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EVALUATION OF ANTIDIABETIC ACTIVITY OF AERIAL PARTS OF *THUJA OCCIDENTALIS*

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Abstract

The present research investigations were designed to develop the antidiabetic profile of aerial parts of *Thuja occidentalis* collected from Himachal Pradesh, Rajasthan and Tamil Nadu (Thuja; family – Cupressaceae) using in vivo alloxan monohydrate induced diabetic model in rats at the dose of 100 mg/kg, *p.o*. The hydroalcoholic extracts of *Thuja occidentalis* collected from different regions were prepared separately as per standard procedure described in literature. Amongst various extracts, hydroalcoholic extract of *Thuja orientalis* aerial parts collected from Tamil Nadu exhibited strong significant antidiabetic activity followed by plant collected from Rajasthan and Himachal Pradesh as compared to standard antidiabetic drug Glibenclamide (0.5 mg/kg, *i.p.*). Further, biochemical estimations in most bioactive hydroalcoholic extract showed decreased levels of serum glucose, HOMA-IR, total cholesterol, triglycerides, low density lipid cholesterol, very low density lipid, alanine amino transaminase, aspartate amino transaminase, lactate dehydrogenase, alkaline phosphatase, acid phosphatase, albumin, creatinine, urea and uric acid and increased levels of serum insulin, HOMA-β, high density lipid cholesterol, total protein and impairment in pancreatic β-cell functioning. General chemical screening of hydroalcoholic extracts showing presence of flavonoids and phenolic compounds. The exhaustive survey of literature suggested that flavonoids and phenolic compounds such as quercetin, rutin, luteolin and naringenin have been scientifically claimed as potential antidiabetic drugs. Thus, finally it can be concluded that antidiabetic activity of hydroalcoholic extract of *Thuja orientalis* aerial parts may be due to presence of flavonoids and phenolic compounds.

Key words : Alloxan monohydrate, Antidiabetic, Glibenclamide, *Thuja occidentalis*.

Introduction

Diabetes is the world's biggest endocrine ailment. As indicated by W.H.O. projection, the predominance of diabetes is probably going to increment by 35% (Boon *et al.,* 2006). Recent exhaustive survey of literature demonstrate there were 171 million diabetics worldwide in the year 2000 and this would increment to 366 million constantly 2030 (Patel *et al.,* 2009). The diet restriction and physical exercise uncontrolled diabetes is treated by oral antidiabetic drugs. These drugs are classified under six classes such as biguanides, sulfonylureas, meglitinides, thiazolidinediones, alpha-glucosidase inhibitors and dipeptidyl peptidase-IV inhibitors (Nathan, 2007; Katzung *et al.,* 2012). These medications are costly, produce undesirable reactions, and also they are not viewed as safe for use during pregnancy (Coetzee and Jackson, 1984; Gilbert *et al.,* 2006; Gutzin *et al.,* 2006). Therefore, it is important to search for new answer for deal with this medical issue. The utilization of herbal medications for the treatment of diabetes mellitus has picked up significance all through the world. The W.H.O. additionally prescribed and empowered this training particularly in nations where access to the traditional treatment of diabetes isn't adequate (Egede *et al.,* 2002). Hence, natural product researchers are exploring natural resources to find out more efficacious and safer drugs for the treatment of diabetes. *Thuja occidentalis* is one of such plants.

Thuja occidentalis (Thuja; family – Cupressaceae) is widely distributed throughout the Canada - Ontario, New Brunswick, Nova Scotia; USA - Minnesota, Virginia, New York, New Hampshire; United States - south of the Great Lakes, southern New England. The main habitat grown areas of *T. occidentalis* are wet forests, coniferous swamps, south manitou island and cliffs

