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EVALUATION OF ANTIDIABETIC ACTIVITY OF *HETEROPHRAGMA QUADRILOCULARE* (ROXB.) K. SCHUM. LEAVES

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Heterophragma quadriloculare (Roxb.) K. Schum, Anti-diabetic, Oral Glucose Tolerance Test, Streptozotocin Nicotinamide induced type 2 Diabetes

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ABSTRACT: *Heterophragma quadriloculare* (Roxb.) K. Schum. (HQ) belongs to Bignoneace family and known as Warras. Usage of HQ plant in diabetes has been claimed traditionally but scientific documentation is not yet available. So it was our interest to develop scientific database for HQ leaves. HQ leaves were studied for its traditional claim of antidiabetic activity by oral glucose tolerance test (OGTT) model using normal rats. All possible phytoconstituents were extracted into selected range of solvents based on its polarity *i.e.* petroleum ether, chloroform, methanol, water. The extracts were screened for OGTT at dose level of 200 and 400 mg/kg per oral. In OGTT study though all extracts found to reduce blood glucose level, petroleum ether extract and chloroform extract showed dose dependent activity. These results provided the clue for presence of anti-diabetic constituents in petroleum ether extract. Thus petroleum ether extract (200 mg/kg), unsaponifiable fraction of petroleum ether extract (100 mg/kg) and saponifiable fraction of petroleum ether extract (100 mg/kg) of HQ were studied in rats by STZ-NAD induced type-II diabetic model up to 21 to find out most potent anti-diabetic fraction. Blood glucose and lipid profile were recorded on 0^{th} and 21^{st} day. Oral administration of PEHQ and UPEHQ resulted in significant weight gain, reduction of blood glucose, serum cholesterol, serum VLDL and triglycerides in diabetic rats as compared to diabetic control rats. Unsaponifiable fraction of petroleum ether extract at the dose of 100 mg/kg showed significant antidiabetic activity. The present study thus justifies the traditional claim of *Heterophragma quadriloculare* (Roxb.) K. Schum. for diabetes. Unsaponifiable fraction of petroleum ether extract of HQ needs further attention for separation and identification of biologically active compounds that are unidentified yet.

INTRODUCTION: *Heterophragma quadriloculare* (Roxb.) K. Schum. (HQ) belongs to Bignoneace family and known as Warras [1].

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In India it is found in different regions of Madhya Pradesh, Maharashtra, Tamil Nadu, Karnataka, Gujarat and Andhra Pradesh [2 - 4]. This plant is utilized as anti-diabetic, antifungal, antiseptic and in skin disease like toe sores and in chilblain [5]. Utility of HQ plant material has been claimed traditionally by many authors but scientific documentation is not yet available. So it was our thought of interest to develop scientific database for HQ leaves.

With this objective antidiabetic activity of HQ leaves has been evaluated by *in vivo* screening of different extracts of leaves for oral glucose tolerance test in normal rats followed by *in vivo* screening of different extracts of leaves for anti-diabetic activity in streptozotocin (STZ) - nicotinamide (NAD) induced type-II diabetic rat.

MATERIALS AND METHODS: Petroleum ether extract (PEHQ), chloroform extract (CHQ), aqueous extract (AqHQ) and methanolic extract (MeHQ) obtained by successive solvent extraction of HQ leaves have been screened for oral glucose tolerance test (OGTT) and Petroleum ether extract (PEHQ), Unsaponifiable fraction of petroleum ether extract (UPEHQ) and Saponifiable fraction of petroleum ether extract (SPEHQ) of HQ leaves have been screened by streptozotocin-nicotinamide induced type-II diabetic rat model. Whenever required, 0.5 % Tween 80 in normal saline solution was used as a vehicle.

Chemicals: Streptozotocin, Nicotinamide and Metformin hydrochloride was obtained from HiMedia laboratories (Mumbai, India). Kit for biochemical estimations were procured from Span Diagnostics Ltd., India and Reckon Diagnostics Pvt. Ltd., India. Other experimental chemicals used were of analytical grade and purchased from Loba Chemie Pvt. Ltd. (Mumbai, India).

