract: In this study, the effect of rate-limiting enzymes involved in degradation of hepatic adenosine and intracellular sorbitol was investigated in rats exposed to haloperidol (HAL) and treated with functional oil (FO), containing principal active phytochemicals from black seed. Animals were divided into six groups (n=10): Distilled water, HAL 15 mg/kg, pre-administration/HAL 15 mg/kg, co-administration/HAL 15 mg/kg, post-administration/HAL 15 mg/kg, FO 150 mg/kg. The results of this study revealed that the activities of ectonucleotidase and aldose-reductase were significantly increased in HAL-treated rats when compared with the control (p < 0.05). However, differential treatments (pre, co and post) with FO depleted the activities of these enzymes compared with HAL-treated rats. Furthermore, therapeutic HAL administration increased the levels of key hepatic biomarkers (ALT, AST, and ALP) and malondialdehyde level with a concomitant decrease in functional hepatic cellular ATP. However, differential treatment with FO increases hepatic ATP and non-enzymatic antioxidant status, with a concomitant decrease in the levels of malondialdehyde and liver biomarkers. Therefore, results of this finding underlined the importance of aldose-reductase and ectonucleotidase activities in HAL induced toxicity and suggest some possible mechanisms of action by which FO prevent HAL-induced hepatic toxicity in rats.

Key words: differential treatment, essential oil, hepatic adenosine, biomarkers, aldose-reductase

1 Introduction

One of the major causes of hepatocyte adenosine triphosphate (ATP) depletion during in vivo drug biotransformation (IVDB) is oxidative stress¹, ⁵. The in vitro treatment has also been implicated in the production of reactive oxygen species (ROS), which is one of the main contributing factors of hepatic toxicity or dysfunction⁶. The increased ROS levels principally may arise from events such as pharmacologic inactivation of the active metabolites, bio-activation of inactive metabolites and when metabolite retain the pharmacological activity of their parent compounds to a greater or lesser degree⁷. Added to that, synthetic drugs cause lipid peroxidative damage, as well as compromising the membrane integrity of liver functions⁸. ROS and oxidative stress can also initiate mitochondrial dysfunction of the hepatocytes via low cellular ATP, which eventually inhibits its wellness¹, ⁵. This has been implicated as one of the risk factors behind the low performance or absolute failure of the liver cells during in vivo metabolism of xenobiotic⁹.

One basic technique that may help to overcome this global problem is the differential treatment of functional oil (FO) with antioxidant molecules. The addition of antioxidants to prevent or cure hepatic damage has been reported to decrease ROS levels and increase ATP production levels⁶, ⁶. Functional oil from black seeds contain potent antioxidants particularly thymoquinone and dithymoquinone, which has been reported to enhance normal hepatic functioning of many living subjects⁶. The volatile oil from black seeds could be used by patients to delay carcinogenic process, anti-inflammation and hepatic physiology⁷. Additionally, both thymoquinone and dithymoquinone can di-
rectly scavenge ROS\textsuperscript{8}. Furthermore, FO from black seed was shown to act as neuron-stimulant and cytoprotector agent, protecting aminergic metabolizing enzymes and membrane lipids against oxidative damage both in vivo and in vitro\textsuperscript{9}.

Haloperidol (HAL), an antipsychotic drug is commonly used in neuropathology and neuropsychology\textsuperscript{10}. When HAL is administered, its beneficial effects are highly obvious and can be associated to the phenolic moiety, particularly with regards to its ability to suppress the incoherent speech. However, there are limitations to using HAL due to its hepatic toxicity and chemical properties including low and variable bioavailability, short biological half-life, presence of chloride ion at its active site\textsuperscript{11, 12}. These characteristics likely to limit the activity of exogenously applied HAL as an anti-neurodegenerative disease molecule. Differential hepatic pharmacology is a promising approach that may solve the above-detailed limitations by enhancing the stability of HAL and apparent removal of chloride ion in the liver\textsuperscript{9}, thus ensuring harmlessness during its metabolic biotransformation. In xenobiology, pharmacology has provided a healthy substitute for drug release as it can diminish cytotoxicity, other adverse effects and can therefore increase bioavailability\textsuperscript{13}. Also, the conventional mechanism of the hepatic disease was basically associated with excessive ROS generation by antipsychotic drugs. This could limit clinical application if other mechanistic processes are not known. In light of this, the objective of this study was to examine the differential pharmacologic effect of functional oil from black seeds on hepatic aldose-reductase, ectonucleotidase, lactate dehydrogenase (LDH) activities and some key biochemistry markers of liver damage in rats treated with therapeutic haloperidol-antipsychotic drug.