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(Dhiman *et al.,* 2012). The plant has been traditionally used in the treatment of arthritis pain, rheumatism, skin fungal infections, cancer, insomnia, heart problems, headaches, diabetes, vertigo, emotional depression, restlessness, scalp itching, throat pain, stomach cramps, breathing problem, anxiety, asthma, colds, congestion, acne and dandruff (Kumar *et al.,* 2012). The phytoconstituents have been scientifically reported from the plant - essential oil (0.6%), water-soluble polysaccharides (4.9%), 2.07% reducing sugar, water-soluble minerals (2.11%), tannic agents (1.31%) and free acid (1.67%). The essential oils present in plant are the types of monoterpene i.e., thujone (65%), sabines (5%), á-pinen (2%), fenchone (8%), isothujone (8%), origanes, origanol, camphen, carvotanacetone, and myrcen (Kumar *et al.,* 2012). The pharmacological activities scientifically reported from this plant are antimicrobial (Olonisakin *et al.,* 2017), neuroprotective (Revathy *et al.,* 2016), antiulcer (Deb *et al.,* 2009), antioxidant (Dubey and Batra, 2009), hepatoprotective (Dash, 2014), antiviral (Elbeshehy *et al.,* 2015), anticancer (Biswas *et al.,* 2011), anti-HIV, antispasmodic, insecticidal, anti-atherosclerosis (Kumar *et al.,* 2012), molluscicidal, nematicidal, antipyretic, and anti-inflammatory activities (Jain and Sharma, 2017). A complete survey of literature suggested that the selected plant has not been systematically investigated for antidiabetic profile to justify its traditional claims. Therefore, the present investigations were designed to evaluate the antidiabetic profile of selected plant.

Materials and Methods

Collection of plant materials

The dried aerial parts of *Thuja occidentalis* were collected from wild regions of different states such as Himachal Pradesh, Rajasthan and Tamil Nadu. The identity of collected dried aerial parts of *Thuja occidentalis* were also confirmed from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi by Dr. Sunita Garg, Emeritus Scientist, CSIR-NISCAIR with reference no. – NISCAIR/RHMD/Consult/ 2018/3203-04-02 dated 27/ 04/ 2018).

Chemicals, reagents and solvents

The various chemicals, reagents and solvents (Laboratory and analytical grade) used in present research work were procured from authentic sources such as E Merck, Delhi, India and S.D. Fine Chemicals, Mumbai, India.

Preparation of various extracts

The hydroalcoholic extracts of *Thuja occidentalis* collected from different regions were prepared separately as per standard procedure described in literature (Richa *et al.,* 2017). Further, the hydroalcoholic extracts were secreened for presence of bioactive phytoocnstituents uisng gerenal chemical tests (Farnsworth, 1966).

Antidiabetic studies

Animals

Wistar Albino rats weighing (180-220 g) maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India). They were housed in the departmental animal house and were exposed to a regular 12 h cycle of light and dark. The experimental protocol was approved by the institutional animal ethical committee as per CPCSEA guidelines by Ministry of Environment and Forests, Government of India (Reg. No. 1181/PO/Ebi/08/ CPCSEA).

Drugs and chemicals

The mixture of distilled water and 2% of Tween 80 (0.5 ml/kg) was used as vehicle for preparing various test doses of hydroalcoholic extracts of aerial parts of *Thuja occidentalis*. Glibenclamide (0.5 mg/kg) was used as standard antidiabetic drug. Test samples and standard drug were administered orally to rats. The alloxan monohydrate (150 mg/kg, *i.p.*) was used to induction of diabtes in rats.

Experimental setup

Six groups of animals were made, and each group comprised six animals. The standard drug and test extracts were administered orally to rats once daily at 9:00 AM for 15 days to diabetic rats. The blood glucose concentration was determined in groups of rats treated with crude extracts on 0 day, 5th day, 10th day and 15th day.

Group 1 – Control group received vehicle (0.5 ml, *p.o.*); Group 2 – Standard group received Glibenclamide (0.5 mg/kg, *p.o.*); Groups 3 – Diabetic control received alloxan (150 mg/kg, *i.p.*); Groups 4 – Test groups received 100 mg/kg dose of hydroalcoholic extract *Thuja orientalis* collected from Himachal Pradesh (100mg/kg); Groups 5 – Test groups received 100 mg/kg dose of hydroalcoholic extract *Thuja orientalis* Rajasthan (100mg/kg) and Groups 6 – Test groups received 100 mg/kg dose of hydroalcoholic extract *Thuja orientalis* Tamil Nadu (100mg/kg).

Hypoglycemic study in normal rats

The fasting blood glucose level was monitored in blood sample collected from the ear vein, using the glucose oxidase method. The blood glucose level of the different groups was estimated 2 h after the

administration of the drug. The period of 2 h is based on the finding that the maximum hypoglycemic effect of glibenclamide was found around two hours of administration.