Plant Material Collection, Processing and Preparation of Extracts: The fresh leaves of wildly growing HQ was collected from the outskirts of village Mota Randha, Dadara and Nagar Haveli, during April-May 2010 and authenticated Dr. J K Vanparia, Botanist, VNSGU, Surat. A voucher specimen of sample (No. Pharmacy / HDT/HQ/09-10/01/BS) has been deposited in Herbal Drug Technology Laboratory, Pharmacy Department, The M. S. University of Baroda, Gujarat, India for database. Collected leaves were shed dried for two week, powdered in mechanical grinder and stored in air tight container. HQ leaves (2000 g) was extracted with petroleum ether followed by chloroform and methanol using soxhlet apparatus. Remaining plant material was extracted with water by decoction. Each extract was dried (45 °C) in a rotary evaporator (Heidolph, Germany). The dried extracts were stored in vacuum desiccator until further use. PEHQ (20 g) was saponified by refluxing it with 10% alcoholic KOH for 2 hrs. After saponification saponifiable fraction (SPEHQ) was separated and unsaponifiable matter (UPEHQ) was extracted in Diethylether (3 x 100 ml). Unsaponifiable matter was collected as orange solid mass with sticky nature.

Experimental Animals: Healthy female Wistar albino rats weighing 200 - 250 gm, procured from Zydus Cadilla Research Laboratory, Ahmedabad maintained under standard husbandry conditions (Temperature 23 ± 2 °C, relative humidity 55 ± 10% and 12 hrs light dark cycle). The animals were fed with standard rat pellet diet and had free access to water. The experimental protocols were approved by the Institutional Animal Ethics Committee, The M. S. University of Baroda, Vadodara, Gujarat (CPCSEA Reg. No. 404/PO/Re /S/01/CPCSEA). All studies were conducted as per the National Institute of Health's Guide for the Care and Use of Laboratory Animal.

***In vivo* Screening for Oral Glucose Tolerance Test in Normal Rats:**

Experimental Design: The oral glucose tolerance test for selected extracts was performed on overnight (18 hr) fasted normal rats. In this experiment 60 normal rats were used. They were separated in to ten groups of 6 rats each. Extracts were dissolved in vehicle.

- **Group I (P1):** Rats were given PEHQ (200 mg/kg b.w.) orally.
- **Group II (P2):** Rats were given PEHQ (400 mg/kg b.w.) orally.
- **Group III (C1):** Rats were given CHQ (200 mg/kg b.w.) orally.
- **Group IV (C2):** Rats were given CHQ (400 mg/kg b.w.) orally.
- **Group V (M1):** Rats were given MeHQ (200 mg/kg b.w.) orally.
- **Group VI (M2):** Rats were given MeHQ (400 mg/kg b.w.) orally.
- **Group VII (A1):** Rats were given AqHQ (200 mg/kg b.w.) orally.
- **Group VIII (A2):** Rats were given AqHQ (400 mg/kg b.w.) orally.
- **Group IX (control):** Rats were given Vehicle (quantity sufficient) orally.

- **Group X (metformin):** Rats were given metformin HCl (15 mg/kg b.w.) orally.

Glucose (3 g/kg) was fed orally in solution form to each group 30 min after the administration of extracts and metformin HCl [6].

Biochemical Analysis: Blood was withdrawn using haematocrit capillary from the retro orbital plexus under ether inhalation anesthesia at - 30, 0, 30, 60, and 120 minutes of glucose administration. Collected blood was allowed to coagulate and centrifuged at 5,000 RPM for 15 minutes. Glucose level in serum was estimated by glucose oxidase-peroxidase method using reagent kit.

Statistical Analysis: All results were reported as mean ± SEM. The variation in a set of data has been estimated by performing Bonnferroni repeated measures one-way ANOVA using non-parametric methods in Graph pad prism.