2 Material and Methods

2.1 Chemicals and Reagents

Haloperidol, substrates adenosine monophosphate (AMP), glyceraldehydes, reduced nicotinamide adenine diphosphate (NADPH), 5, 5 -dithio-bis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), hydrogen peroxide, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Sigma. All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK). Kits for lactate dehydrogenase (LDH), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities were purchased from Random Laboratory Limited, United Kingdom.

2.2 Sample collection

The fresh seeds of black seeds (Nigella sativa) were obtained from Al-Medinat ventures, Kwara State, Ilorin, Nigeria. Authentication of the plants was carried out at the Department of Botany, University of Ilorin, Nigeria. The seeds were sorted out to remove all the possible stones and dirty materials and grounded into powder to enhance the efficiency of extraction of the active component(s). Quantification of phenolic compounds by HPLC-DAD Reverse phase chromatographic analyses were carried out under gradient conditions\textsuperscript{20}.

2.3 Extraction of the oil

Pulverized ten (10 g) grams of the black seed was extracted by steeping in 100 mL of methanol overnight, for 24hrs at 25°C. Thereafter, the mixture was filtered through Whatman No. 1 filter paper. The filtrate, subsequently known as oil was concentrated and stored at −4°C for further analysis. About 5 mL of the oil was obtained after methanol removal.

2.4 Animals

Adult male Wistar rats (125 ± 4.23 g) from University of Ibadan, Nigeria were used in this experiment. They were housed in the metallic cage at the central Animal House of Kwara State University, Malete. The animals were maintained at a constant temperature (30 - 32°C) on a 12 h light/dark cycle with free access to food and water.

2.5 Experimental design

The rats were acclimatized for two weeks and randomly divided into six groups of ten animals each (n = 10). Group 1 (Control) was given distilled water only via oral route; Group 2 (Induced) was given therapeutic dose (15 mg/kg body weight) of Haloperidol for 7 days. 15 mg/kg body weight is the conventional therapeutic dose for patients; Group 3 (Pre-treatment) was given 150 mg/kg body weight of functional oil (FO before) for 7 days plus therapeutic dose (15 mg/kg body weight) of Haloperidol (HAL after) for 7 days; Group 4 (Co-treatment) was given therapeutic dose (15 mg/kg body weight) of Haloperidol (HAL) plus 150 mg/kg body weight of essential oil (EO) for 7 days. Group 5 (Post-treatment) was given therapeutic dose (15 mg/kg body weight) of Haloperidol for 7 days (HAL before) plus 150 mg/kg body weight of essential oil for 7 days (FO after); Group 6 (Oil only) was given 150 mg/kg body weight of functional oil (FO only) for 7 days. The rats were fed with the same standard food and had free access to drinking water throughout the entire experiment. The duration for the experiment was two weeks (14 days). These rats were euthanized 24 h after the last treatment. All the animals received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institute of Health. Ethic regulations have been followed in accordance with National and insti-
tutional guidelines for the protection of animal welfare during experiments\textsuperscript{19}.

2.6 Hepatic post mitochondrial homogenate

After the treatment, the animals were submitted to euthanasia being previously anesthetized with ethyl acetate and liver was removed for hepatic post mitochondrial homogenate. The liver was homogenized in 4 volumes of an ice cold medium, consisting of 1.15% potassium chloride and 50 mM Tris-HCl buffer with a pH 7.4 in a motor driven Teflon-glass homogenizer. The supernatants were isolated at 4°C and used for biochemistry and enzymatic assays.

2.7 Aldose Reductase (AR) Assay

Aldose reductase activity was assayed by estimating the consumption of NADPH as described by Da Settimo et al.\textsuperscript{20}. Briefly, the reaction mixture contained 4.67 mmol/L D,L-glyceraldehyde as substrate, 0.11 mmol/L NADPH, 0.067 mol/L phosphate buffer (pH 6.2), and 0.05 mL of the enzyme preparation (rat liver homogenate) in a total volume of 1.5 mL. The enzyme reaction was initiated by addition of D, L-glyceraldehyde and absorbance of sample test at 340 nm which is a functional amount of NADPH consumed was measured.