Hypoglycemic study in alloxan induced diabetic rat

Alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline and injected i.p. in 18 h previously fasted animal to induce diabetes. After one hour of alloxan administration, the animals were fed standard pellets and water at libitum. After 72 h, the blood glucose levels were estimated, applying the glucose oxidase method and rats having blood glucose level more than 150 mg/dl were selected for the study. Fasting blood glucose level before and 2 h after the administration of the drug were estimated (Rohilla and Ali, 2012). Blood glucose was estimated by autoanalyser using a commercial assay kit (ERBA diagnostics mannchim GmbH, Germany). The blood sample was centrifuged at 3000.

Biochemical estimations

Collection of blood

The most bioactive extract was further subjected to biochemical estimations. Rats were euthanized by diethyl ether. The blood tests for biochemical estimation were gathered through cardiovascular cut and put on ice for 2 hr. Blood tests were gathered in cylinder containing EDTA for plasma examination and without EDTA for serum investigation. Serum is acquired after centrifugation at 3000 rpm for 15 min and put away at 25°C. These blood samples were used to determination of plasma glucose, insulin HOMA-IR and HOMA-β (Wilson and Islam, 2012); lipids – triglycerides, complete cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol (Friedewald *et al.,* 1972); hepatic marker enzymes - AST (Aspartate amino transaminase), ALT (Alanine amino transaminase), ALP (Alkaline phosphatase), LDH (Lactate dehydrogenase) and ACP (Acid phosphatase) (Bergmeyer *et al.,* 1986); kidney function markers – creatinine, urea and uric acid (Toro and Ackerman, 1975); total protein and albumin (Gornall *et al.,* 1949) using well established standard procedures.

Statistical analyses

The information has been communicated as the mean ± S.D. The statistical analyses are done by utilizing Sigma details adaptation 3.5. The acquired outcomes are broke down by one way ANOVA followed by Student Newman Keul's test.

Results and Discussion

Antidiabetic activity

The hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh were investigated for *in vivo* antidiabetic activity at the dose of 100 mg/kg, *p.o.* in rats using alloxan monohydrate induced diabetic model. Glibenclamide was used as a standard drug.

Amongst various extracts, hydroalcoholic extract of *Thuja orientalis* aerial parts collected from Tamil Nadu exhibited strong significant antidiabetic activity followed by plant collected from Rajasthan and Himachal Pradesh as compared to glibenclamide (0.5 mg/kg, *i.p.*). The results are presented in (Table 1).

The results of preliminary phytochemical profiling of various hydroalcoholic extracts of *Thuja orientalis* aerial parts showed presence of phenolic and flavonoids as major classes of phytoconstituents. The exhaustive survey of literature suggested that flavonoids such as quercetin, rutin (Jadhav and Puchchakayala, 2012), luteolin (Josline *et al.,* 2013) and naringenin (Ortiz-Andrade *et al.,* 2008) have been scientifically claimed as potential antidiabetic drugs. Thus, finally it can be concluded that antidiabetic activity of hydroalcoholic extract of *Thuja orientalis* aerial parts may be due to presence of phenolic and flavonoids.

Biochemical estimations

The effect of hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild area of Tamil Nadu on serum glucose and insulin levels were estimated using alloxan monohydrate induced diabetes model on the last day of test. The homeostatic model assessment-insulin resistance (HOMA-IR) and HOMA-β (pancreatic β-cell function) scores were also calculated. The results of hydroalcoholic extract of *Thuja orientalis* aerial parts showed significant decreased level of serum glucose, HOMA-IR and increased level of serum insulin, HOMA-β and improvement in pancreatic β-cell functioning as compared to standard drug. The results are presented in table 2. The increased levels of serum glucose, HOMA-IR and decreased levels of serum insulin, HOMA-β and impairment in pancreatic β-cell functioning was observed in diabetic animals. The levels of above mentioned parameters are reversed via using antidiabetic drugs. Therefore, our findings are in accordance with the literature review (Chen *et al.,* 2015; Masiello *et al.,* 1998; Qu *et al.,* 2011; Tahara *et al.,* 2008; Turner *et al.,* 1979; Wallace *et al.,* 2004).

The effect of hydroalcoholic extract of *Thuja*

Table 1: Antidiabetic activity of hydroalcoholic extracts of *Thuja orientalis* aerial parts.