***In vivo* Screening for Anti-Diabetic Activity on STZ - NAD Induced Type-II Diabetic Rat:**

Experimental Induction of Diabetes in Rats: Diabetes was induced by a single intraperitoneal injection of 230 mg/kg nicotinamide prepared in normal saline followed by freshly prepared streptozotocin (65 mg/kg) in 0.1 M citrated buffer (pH = 4.5) to overnight starved rats. Diabetic rats were provided with 20% glucose solution to drink overnight to prevent the initial drug induced hypoglycaemic death. Blood glucose was estimated after 7 days and animals with glucose level >180 ± 8 mg/dl were only selected for the study [6 - 9].

Experimental Design: In this experiment 30 diabetic rats were used. They were separated in to five groups of 6 rats each. Extracts and fractions were dissolved in vehicle for administration.

- **Group I (Diabetic Control):** Rats were given Vehicle (quantity sufficient) orally for 21 days.
- **Group II (STD):** Rats were given metformin HCl (15 mg/kg b.w.) orally for 21 days.
- **Group III (PEHQ):** Rats were given PEHQ (200 mg/kg b.w.) orally for 21 days.
- **Group IV (UPEHQ):** Rats were given UPEHQ (100 mg/kg b.w.) orally for 21 days.
- **Group V (SPEHQ):** Rats were given SPEHQ (100 mg/kg b.w.) orally for 21 days.

Blood was withdrawn using haematocrit capillary from the retro orbital plexus under ether inhalation anesthesia. Fasting blood glucose, total cholesterol, VLDL, triglyceride and body weight were recorded on 0^{th} and 21^{st} day.

Biochemical Analysis: Glucose level in serum was determined by glucose oxidase / peroxidase method using reagent kit from Span Diagnostic [10]. Total cholesterol, serum VLDL and triglyceride were determined by standard methods using regent kit from Reckon Diagnostics [11].

Statistical Analysis: All results were reported as mean ± SEM. The variation in a set of data has been estimated by performing Bonnferroni repeated measures one-way ANOVA using non-parametric methods in Graph pad prism.

RESULTS AND DISCUSSION:

***In vivo* Screening for Oral Glucose Tolerance Test in Normal Rats:** Antidiabetic activity of aerial part of the plant *Heterophragma quadriloculare* (Roxb.) K. Schum. (HQ) is traditionally reported by Soumyanath Amla [12]. While, phytochemical study showed presence of carbohydrates in leaves. So there was a question that despite presence of carbohydrates in leaves how aerial part could be used as an anti-diabetic agent? But this ambiguity was little cleared by results of OGTT study (**Fig. 1**).

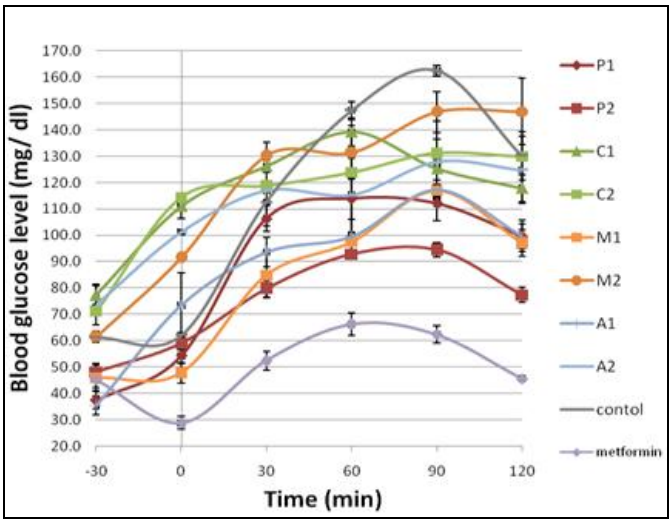

FIG. 1: BLOOD GLUCOSE LEVEL OBTAINED IN OGTT STUDY. P1- PEHQ (200 mg/kg), P2- PEHQ (400 mg/kg), C1- CHQ (200 mg/kg), C2- CHQ (400 mg/kg), M1- MEHQ (200 mg/kg), M2- MEHQ (400 mg/kg), A1- AQHQ (200 mg/kg), A2- AQHQ (400 mg/kg)

Though all extracts found to reduce blood glucose level, PEHQ and CHQ showed dose dependent activity. We have found that MeHQ and AqHQ did not reduced blood glucose level significantly as reduced by PEHQ and CHQ; this might be due to presence of high amount of carbohydrates in leaves and carbohydrates come out with aqueous and alcoholic extracts.