2.8 Hepatic ecto-5′-nucleotidase assay

The hepatic ecto-5′-nucleotidase activity was determined essentially by the method of Heymann et al.\textsuperscript{22} with little modification. Briefly, a reaction medium containing 0.1 mL of 10 mM MgCl\textsubscript{2} and 0.1 mL of 5 mM Tris-KCl little modification. Briefly, a reaction medium containing 0.1 mL of 10 mM MgCl\textsubscript{2} and 0.1 mL of 5 mM Tris-KCl buffer, pH 7.6 to final volume of 500 mL was prepared. The reaction was initiated by the addition of 0.15 mL 10mM AMP. 0.15 mL of the homogenate was added to the reaction mixture and incubated at 37°C for 20 min. Reaction was stopped in all cases by the addition of 0.5 mL of 10% TCA and the protein precipitated was removed by centrifugation for 10 min. 0.5 mL of the supernatant was added to 0.5 mL of 1.6% ammonium molybdate, then 0.2 mL of sodium acetate and 0.8 mL of 10% ferrous sulphate. The released inorganic phosphate(Pi) was assayed at 700 nm using colorimetric reagent and KH\textsubscript{2}PO\textsubscript{4} as standard. Controls were carried out by adding liver homogenate fraction after TCA addition to correct for non-enzymatic nucleotide hydrolysis. Enzyme activities are reported as mmol Pi released/min/mg of protein.

2.9 LDH assay

The hepatic homogenate was assayed for LDH activity using commercially available kit (Randox Laboratories UK). Assay was carried out according to the manufacturer’s instructions\textsuperscript{23}.

2.10 Lipid peroxidation assay

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Ohkawa et al.\textsuperscript{24} and expressed as μmoles/mg protein.

2.11 Hepatic toxicological analysis

The activities of plasma AST, ALT and ALP were measured using available commercially kits (Randox Laboratories Kits, St Louis, MO, USA).

2.12 Hepatic antioxidant assay

The catalase (CAT) activity was estimated using hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) as substrate according to the method of Clairborne\textsuperscript{19}. Briefly, the method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H\textsubscript{2}O\textsubscript{2}, with the formation of perchormic acid as an unstable intermediate. The chromic acetate produced was measured spectrophotometrically at 570 nm. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30 ± 1°C according to Misra and Fridovich\textsuperscript{25}. GSH was determined at 412 nm using the method described by Jollow et al.\textsuperscript{26}. Protein concentration was determined by the method of Gornall et al.\textsuperscript{27}. GST was assayed using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate according to the method of Habig\textsuperscript{28} and expressed as mole CDNB-GSH complex formed/min/mg protein.

2.13 Histopathological examination

After the treatment, the livers were separated and placed in 4% para-formaldehyde at 4°C for 48 hours. After dehydration, transparency, paraffin immersion and paraffin embedding, the liver was sliced along median antero-posterior axes at a thickness of 6 mm. The section was stained with hematoxylin and eosin for morphological observation and defining positions. Sections were read and images were captured using an Olympus microscope (BX51).

2.14 Statistical analysis

The data in each group were expressed as mean ± standard deviation. A one way analysis of variance (ANOVA) was used to analyse the results and Duncan multiple test was used for the post hoc\textsuperscript{29}. Statistical package for Social Science (SPSS) 17.0 for windows was used for the analysis and the least significance difference (LSD) was accepted at \( p < 0.05 \).

3 Results

3.1 Chemical characterization of the functional oil from black seed

In order to identify the phenolic compounds responsible for their action, we characterized the black seed extract with reversed-phase HPLC using their retention time and
UV spectra in comparison with standard phenolic compounds. This result has already been published according to the previous work by Akintunde and Irechukwu\(^9\). HPLC phenolic profile of the extract revealed the presence of the gallic acid, catechin, chlorogenic acid, caffeic acid, orientin, rutin, quercitrin, quercetin and luteolin\(^9\). Also, gas chromatography-mass spectrum (GC-MS) had formerly indicated the presence of carvone, alfa-pinene, sabinene, p-cymene, \(\beta\)-pinene, and carotene which could be biotransformed into vitamin A\(^25\). Similarly, the oil was regarded as the key source of protein, thiamin, riboflavin, pyridoxine, niacin, folacin, calcium, iron, sodium, phosphorous, copper, zinc and potassium\(^26\). Furthermore, it was rich in essential amino acids including arginine, cysteine, lysine, carnitine fatty acids, particularly the unsaturated and essential fatty acids\(^26\).