Groups	Dose (mg/kg)	Blood glucose concentration (mg/dL)			
		0 day	5th day	10th day	15th day
Control(0.5% Tween 80)	Vehicle (1ml)	80.25 ± 8.25*[a]	81.22 ± 6.25*[a]	81.75 ± 8.25*[a]	80.21 ± 8.25*
Alloxan	150	160.25 ± 16.33	178.23± 14.22[a]	191.25 ± 17.25[a]	205.25 ± 10.25[a]
Glibenclamide	0.5	156.22 ± 17.25	145.11± 11.25*	110.22 ± 11.00*	82.25 ± 8.25*
Thuja orientalis (Tamil Nadu)	100	170.25 ± 15.54	148.25±11.25*	116.25±15.25*	89.25±8.25*
Thuja orientalis (Rajasthan)	100	162.25 ± 16.24	152.25±15.25*	135.50 ±16.25*[a]	99.25±8.25*[a]
Thuja orientalis (Himachal Pradesh)	100	165.25 ± 16.54	156.25±12.58*[a]	145.25±12.25*[a]	105.25±13.25*[a]

The observations are presented in the form of Mean ± S.D. *$P<0.05$ vs Alloxan control; [a]$P<0.05$ vs. Glibenclamide (Standard drug); one-way ANOVA followed by Student-Newman-Keul's test.

Table 2: Effect of bioactive hydroalcoholic extracts of *Thuja orientalis* aerial parts on serum glucose, insulin, HOMA-IR and HOMA-β in alloxan induced diabetic test in rats.

Groups	Dose (mg/kg)	Glucose(mg/dL)	Insulin(μIU/mL)	HOMA-IR	HOMA-β
Control(0.5% Tween 80)	Vehicle (1ml)	80.21 ± 8.25*	20.25±0.12*	4.25±0.10*	56.25±2.25*
Alloxan	150	205.25 ± 10.25[a]	13.11±0.30[a]	6.58±0.12[a]	20.25±0.25[a]
Glibenclamide	0.5	82.25 ± 8.25*	18.25±0.65*	4.10±0.25*	54.25±1.99*
Thuja orientalis (Tamil Nadu)	100	89.25±8.25*	19.80±0.75*	3.99±0.11*	55.25±1.85*

The observations are presented in the form of Mean ± S.D. *$P<0.05$ vs Alloxan control; [a]$P<0.05$ vs. Glibenclamide (Standard drug); one-way ANOVA followed by Student-Newman-Keul's test.

Table 3: Effect of bioactive hydroalcoholic extracts of *Thuja orientalis* aerial parts on serum lipid levels in alloxan induced diabetic test in rats.

Groups	Dose(mg/kg)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	VLDL (mg/dL)
Control(0.5% Tween 80)	Vehicle (1ml)	60.25±1.55*	70.25±1.11*	60.25±1.25*	15.25±0.58*	16.01±0.12*
Alloxan	150	110.25±1.60[a]	180.25±2.25[a]	30.25±1.01[a]	50.25±0.55[a]	46.25±0.25[a]
Glibenclamide	0.5	63.25±1.88*	75.25±1.25*	66.25±1.11*	17.25±0.99*	18.24±0.30*
Thuja orientalis (Tamil Nadu)	100	66.24±1.65*	80.25±1.34*	68.25±1.58*	15.55±0.66*	18.65±0.48*

The observations are presented in the form of Mean ± S.D. *$P<0.05$ vs Alloxan control; [a]$P<0.05$ vs. Glibenclamide (Standard drug); one-way ANOVA followed by Student-Newman-Keul's test.

orientalis aerial parts obtained from wild area of Tamil Nadu on serum lipid levels were estimated using alloxan monohydrate induced diabetes model on the last day of test. The results of hydroalcoholic extract of *Thuja orientalis* aerial parts showed significant decreased levels of total cholesterol, triglycerides, LDL-cholesterol, VLDL and increased level of HDL-cholesterol as compared to standard drug. The results are presented in table 3. The higher levels of total cholesterol, triglycerides, LDL-cholesterol, VLDL and lower level of HDL-cholesterol are observed in diabetic animals. On the other side, antidiabetic drugs reverse the levels of parameters involved in lipid profile. Therefore, our results are in accordance with literature (Daniel, 2011; Garvey *et al.,* 2003; Hermayer, 2004; Krauss, 2004).