This experiment showed probability of presence of some anti-diabetic constituents in PEHQ thus PEHQ, UPEHQ and SPEHQ have been selected for chronic study to find most potent anti-diabetic fraction.

***In vivo* Screening for Anti-Diabetic Activity on STZ - NAD Induced Type-II Diabetic Rat:** Chronic anti-diabetic study was carried out using STZ-NAD induced diabetic rats. Duration of study was 21 days. Change in body weight, blood glucose, serum cholesterol, serum VLDL and serum triglyceride observed at 0 and 21st day of study is explained in this section.

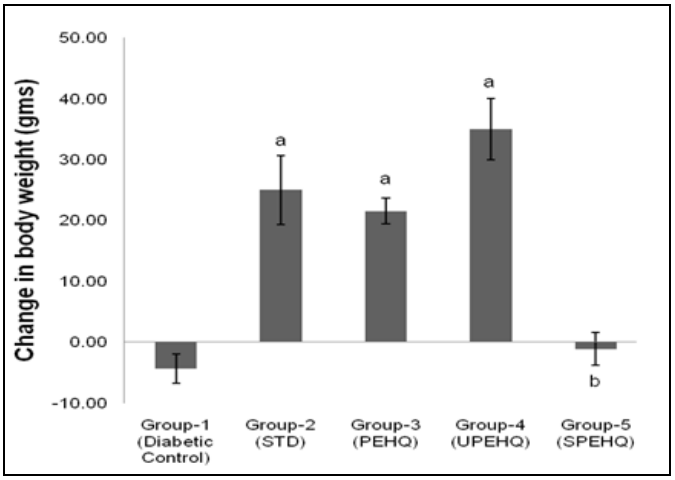

FIG. 2: CHANGE IN BODY WEIGHT OF RATS OBSERVED AT 0th AND 21st DAY OF TREATMENT. AP < 0.0001 AS COMPARED TO DIABETIC CONTROL RAT. BP > 0.05 AS COMPARED TO DIABETIC CONTROL RATS. STD - METFORMIN HCl (15 mg/kg), PEHQ- PEHQ (200 mg/kg), UPEHQ- UPEHQ (100 mg/kg), SPEHQ- SPEHQ (100 mg/kg)

Loss in body weight was observed in STZ induced diabetic rats. STZ partly destroys the beta cells which decrease insulin secretion and produce type-II diabetes [13]. Reduced body weight in diabetic rats affirms a corruption of basic proteins because of diabetes [14]. The deficit in body weight noticed in STZ induced diabetic control rats may be due to muscle squandering [15-16]. Oral administration of PEHQ and UPEHQ resulted in significant weight gain in diabetic rats while SPEHQ prevented further loss in body weight of diabetic rats as compared to diabetic control (**Fig. 2**). Improvement in body weight by PEHQ and UPEHQ was comparable to the standard drug metformine HCl.

In diabetes glucose metabolism is impaired and resulted in increased blood glucose level. Similarly increased blood glucose was observed in STZ induced diabetic rats. Oral administration of PEHQ and UPEHQ significantly reduced blood glucose as compared to diabetic control and was observed to comparable with standard drug metformin HCl. SPEHQ was not able to reduce blood glucose suggest that antidiabetic phytochemicals are not present in this fraction (**Fig. 3**).