3.2 Effect of functional oil on hepatic AR and ecto-5\(^1\)-nucleotidase activities in HAL-induced hepatic toxicity in rat liver

AR and ecto-5\(^1\)-nucleotidase activities in liver tissues of the experimental animals are shown in Figs. 1 and 2. The results revealed that therapeutic oral HAL administration significantly hiked the activities of AR and ecto-5\(^1\)-nucleotidase enzymes compared with the control rats\((p<0.05)\). However, differential (pre, co and post) treatment with functional oil (150 mg/kg) prevented these hikes by inhibiting the activities of AR and ecto-5\(^1\)-nucleotidase enzymes, compared with the HAL-induced liver toxicity. Similarly, the results demonstrated that the rats treated with EO depleted the activities of AR and ecto-5\(^1\)-nucleotidase proteins, compared with the HAL-induced liver toxicity\((p<0.05)\).

3.3 Effect of functional oil on key index of cellular ATP in HAL-induced liver toxicity

Figure 3 depicts the effect of HAL administration on functional marker of cellular ATP. Administration of therapeutic HAL caused a significant decrease\((p<0.05)\) in lactate dehydrogenase (LDH) activity when compared with the control (Fig. 3). However, the activity of LDH was increased in FO treated rats when compared with the control. Additionally, pre and co-treatment with functional oil from black seed (150 mg/kg) significantly\((p<0.05)\) up-
regulated the reduced activity of LDH when compared with the HAL-induced liver toxicity ($p < 0.05$) (Fig. 3).

### 3.4 Effect of functional oil on HAL-induced changes in hepatic function indices

Figure 4 showed the effect of HAL administration on key biomarkers of liver toxicity in animal model. Therapeutic administration to HAL caused a significant alteration ($p < 0.05$) in plasma ALT, AST and ALP activities when compared with the control (Figs. 4A, B and C). However, differential (pre, co and post) treatment with EO from black seed (150 mg/kg) significantly ($p < 0.05$) prevented the alteration by reducing the activities of ALT, AST and ALP (Figs. 4A, B and C) when compared with HAL-induced liver toxicity.

### 3.5 Effect of functional oil on HAL-induced changes in hepatic oxidative stress biomarkers

As presented in Fig. 5, the hepatic MDA level in HAL-treated rats was significantly ($p < 0.05$) elevated when compared with the control, indicating that the exposure to HAL caused noticeable oxidative damage in rats. However, it was discovered that the increase was reduced by pre, co,

**Fig. 5** Effect of oil from black seed on malondialdehyde (MDA) level in haloperidol-induced hepatic cell toxicity. Data are presented as mean ± SD ($n = 10$). Bars with different letters are statistically significant.

**Fig. 4** Effect of oil from black seeds on ALT, AST and ALP activities (toxicology analysis) in haloperidol-induced liver toxicity. Data are presented as mean ± SD ($n = 10$). Bars with different letters are statistically significant.
and post-treatment with FO (150 mg/kg) when compared with the HAL-induced liver toxicity. Also, administration of FO only significantly \(p < 0.05\) lowered the level of MDA in relation to HAL-induced liver toxicity. In addition, oral administration of therapeutic HAL caused significant alterations in enzymatic and non-enzymatic proteins when compared with the control \(p < 0.05\), indicators of depletion of endogenous antioxidant molecules (Figs. 6A, B, C and D). However, differential (pre, co and post) treatments including functional oil group were able to prevent/reverse the alterations in both enzymatic and non-enzymatic antioxidants when compared with the control \(p < 0.05\) (Figs. 6A, B, C and D).