The effect of hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild area of Tamil Nadu on level of hepatic marker enzymes levels were estimated using alloxan monohydrate induced diabetes model on the last day of test. The results of hydroalcoholic extract of *Thuja orientalis* aerial parts showed significant decreased levels of ALT, AST, LDH, ALP, ACP, albumin and increased level of total protein (TP) as compared to standard drug. The results are presented in table 4. The levels of ALT, AST, LDH, ALP, ACP, albumin are increased and TP are decreased in diabetic animals. The antidiabetic drugs act via decreased levels of ALT, AST, LDH, ALP, ACP, albumin and increased level of TP. Therefore, our results are in agreement with literature review (Harris, 2005).

The effect of hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild area of Tamil Nadu on level of kidney function markers levels were estimated using alloxan monohydrate induced diabetes model on the last day of test. The results of hydroalcoholic extract of *Thuja orientalis* aerial parts showed significant mild decreased levels of creatinine, urea and uric acid as compared to standard drug. The results are

Table 4: Effect of bioactive hydroalcoholic extracts of *Thuja orientalis* aerial parts on hepatic marker enzymes levels in alloxan induced diabetic test in rats.

Groups	Dose(mg/kg)	ALT(U/L)	AST(U/L)	LDH(U/L)	ALP(U/L)	ACP(U/L)	TP(g/dL)	Albumin(g/dL)
Control(0.5% Tween 80) Vehicle (1ml)	25.56±0.25*	50.25±1.87*	1454.11±3.25*	26.25±0.11*	8.25±0.11*	6.25±0.01*	3.25±0.01*	
Alloxan	150	70.25±0.85[a]	130.25±1.47[a]	3011.25±2.48[a]	80.45±023[a]	22.24±0.12[a]	4.55±0.11[a]	5.45±0.05[a]
Glibenclamide	0.5	27.25±0.40*	52.25±1.65*	1324.45±3.47*	29.44±0.45*	10.24±0.16*	7.25±0.15*	3.45±0.07*
Thuja orientalis (Tamil Nadu)	100	35.25±0.69*	56.60±1.62*	1445.99±2.69*	27.88±0.78*	9.87±0.54*	6.89±0.05*	3.98±0.12*

The observations are presented in the form of Mean ± S.D. **P<0.05* vs Alloxan control; [a]*P<0.05* vs. Glibenclamide (Standard drug); one-way ANOVA followed by Student-Newman-Keul's test.

Table 5: Effect of bioactive hydroalcoholic extracts of *Thuja orientalis* aerial parts on kidney function markers levels in alloxan induced diabetic test in rats.

Groups	Dose (mg/kg)	Creatinine(mg/dL)	Urea(mg/dL)	Uric acid(mg/dL)
Control(0.5% Tween 80)	Vehicle (1ml)	0.52 ± 0.05*	40.12±0.55*	3.58±0.29*
Alloxan	150	1.25±0.15[a]	61.25±0.60[a]	6.25±0.31[a]
Glibenclamide	0.5	0.60±0.07*	42.25±0.45*	3.85±0.56*
Thuja orientalis (Tamil Nadu)	100	0.60±0.07*	48.54±0.90*	3.26±0.67*

The observations are presented in the form of Mean ± S.D. **P<0.05* vs Alloxan control; [a]*P<0.05* vs. Glibenclamide (Standard drug); one-way ANOVA followed by Student-Newman-Keul's test.

presented in (Table 5). The levels of creatinine, urea and uric acid are increased in diabetic animals. The antidiabetic drugs decline the higher levels of levels of creatinine, urea and uric acid. Therefore, our results of kidney function marker level are in agreement with literature (Gross *et al.,* 2005).

Conclusion

The hydroalcoholic extracts of *Thuja orientalis* aerial parts collected from different regions exhibited significant antidiabetic activity in comparison to glibenclamide (0.5 mg/kg, *i.p.*). The results of preliminary phytochemical screening showing presence of flavonoids and phenolic compounds as major classes of phytoconstituents. Thus, finally it can be concluded that antidiabetic activity of hydroalcoholic extract of *Thuja orientalis* aerial parts may be due to presence of flavonoids and phenolic compounds. Further, the bioactive extract will be subjected to column chromatography to isolate potent antidiabetic constituents.

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