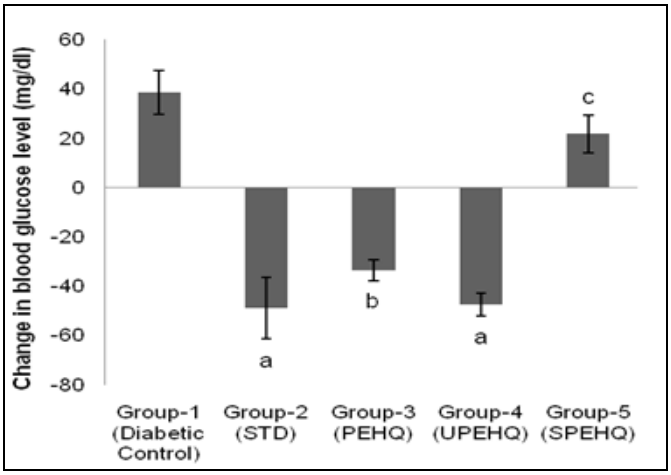

FIG. 3: CHANGE IN BLOOG GLUCOSE LEVEL OF RATS OBSERVED AT 0th AND 21st DAY OF TREATMENT. AP < 0.0001 AS COMPARED TO DIABETIC CONTROL RAT. BP < 0.05 AS COMPARED TO DIABETIC CONTROL RATS. CP > 0.05 AS COMPARED TO DIABETIC CONTROL RATS. STD-METFORMIN HCl (15 mg/kg), PEHQ- PEHQ (200 mg/kg), UPEHQ - UPEHQ (100 mg/kg), SPEHQ- SPEHQ (100 mg/kg)

It is hypothesized that antidiabetic activity may be due to presence of lupeol, ursolic acid, β-sitosterol and some other unidentified compounds [17-20]. The diabetic rats had found to have significantly higher chylomicron, VLDL, cholesterol, and triglyceride [21]. In diabetes mellitus metabolic derangements resulting from the absolute lack of insulin or from resistance to the actions of insulin can affect VLDL and triglyceride metabolism. It has been reported that the outcome of this interplay between diabetes and VLDL metabolism is the common occurrence of elevated plasma VLDL and triglyceride

concentrations in individuals with both Type 1 and Type 2 diabetes mellitus [22]. In this study, serum cholesterol level was reduced by PEHQ and UPEHQ where as SPEHQ was unable to even hinder increment of serum cholesterol level which was raised due to diabetic condition (**Fig. 4**).

Similarly reduction in VLDL and in serum triglyceride level was observed by PEHQ and UPEHQ as compared to diabetic control. SPEHQ administration leads to increment in VLDL level as well serum triglyceride level (**Fig. 5** and **6**).

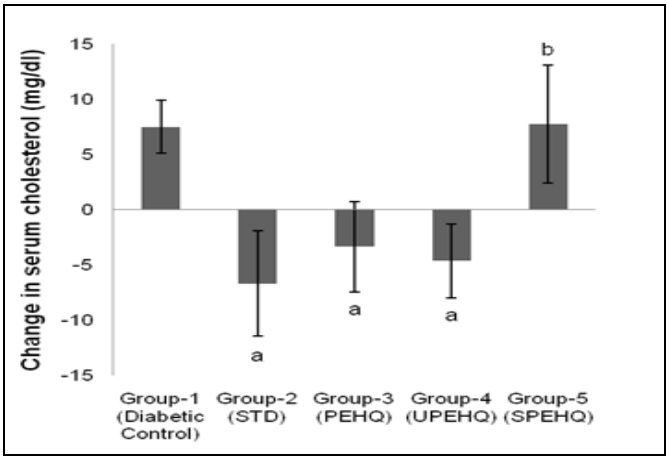

FIG. 4: CHANGE IN SERUM CHOLESTEROL LEVEL OF RATS OBSERVED AT 0th AND 21st DAY OF TREATMENT. AP < 0.05 AS COMPARED TO DIABETIC CONTROL RAT. BP > 0.05 AS COMPARED TO DIABETIC CONTROL RATS. STD- METFORMIN HCl (15 mg/kg), PEHQ- PEHQ (200 mg/kg), UPEHQ- UPEHQ (100 mg/kg), SPEHQ- SPEHQ (100 mg/kg)