3.6 Effect of functional oil on hepatic kupffer cell in HAL-induced liver toxicity

As observed in Fig. 7, control animal indicated little or no visible lesions to the hepatocytes. HAL induced rats showed widespread marked thinning of hepatic cords. Also, increased production or growth (hyperplasia) of the Kupffer cells was markedly observed. Pre-treated group showed moderate thinning of hepatic cords. Similarly, co-treated group revealed moderate thinning of hepatic cords. Post-treated animals showed adequate thinning of liver cords. Finally, the group administered with essential oil depicted moderate growth of Kupffer cells (moderate hyperplasia). Although, the tissue may be larger but still retains its normal functioning i.e. no visible lesions to the hepatocytes.
4 Discussion
The current study confirms that ingestion of essential oil from black seed differentially prevents/reverses the rat’s liver toxicity induced by haloperidol-antipsychotic drug. The present study used the innovative approach of differential hepatic pharmacology to allow the prevention of liver toxicity/ and or hepatic failure to the beneficial activities of essential oil from black seed. Related effect has been demonstrated in our previous work using essential oil as neuron-stimulant, cyto-protector agent which protect aminergic metabolizing enzymes and mediation of neuronal antioxidative damage both in vivo and in vitro. Excitingly, in the current study, differential treatment with functional oil from black seed significantly lowered AR activity than HAL induced liver toxicity group, indicating that ability to inhibit AR was most efficacious by co-treatment. This inhibition is significant, as treatment with essential oil can reduce hepatic sorbitol and fructose levels (type 1 and type 2 diabetic) via polyol pathway which in turn prevents hyperglycemia. It is observed that rutin detected by HPLC in our present study inhibited aldose-reductase activity. Boligon et al. established that rutin isolated from the extract obtained from Scutia buxifolia Reissek inhibited aldose-reductase, adenosine deaminase and 5’-nucleotidase activities. This observation was further substantiated by ref. which reported that hike hepatic glucose level as a result of increased activity of AR could cause high NF-KB binding activities, leading to hepatic pathology and consequently result in unhealthy kupffer cells development. The study also demonstrated that, the activity of ecto-5’-nucleotidase was higher in the HAL treatment group versus both differential treatment and the control group. The lowest rate of hepatic ATP hydrolysis to form adenosine was found in the differential treatment with functional oil than HAL induced liver toxicity group. Elevations of these enzymes have previously been discovered in the livers of compromised patients when compared with normal individuals. Additionally, the current investigation shows that differential (pre, co and EO) treatments up-regulated the activity of LDH- marker of ATP production in liver when compared with HAL induced liver toxicity group. The hepatic high activity of LDH can be a consequence of the luteolin and quercitin action from the oil in the cell, preventing ATP hydrolysis or blocking the breakdown of the liver tissue as well as depleting eco-5’-nucleotidase activity. This finding supports the previous discovery that patients with hepatic dysfunctions displayed low level of ATP molecules. Also, recent finding reported that quercitrin, quercetin and luteolin have been implicated against ATP drop in hepatic cells.

The central site of xenobiotic metabolism known as liver is at risk to injury. Also, one of the adverse effects of antipsychotic drugs is hepatotoxicity. However, natural antioxidants had been implicated to elicit protective potentials by interacting with biomolecules both at cellular and molecular levels to inhibits those involve in carcinogen activation. The biomarkers of hepatic toxicity (ALT, AST and ALP) that were produced following the differential treatments had lower activities than that produced by the HAL induced group. This effect is due the synergistic properties of antioxidants- gallic acid, catechin, chlorogenic acid, caffeic acid, orientin, rutin, quercitrin, quercetin, luteolin. It was reported that luteolin caused the production of high quality hepatocytes and ultimately preserving the functionality of the liver cells. Increased activities of liver marker enzymes as alkaline phosphatase (ALP) , aspartate aminotransferase (AST)and alanine aminotransferase (ALT) indicates serious damage of the hepatocytes and compromised integrity as well as permeability of its membrane. A previous study on hepatic enzymes demonstrated that the administration of silymarin and/or vitamin C led to a reduction in the activities of liver enzymes in comparison to the control. Also, these data support the beneficial effect of functional oil, as down-regulator of hepatic genes in relation to elevated oxidative stress and free radicals prophylactics in animals.
for toxic effect of HAL. It was recently reported that HAL could increase the level of MDA indicates lipid peroxidation. Hence, the level of MDA in HAL-administered rats was measured. The result indicated that oxidative stress plays an important role in HAL-induced hepatic damages. The tested antipsychotic drug disrupted the normal working process of the liver membrane leading to its failure. It is possible that the HAL was able to penetrate the cellular membrane of the hepatocytes. However, differential treatments prevented this oxidative-mediated action. It could be believed that EO prevents the release of HAL into the hepatocellular membrane thereby retaining its structural integrity as well as blocking membrane permeability transition pore (MPTP). This effect was linked to the synergy or competitive interactions of functional components predominantly thymoquinone and dithymoquinone in the oil of black seed.

