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Effects of Essential Oil Extracted from the Leaves of *Hoslundia opposita* V. on Selected Biochemical Indices in Rats

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Abstract: Biochemical effect of essential oil extracted from the leaves of *Hoslundia opposita* on selected toxicological indices was evaluated. Twenty four *Rattus norvegicus* were randomly selected into four groups. The rats were treated with 110 and 220 mg/kg body weight (b.wt.) of the essential oil. All treatments were administered intraperitoneally, once a day for four days. The Alkaline phosphatase (ALP), alanine (ALT) and aspartate transaminases (AST) activities in the liver as well as kidney ALP and AST of the rats treated with the 110 and 220 mg/kg b. wt. of oil were significantly ($p<0.05$) lower, while the serum ALP and AST activities were higher compared to values in the non-treated rats. More so, the heart AST activities of rats treated with 220 mg/kg b. wt. of the oil were significantly ($p<0.05$) lower. However, serum protein, albumin, urea, total, conjugated bilirubin, ALT/AST and organ-body weight ratios were not significantly ($p>0.05$) altered, whereas serum creatinine concentrations were significantly ($p<0.05$) lower compared to those of non-treated control. Biochemical alterations observed in this study showed that the oil may not be completely safe when administered.

Keywords: Enzymes, essential oil, *Hoslundia opposita*, rats, toxicity

INTRODUCTION

In recent times, phytotherapy which dates back to antiquity has generated interest in the research niche as alternative to the conventional chemotherapies. The use of these herbs and their bioactive constituents either as neutraceuticals or for treatment of wide range of ailments is on the increase.

Essential oils are volatile constituents that give plants their characteristic aroma. These oils are natural, complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components (Edris, 2007). They are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, nutrition, spices and phytotherapy (Buchbauer, 2000). The role and mode of action of these natural products in prevention and treatment of cancer and cardiovascular diseases as well as their bioactivity as antimicrobial, antioxidants and antidiabetic agents have been investigated. Edris (2007) and Bakkali *et al*. (2008) detailed a review on their biological activities.

Hoslundia opposita Vahl. (Lamiaceae) is an herbaceous perennial shrub, wildly grown in Nigeria (Iwu, 1993); a common herb used in the treatment of diabetes by natives in Africa (Abbiw *et al*., 2002). Infusions of its leaves are widely used in traditional medicine as purgative, diuretic, febrifuge, antibiotic and antiseptic. Biological activities of the plant extracts such as antimalarial, anticonvulsant and antimicrobial have been established to confirm its use in folk (Anchebach *et al*., 1992; Gundiza *et al*., 1992; Ojo *et al*., 2010). Numerous bioactive compounds such as of alkaloids, tannin, flavonoids, cardiac glycoside and essential oils have been identified as principles responsible for its pharmacological actions (Olajide *et al*., 1999).

Table 1: Major chemical constituents of leaf essential oil of *Hoslundia opposita*

Compound	% Composition
1, 8-Cineole	72.3
Terpineol	7.2
Sabinene	4.5
Thymol	4.2
Car-3-ene	3.7
Terpine-4-ol	1.1
Cubebene	1.1

Usman *et al*. (2010)

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Previous documented studies on *H. opposita* Leaf Essential Oil (HOLEO) by our research team revealed that the oil is 1, 8-cineole chemotype (Table 1; Usman *et al*., 2010), its anti-dyslipidemic potentials (Akolade *et al*., 2011) and ameliorative effects on alloxan-induced anaemia (Muhammad *et al*., 2012). Therapeutic effects of aromatic plants have been attributed to their essential oil composition. However, scarcity of documented scientific studies on their safety, have raised toxicological fears. There is the need to assess the potential toxic effects of the plants and/or their phytoconstituents. Thus, the study was designed to evaluate the biochemical effect of essential oil extracted from the leaves of *Hoslundia opposita* (HOLEO) on selected toxicological indices in rats.

MATERIALS AND METHODS

Source of materials: The assay kits for creatinine, urea, calcium, albumin, bilirubin, alanine and aspartate transaminases were obtained from Randox Laboratories (Antrim, UK). ρ-Nitrophenyl phosphate was a product of Sigma-Aldrich (St. Louis, MO, USA). Dimethysulphoxide and all other reagents used were of analytical grade and were supplied by BDH Laboratories Ltd. (Poole, UK). Apparatus used include UV-Vis spectrophotometry (Lab-kits, China) and OHAUS analytical balance (Ohaus Corporation, NJ, USA).

Fresh leaves of *Hoslundia opposita* were obtained from the Parks and Gardens Unit of the University of Ilorin, Nigeria. Identification of the leaf was carried out at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, where a voucher specimen was deposited.

Albino rats (*Rattus norvegicus*) were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria.