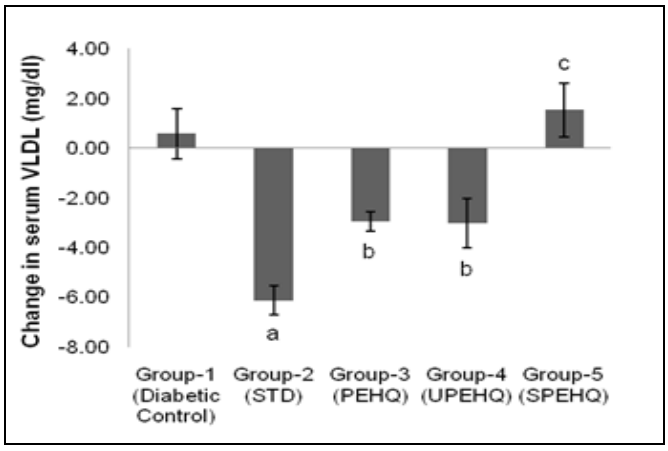

FIG. 5: CHANGE IN SERUM VLDL LEVEL OF RATS OBSERVED AT 0th AND 21st DAY OF TREATMENT. AP < 0.0001 AS COMPARED TO DIABETIC CONTROL RAT. BP < 0.05 AS COMPARED TO DIABETIC CONTROL RATS. CP > 0.10 AS COMPARED TO DIABETIC CONTROL RATS. STD - METFORMIN HCl (15 mg/kg), PEHQ- PEHQ (200 mg/kg), UPEHQ- UPEHQ (100 mg/kg), SPEHQ- SPEHQ (100 mg/kg)

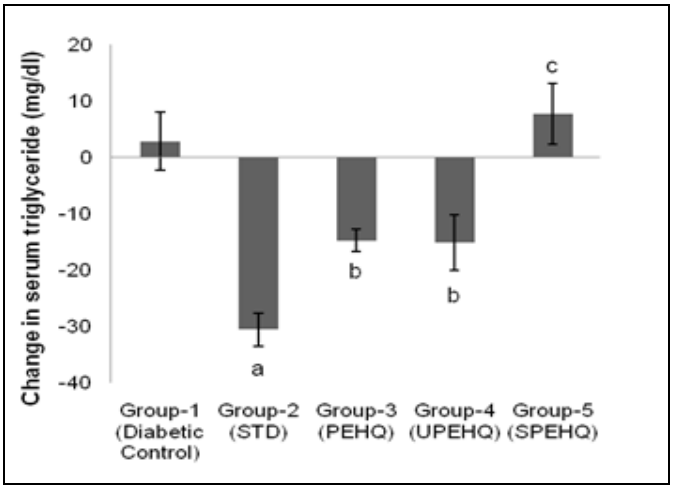

FIG. 6: CHANGE IN SERUM TRIGLYCERIDE LEVEL OF RATS OBSERVED AT 0th AND 21st DAY OF TREATMENT. AP < 0.0001 AS COMPARED TO DIABETIC CONTROL RAT. BP < 0.05 AS COMPARED TO DIABETIC CONTROL RATS. CP > 0.10 AS COMPARED TO DIABETIC CONTROL RATS. STD- METFORMIN HCl (15 mg/kg), PEHQ- PEHQ (200 mg/kg), UPEHQ- UPEHQ (100 mg/kg), SPEHQ- SPEHQ (100 mg/kg)

CONCLUSION: The result of OGTT study showed highly active fraction was petroleum ether extract followed by chloroform. Activity of both these extract was observed to be in dose dependant manner. While activity shown by methanol and water extract was inversely proportional to the dose. It was due to presence of carbohydrates in plants. The petroleum ether extract (PEHQ), unsaponifiable fraction of petroleum ether extract (UPEHQ) and saponifiable fraction of petroleum ether extract (SPEHQ) of HQ were studied *In vivo* for anti-diabetic activity in rats by STZ-NAD induced type-II diabetic model up to 21 days. Results of the study demonstrated that unsaponifiable fraction of petroleum ether extract (UPEHQ) at the dose of 100 mg/kg possesses antidiabetic activity. The present study thus justifies the traditional claim of *Heterophragma quadriloculare* (Roxb.) K. Schum. for treatment of diabetes and points out that it requires future detail investigation. Unsaponifiable fraction of petroleum ether extract of HQ also needs further attention for separation and identification of biologically active compounds that are unidentified yet.

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CONFLICTS OF INTEREST: Nil.

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