Furthermore, phenolic compound plays an important role in activating antioxidant defenses, including GSH. A high GSH content in mammalian hepatocytes has been found to prevent an exacerbated production of ROS. Intracellular GSH level was higher in animal groups differentially treated with functional oil than HAL-induced liver toxicity. It was found that co-treatment with functional oil does not increase the levels of GSH in comparison with HAL-induced liver toxicity group. A study under differential psychopharmacology, also presented that co-treatment does not affect GSH level in relation to HAL-induced neuronal toxicity. Based on the above finding, it seems that differential treatments with functional oil regulate the cellular defense system against ROS via the enzymatic system. When measuring the activities of the antioxidant enzymes that were tested in this work (GST, SOD, and CAT), significant changes were observed following differential treatments with functional oil compared to HAL-induced liver toxicity group. The study may also suggest that post-treatment does not affect SOD activity in relation to HAL-induced liver toxicity. This effect may be linked to irreversible action caused by SOD in presence of antipsychotic drug-HAL. The observation is in line with our previous work. Differential treatments with FO produced nearly equivalent effects on both GST and CAT activities, whereas the activities of the GST and CAT were higher following treatment with EO. Functional oil from black seed always induced higher activities of the above-detailed antioxidant enzymes compared to the HAL-induced group.

Finally, the hepatocellular toxicity effect of HAL-administered rat showed widespread marked thinning of hepatic cords with growth (hyperplasia) in the Kupffer cells. This could be linked to the direct interface of the antipsychotic drug with hepatic kuffer cells, thereby slowing down its normal functionality. The continuous deactivation of these cells may trigger irreparable liver failure. The observation was supported by the investigation which reported that hepatomegaly as well as hepatitis (inflammation of the liver) was obvious in patients chronically exposed to antipsychotic drugs. Differential treatments with functional oil from black seed moderately prevented these abnormalities. Therefore, based on the hepatic protective potential in rats exposed to HAL, EO could be employed as hepatic pharmacology particularly when differential approaches are adopted.

**Conclusion**

This investigation represents the use of differential pharmacologic approaches of the functional oil to reverse hepatic functionality in HAL-induced liver toxicity. It was demonstrated that therapeutic administration of HAL increased the activities of hepatic aldose-reductase and ecto-5'-nucleotidase as well as MDA level with corresponding decrease in the activity of LDH rats-marker of ATP. Differential treatments with functional oil prevented and reversed these alterations by depleting the activities of hepatic aldose-reductase and ecto-5'-nucleotidase as well as MDA level with concomitant increase in the activity of LDH. It was also found that differential treatment with functional oil regulates the cellular defense system against ROS via the enzymatic system. It is then recommended that differential treatment with functional oil from black seed provides natural pharmacologic care for patients predisposed to hepatic dysfunctions and ailments associated with antipsychotic drugs.

**References**

1. Akintunde, J.K.; Oboh, G. Depletion of cellular adenosine triphosphate and hepatocellular damage in rat after sub-chronic exposure to leachate from anthropogenic recycling site. *Hum. Exp. Toxicol.* 31, 1-13 (2015).
2. Akintunde, J.K.; Bolarin, E.O.; Akintunde, D.G. Garlic capsule and selenium-vitamins ACE combination therapy modulate key antioxidant proteins and cellular adenosine triphosphate in lisinopril-induced lung damage in rats. *Drug Metab. Pers. Ther.* 31, 47-54 (2016).
3. Frank, H.; Joep, B.; Andreas, L. The role of oxidative stress in carcinogenesis induced by metals and xenobiotics. *Cancers (Basel)* 2, 376-396 (2010).
4. Takemoto, K.; Hatano, E.; Iwaisako, K. et al. Necrostatin-1 protects against reactive oxygen species (ROS)-induced hepatotoxicity in acetaminophen-induced acute liver failure. *FEBS Open Bio.* 4, 777-787 (2014).
5. Ilker, O.A.; Adman, T.; Orhan, T. Thymoquinone treatment against acetaminophen-induced hepatotoxicity
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6) Eda, O.; Sema, U.; Niufer, E.; Hadi, K. Protective effect of chitosan treatment against acetaminophen-induced hepatotoxicity. *Kaohsiung J. Med. Sci.* 30, 286-90 (2014).