Oil isolation, characterization and standardization: Pulverized leaves of *Hoslundia opposita* (500 g) was hydrodistilled for 3 h in a Clevenger-type apparatus according to the British Pharmacopoeia (1980). The resulting oils were characterized using Gas Chromatography and Mass Spectrometry (GC/MS; Table 1) and prepared in 5 and 10% v/v saline solution of dimethylsulphoxide (DMSO; Lahlou, 2004).

Animal grouping, management and treatment: Twenty-four albino rats (*Rattus norvegicus*) were maintained under standard laboratory conditions (12-h light/dark cycle, 25±2°C). Prior to experimentation, the rats were acclimatized to laboratory conditions for one week. They were then randomly selected into four groups:

- Rats treated with 110 mg/kg b. wt. of the essential oil
- Rats treated with 220 mg/kg b. wt. of essential oil
- Non-treated rats as control
- Rats treated with 220 mg/kg body weight (b. wt.) of the vehicle DMSO

All treatments were intraperitoneally (IP) administered, once a day, for four days. This study was carried out following approval from the Ethical Committee on the Use and Care of Laboratory Animals of the Department of Biochemistry, University of Ilorin, Nigeria.

Collection of blood for serum preparation: Blood was collected from the rats by simply incising the neck and evacuating the blood into sample bottles without anticoagulant for serum separation. Blood samples for serum were allowed to stand at room temperature for 30 min to form clot after which it was centrifuged at 1000 g for 10 min. After centrifugation, the supernatant which was the serum was obtained using a Pasteur pipette. The sera obtained were appropriately labeled and stored in the freezer, at 5°C and used for analysis within 24 h (Muhammad and Oloyede, 2010).

Preparation of tissue homogenates: The animals were quickly dissected; the tissues excised and immersed in ice cold 0.25 M sucrose solution (to maintain the integrity of the tissues). Homogenates were prepared for the liver, kidney and heart. This was done by cutting a known weight of the tissue finely with a clean scissors. The tissues were thereafter homogenized in ice-cold, 0.25 M sucrose solution (1:5 w/v) using pestle and mortar. Triton x-100 was added to a final concentration of 1% (Muhammad *et al*., 2006). All operations were carried out at between 0 and 4°C. The homogenates were stored in the freezer (each in a labeled specimen bottle) and used for analysis within 24 h (Muhammad and Oloyede, 2010).

Determination of enzyme activities in the tissues studied: Alkaline phosphatase (ALP) was assayed using the method described by Wright *et al*. (1972) and modified by Muhammad and Oloyede (2010), ALT and AST transaminases were determined following the method reported by Reitman and Frankel (1957) as modified by Schmidt and Schmidt (1963).

Determination of biochemical parameters: Protein concentration of the homogenates and serum were determined by the Biuret's reaction (Plummer, 1978), serum albumin concentration (Doumas *et al*., 1971), bilirubin (Tolman and Rej, 1999) and the levels of urea and creatinine by Tietz *et al*. (1994).

Statistical analysis: Data were expressed as the mean of six replicates±Standard Error of Mean (S.E.M). Statistical evaluation of data was performed by Graph

pad prism version 5.02 using one way Analysis of Variance (ANOVA), followed by Dunett's posthoc test for multiple comparism. Values were considered statistically significant at $p<0.05$ (confidence level = 95%).

RESULTS

Effect of leaf essential oil of *Hoslundia opposita* on selected serum enzyme activities:

Alkaline phosphatase (ALP): The alkaline phosphatase (ALP) activity in the serum and selected tissues of the rats treated with *Hoslundia opposita* Leaf Essential Oil (HOLEO) are shown in Table 2.

The ALP activity in the liver, kidney and heart of the rats treated with the 110 and 220 mg/kg b. wt of HOLEO were significantly low ($p<0.05$) compared to the control. Whereas, the activity of ALP in the serum of the rats treated with 110 mg/kg b. wt. HOLEO were significantly higher ($p<0.05$) than those treated with 220 mg/kg b. wt. HOLEO or the non-treated rats.

Aspartate transaminase (AST): Table 3 shows the aspartate transaminase (AST) activities in the serum and selected tissues of the diabetic and rats treated with HOLEO.

The AST activity was significantly lower ($p<0.05$) in the liver and kidney of the rats treated with 110 and 220 mg/kg b. wt. of HOLEO, while it was significantly higher ($p<0.05$) in the serum when compared with the non-treated control. Likewise, the heart AST activities of the rats treated with 110 mg/kg b. wt. of HOLEO were significantly lower ($p<0.05$), but those treated with 220 mg/kg b. wt. of HOLEO were significantly higher ($p<0.05$) when compared with those of non-treated rats.

Alanine transaminase (ALT): The alanine transaminase (ALT) activity in the serum and selected tissue homogenates of the rats treated with HOLEO are shown in Table 4.

The ALT activities was significantly lower ($p<0.05$) in the liver of the rats treated with 110 and 220 mg/kg b. wt. of HOLEO, while it was not significantly different ($p<0.05$) in the serum, kidney and heart when compared with the non-treated control.

Effect of leaf essential oil of *Hoslundia opposita* on selected serum metabolites: Table 5 show the effects of HOLEO on selected biochemical indices in serum samples of rats.

Table 2: Effect of intraperitoneal administration of leaf essential oil of *Hoslundia opposita* on alkaline phosphatase in serum and tissue homogenates (nm/min/mg protein) of rats

Treatment	Liver	Kidney	Heart	Serum
Control	7155±143.90^{c}	2159±60.88^{c}	722.0±29.16^{b}	2208±7.33^{ab}
DMSO	6463±59.06^{c}	1497±84.16^{ab}	734.8±14.97^{b}	2093±37.31^{a}
110 mg/kg	5535±189.70^{b}	1365±20.60^{a}	647.3±4.52^{a}	2598±8.46^{c}
220 mg/kg	4868±208.60^{a}	1581±23.64^{b}	733.8±16.18^{b}	2397±43.71^{b}

Values are expressed as mean of six replicates ± S.E.M; values with different superscripts along a column are statistically different ($p<0.05$)

Table 3: Effect of intraperitoneal administration of leaf essential oil of *Hoslundia opposita* on aspartate transaminase in serum and tissue homogenates (U/L) of and rats

Treatment	Liver	Kidney	Heart	Serum
Control	91.82±2.20^{c}	61.07±2.03^{b}	77.70±1.35^{b}	67.17±1.55^{ab}
DMSO	95.80±1.75^{c}	34.17±1.42^{a}	70.57±2.93^{ab}	71.57±2.16^{bc}
110 mg/kg	46.46±3.17^{a}	31.17±1.19^{a}	66.18±1.84^{a}	73.17±1.09^{c}
220 mg/kg	55.90±1.77^{b}	26.33±1.05^{a}	100.03±1.06^{c}	94.06±1.05^{d}

Values are expressed as mean of six replicates ± S.E.M; values with different superscripts along a column are statistically different ($p<0.05$)

Table 4: Effect of intraperitoneal administration of leaf essential oil of *Hoslundia opposita* on alanine transaminase in serum and tissue homogenates (U/L) of rats

Treatment	Liver	Kidney	Heart	Serum
Control	27.95±1.89^{b}	36.47±2.12^{ab}	74.53±2.80^{ab}	27.42±0.73^{a}
DMSO	20.97±0.39^{a}	35.97±1.37^{ab}	75.59±2.50^{ab}	27.53±1.67^{a}
110 mg/kg	17.80±0.68^{a}	34.11±1.22^{a}	70.98±0.31^{a}	27.94±0.62^{a}
220 mg/kg	21.28±0.20^{a}	40.64±3.03^{ab}	75.03±0.68^{ab}	29.06±1.56^{a}

Values are expressed as mean of six replicates ± S.E.M; values with different superscripts along a column are statistically different ($p<0.05$)

Table 5: Effect of intraperitoneal administration of leaf essential oil of *Hoslundia opposita* on selected biochemical indices in rat (U/L) of rats

Treatment	Protein*	Albumin*	Total Bilirubin^	Conjugated Bilirubin^	Urea^	Creatinine^
Control	46.37±0.59^{a}	3.42±0.10^{a}	0.82±0.01^{a}	0.24±0.01^{a}	34.50±1.43^{a}	1.86±0.04^{b}
DMSO	46.73±0.43^{a}	3.83±0.10^{a}	0.81±0.01^{a}	0.23±0.01^{a}	35.22±1.36^{a}	1.37±0.06^{a}
110 mg/kg	45.67±0.79^{a}	3.67±0.14^{a}	0.84±0.02^{a}	0.22±0.01^{a}	34.67±0.95^{a}	1.34±0.04^{a}
220 mg/kg	45.43±0.14^{a}	3.83±0.07^{a}	0.83±0.02^{a}	0.24±0.01^{a}	34.65±1.37^{a}	1.36±0.03^{a}

Values are expressed as mean of six replicates ± S.E.M; values with different superscripts along a column are statistically different ($p<0.05$); *Values are g/L; ^Values are mg/dL

Table 6: Effect of intra-peritoneal administration of leaf essential oil of *Hoslundia opposita* on body weight gain and organ-body weight ratio of rats