7) Kapan, M.; Coskun, O.; Uysal, H. The antioxidative and antihistaminic effect of *Nigella sativa* and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage. *Arch. Toxicol.* 80, 217-224 (2006).

8) Kanter, M.; Coskun, O.; Uysal, H. The antioxidative and antihistaminic effect of *Nigella sativa* and its major constituent, thymoquinone on ethanol-induced neuronal damage of male rats. *Ther. Adv. Drug Saf.* 7, 132-146 (2016).

9) Barnes, T.R. Evidence-based guidelines for the pharmacological treatment of schizophrenia: recommendations from the British Association for Psychopharmacology. *J. Psychopharmacol.* 25, 567-620 (2011).

11) Alexander, P.S. Do mollusks use vertebrate sex steroids as reproductive hormones? Part I: Critical appraisal of the evidence for the presence, biosynthesis and uptake of steroids. *Stereoids* 77, 1450-1468 (2012).

12) Miyamoto, S.; Miyake, N.; Jarskog, L.F.; Fleischhaecker, W.W.; Lieberman, J.A. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Mol. Psych.* 17, 1206-1227 (2102).

13) Sabina, E.P.; Rasool, M.; Vedi, M.; Navaneethan, D.; Ravichander, M.; Parthasarthy, P. Hepatoprotective and antioxidant potential of Withania somnifera against paracetamol-induced liver damage in rats. *Int. J. Pharm. Pharmaceut. Sci.* 5, 648-651 (2013).

14) Public Health Service (PHS). Public Health Service Policy on Humane Care and Use of Laboratory Animals. Washington, DC: US Department of Health and Human Services (PL 99-158, *Health Research Extension Act of 1985*) (1996).

15) Da Settimo, F.; Primofoire, G.; La Motta, C.; Sartini, S.; Taliani, S.; Simonini F. Naphtho[1, 2-α]isothiazole acidic acid derivatives as a novel class of selective aldose reductase inhibitors. *J. Med. Chem.* 48, 6897-6907 (2005).

16) Heymann, D.; Reddington, M.; Kreutzberg, G.W. Subcellular localization of 5'-nucleotidase in rat brain. *J. Neurochem.* 43, 971-978 (1984).

17) Weisshaar, H.D.; Prasad, M.C.; Parker, R.S. Estimation of lattice dehydrogenase in serum/plasma. *Med. Welt.* 26, 387-391 (1975).

18) Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid perox-

ides in animal tissues by thiobarbituric acid reaction. *Analyst. Biochem.* 95, 351-358 (1979).

19) Clairborne, A. Catalase activity. in *Handbook of methods for oxygen radical research* (Greweald, A.R. ed.), CRC Press, Florida, pp. 237-242 (1979).

20) Misra, H.P.; Fridovich, I. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay of superoxide dismutase. *Toxicol. Biol. Chem.* 24, 3170-3175 (1989).

21) Jollow, D.J.; Mitchell, J.R.; Zampaglione, N.; Gillette, J.R. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic metabolite. *Pharma.* 11, 151-169 (1974).

22) Gornall, A.G.; Bardlawill, C.J.; David, M.M. Determination of serum proteins by means of the Biuret reagent. *J. Biol. Chem.* 177, 751-756 (1949).

23) Habiq, W.H.; Pabst, M.I.; Jacoby, W.B. Glutathione-transferase the first enzymatic step in mercapturic acid formation. *J. Biochem.* 150, 7130-7139 (1974).

24) Zou, J.H. *Biostatistical Analysis.* Prentice-Hall, Englewood Cliffs, New Jersey (1974).

25) Ali, M.A.; Sayeed, M.A.; Alam, M.S.; Yeasmin, M.S.; Khan, A.M.; Mohamad, I.I. Characterization of oils and nutrient contents of *Nigella sativa* Linn. and *Trigona foenum-graceum* seed. *Bull. Chem. Soc. Ethiop.* 26, 55-64 (2012).

26) Said, G.; Hicham, H.; Dominique, G.; Aziza, R.; Samira, B.; Mohammed, I.; Mushtaq, A.; Shazia, S.; Taibi, B.H.; Imane, C.; Zoubida, C. Chemical composition of *Nigella sativa* L. seed oil produced; Morocco. *J. Saudi Soc. Agric. Sci.* 14, 172-177 (2015).

30) Boligon, A.A.; Pimentel, V.C.; Bagatini, M.D.; Athayde, M.L. Effect of *Scutia buxifolia* Reissek in nucleotide activities and inhibition of platelet aggregation. *J. Natural Med.* 69, 46-54 (2015).

31) Hershfield, M.S. Adenosine deaminase deficiency: clinical expression, molecular basis, and therapy. *Seumin. Hematol.* 35, 291-298 (1998).

32) Michael, B.J.; Ball, J.G.; Wright, M.S.; Van Meter, S.; Valentovic, M.A. Novel protective mechanisms for S-adenosyl-L-methionine against acetaminophen hepatotoxicity: improvement of key antioxidant enzymatic
function. *Toxcol. Lett.* **212**, 320-328 (2012).

33) Akinyemi, J.; Gustavo, R.; Vera, M.; Naiara, S.; Pauline, da C.; Andreia, C. *et al.* Effect of dietary supplementation of ginger and turmeric rhizomes on ectonucleotidases, adenosine deaminase and acetylcholinesterase activities in synaptosomes from the cerebral cortex of hypertensive rats. *J. Appl. Biomed.* **14**, 59-70 (2016).

34) Kobori, M. Dietary quercetin and other polyphenols: Attenuation of obesity. in *Polyphenols in human Health and Disease* (1st ed.), Elsvir, pp. 163-175 (2014).

35) McClanahan, D.; Hillenbrand, K.; Kapur, A.; Carlton, D.; Czuprynski, C. Effects of extracellular ATP on bovine lung endothelial and epithelial cell monolayer morphologies, apoptoses, and permeabilities. *Clin. Vaccine Immunol.* **16**, 43-48 (2008).

36) López-Torres, E.; Süveges, A.; Peñas-LLedó, E.M.; Doña, A.; Dorado, P.; Llerena, A.; Berecz, R. Liver enzyme abnormalities during antipsychotic treatment: a case report of risperidone-associated hepatotoxicity. *Drug Metabol. Drug Interact.* **29**, 123-126 (2014).

37) Liu, W.X.; Jia, F.L.; He, Y.Y.; Zhang, B.X. Protective effects of 5-methoxypsoralen against acetaminophen-induced hepatotoxicity in mice. *World J. Gastroenterol.* **18**, 2197-2202 (2012).

38) Akintunde, J.K.; Oboh, G. Exposure to leachate from municipal battery recycling site: implication as key inhibitor of steroidogenic enzymes and risk factor of prostate damage in rats. *Rev. Environ. Health* **28**, 203-213 (2013).

39) Akintunde, J.K.; Bolarin, O.E. Research on garlic capsule and selenium-vitamin A, vitamin B, vitamin C applied in therapy of acute hepatocellular damage in a rat model. *J. Acute Dis.* **4**, 293-299 (2015).

40) Ajiboye, J.A.; Akintunde, J.K.; Oladejo, O.S.; Sabiu, S.A. Chemoprevention of silymarin and vitamin C on isoniazid-induced hepatotoxicity in experimental rat model. *J. Toxins* **2**, 3 (2015).

41) Wang, A.Y.; Lian, L.H.; Jiang, Y.Z.; Wu, Y.L.; Nan, J.X. Gentiana manshurica Kitagawa prevents acetaminophen-induced acute hepatic injury in mice via inhibiting JNK/ERK MAPK pathway. *World J. Gastroenterol.* **16**, 384-391 (2010).

42) Polydoro, M.; Schroder, N.; Lima, M.N.; Caldana, F.; Laranja, D.C.; Bromberg, E.; Roesler, R.; Quevedo, J.; Moreira, J.C.; Dal-Pizzol, F. Haloperidol- and clozapine-induced oxidative stress in the rat brain. *Pharmacol. Biochem. Behav.* **78**, 751-756 (2004).