Treatment	Liver	Kidney	Heart	Weight gain
Control	0.0411±0.0010[a]	0.0073±0.0003[a]	0.0030±0.0001[a]	17.50±1.31[a]
DMSO	0.0367±0.0012[a]	0.0083±0.0006[a]	0.0035±0.0002[a]	18.33±4.21[a]
110 mg/kg	0.0411±0.0079[a]	0.0087±0.0008[a]	0.0030±0.0001[a]	14.17±1.74[a]
220 mg/kg	0.0372±0.0003[a]	0.0087±0.0005[a]	0.0035±0.0002[a]	10.50±2.01[a]

Values are expressed as mean of six replicates ± S.E.M; values with different superscripts along a column are statistically different ($p<0.05$)

In rats treated with 110 and 220 mg/kg b. wt. of HOLEO, serum protein, albumin, urea, total and conjugated bilirubin were not significantly ($p>0.05$) different, while serum creatinine concentrations were significantly ($p<0.05$) lower when compared to values in the non-treated control.

Effects of leaf essential oil of *Hoslundia opposita* on body weight gain and organ-body weight ratio: As shown in Table 6, the effect of intraperitoneal administration of HOLEO (110 and 220 mg/kg b. wt), on organ weight ratio did not produce any significant ($p>0.05$) change. Although gain in body weight were notice among all treated groups but the increase were not significantly different ($p>0.05$) from the control.

DISCUSSION

There are many enzymes such as phosphatases, dehydrogenases and transferases that are found in the serum which did not originate from the extracellular field. During tissue damage, some of these enzymes find their ways into the serum probably by leakage through disrupted cell membranes (Adeniyi *et al*., 2008). Thus, serum enzymes measurement provides a marker in toxicity studies as well as in clinical diagnosis.

Alkaline phosphatase is a marker ectoenzyme for the plasma membrane and often used to assess the integrity of the plasma membrane (Akanji *et al*., 1993). Of all the tissues studied, alkaline phosphatase activity was highest in the liver and least in the heart. This correlated with previous report by Muhammad and Oloyede (2010), which recorded high activities for tissues involved in active transport or part of the digestive system.

The increase in serum ALP and a concomitant decrease in liver, heart and kidney ALP activities, suggest leakage of the enzymes from the tissues, this implies possible cellular damage to the plasma membrane of such tissues. Liver and heart damages/infections are characterized by elevated ALP concentrations in serum (Singh *et al*., 2011) and these may be associated with inflammation and/or injury to hepatic cell in a condition referred to as apoptosis (Fiordaliso *et al*., 2000). Studies had revealed that, 1, 8-cineole the main constituent of the HOLEO used in this study (Usman *et al*., 2010); a monoterpenoid also referred to as eucalyptol found in high concentrations in the essential oil of eucalyptus (Akolade *et al*., 2012) stimulated induction of apoptosis such as fragmentation of DNA (Moteki *et al*., 2002).

Generally, enzymes such as ALT, AST and ALP are marker enzymes for function and integrity of organs such as liver. The enzymes found within organs and tissues are released into the blood stream following cellular necrosis and cell membrane permeability and are used as diagnostic measure of liver damage (Sanjiv, 2002). The levels of the ALT were not deleteriously altered in tissues of HOLEO treated groups except in the liver where ALT activities were significantly reduced in rats treated with HOLEO. This may be as a result of cellular inhibition of the enzyme activity or molecular inactivation of the enzyme in situ (Muhammad and Oloyede, 2010). Heart AST activities follow different distribution pattern; activities were higher in rats treated with 220 mg/kg b. wt. of HOLEO suggesting *de novo* synthesis of enzyme or response to xenobiotic assaults (Umezawa and Hooper, 1982).

Furthermore, serum ALT/AST has been used as an index to monitor liver pathology (Eteng *et al*., 1998; Akinloye and Olorede, 2000). Ratios higher than unity are indicative of adverse pathological effects on the liver (Edem and Usoh, 2009). Calculated ALT/AST ratios were far below unity in treated rats and values were not significantly different in non-treated rats.

The concentration of total protein, bilirubin and albumin may indicate state of the liver and type of damage (Yakubu *et al*., 2005). HOLEO induce no significant changes in liver functions indices (protein, albumin and bilirubin) assayed in serum of normal rats, suggesting that the secretory functions of the liver were not impaired.

Serum urea and creatinine, indicators of kidney functions are usually required to assess the normal functioning of different parts of the nephrons and could give an insight into the effect of a compound/plant extract on tubular or glomerular part of the kidney (Abolaji *et al*., 2007; Ashafa *et al*., 2008). Urea levels were not altered, while creatinine concentrations were lower in rats treated with the higher dose of HOLEO as well as with the vehicle DMSO. These effects are non-deleterious, suggesting that the normal functioning of the nephrons at the tubular and glomerular level may not be affected.

CONCLUSION

Toxicological indices from this study showed that the *H. opposita* leaf essential oil exhibited functional parameter-specific effects in a dose dependent modus and may not be completely safe at high dose when administered intraperitoneally.

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