CHAPTER 2

Oreocnide integrifolia (Gaud.) Miq leaf water extract improves metabolic alterations in high fructose fed insulin resistant and hypertensive rats.

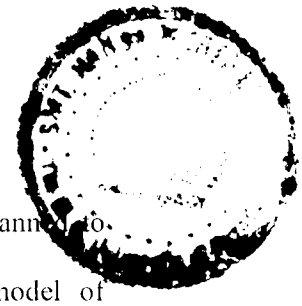
Introduction:

Metabolic syndrome is fast emerging as an epidemic of the new millennium fraught with serious consequences to human health worldwide. Also referred to as "Diabesity" (Astrup and Finer, 2000) it is a combination of diabetic complications co-expressed with obesity resulting from changes in human behavior, life style and food habits. Metabolic syndrome (formerly known as syndrome X or insulin resistant syndrome), represents a cluster of cardiovascular risk factors like, obesity, insulin resistance, dyslipidemia and hypertension. Westernized diets and obesity associated with eating patterns are the main causes for increasing incidence of insulin resistance. Recent evidences tend to identify consumption of carbohydrates more so of refined sugars with high fructose content, as an important culprit in development of metabolic syndrome.

Cardiovascular disease (CVD) is the ultimate cause of mortality in people with metabolic syndrome (Lakka *et al.,* 2002) and one of the associated manifestations leading to CVD could be dyslipidemia. This dyslipidemia is marked by high level of plasma triglyceride (TG) and low level of high density lipoprotein

(HDL-C) (Kohen-Avramoglu *et al.,* 2003). Though dyslipidemia has been tackled very effectively by lipid lowering and anti-diabetic drugs (Krauss and Siri, 2004), there is need for a most composite agent for treating metabolic syndrome as, it is characterized by many etiologically linked or even exclusively or mutually independent manifestations like dyslipidemia, hyperglycemia, hyperinsulinemia, insulin resistance and hypertension. Traditionally, phytotherapy seems to be the best possible alternate medicine for treating many metabolic and vascular disorders as, natural products from herbs/medicinal plants serve as essential arsenal in human endeavor to tackle many diseases, as practiced by tribal's and forest dwellers as folklore medicine. North eastern states of India support a rich biodiversity (biodiversity hotspots) spanning from tropical rainforests to alpine scrubs with geographical and climatic diversity and, holds approximately 50 percent of total flora of India. Plants have found a prime place in the indigenous system of medicine and are in focus for evaluation of their active ingredients. *Oreocnide integrifolia* (*Gaud.*) Miq (OI) (Utricaceae) locally called "U-Khajing" is known as a folk-medicinal plant that has been used as a remedy for Diabetes mellitus in North-eastern parts of India. The infusion prepared from the leaves of this plant is used as a decoction to alleviate diabetic symptoms and hypertension (Kharkongor and Joseph, 1981).

Fructose induced hypertensive rats is a diet induced model, considered equivalent to human metabolic syndrome as marked by the expression of all the manifestations such as dyslipidemia, hyperglycemia, insulin resistance, hyperinsulinemia and hypertension (Reaven *et al.,* 1989; Verma *et al.,* 1994; Verma *et al.,* 1995; Bhanot and McNeill, 1996); and hence, a suitable model for evaluating



the efficacy of preventive/ ameliorative agents. The present study was planned to evaluate the possible amelioration of OI extract in an experimental model of metabolic syndrome.

Material and Methods

Experimental animals

Male *Charles foster* rats (180 to 200 g) were housed and maintained in clean polypropylene cages and fed with either control diet or high fructose diet and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and, approved by the animal ethical committee of the Department of Zoology, The M.S University of Baroda, Vadodara (Approval No.827/ac/04/CPCSEA).

Plant extract preparation: As given in Chapter 1.

Experimental groups

1. Control rats (CON):- Fed with normal diet (Standard laboratory chow) and 0.5% CMC (p.o.) for six weeks

2. Fructose-fed rats (FRU):- Fed with fructose rich diet (60% fructose, Table 1) and 0.5% CMC (p.o.) for six weeks

3. Fructose-fed + OI treated rats (FRU+ OI 250):- Fed with fructose rich diet (60% fructose) and 250mg kg BW (p.o.) OI extract for six weeks.

4. Fructose-fed + OI treated rats (FRU+ OI 500):- Fed with fructose rich diet (60% fructose) and 400mg kg BW (p.o.) OI extract for six weeks.

5. Fructose-fed + Metformin treated rats (FRU+MET):- Fed with fructose rich diet (60% fructose) and Metformin (50mg/kg BW) intra-peritoneally for six weeks.

At the end of the experimental period, overnight fasted animals (for 12 h) were given mild ether anesthesia and blood was collected by micro-heparinised capillary tube from retro orbital sinus in EDTA coated vials and plasma separated.

Biochemical analysis:-

Plasma Glucose (as given in Chapter 1).

Plasma Insulin (as given in Chapter 1).

Fasting insulin resistance index (FIRI) was calculated as: Fasting plasma glucose x Fasting plasma insulin ÷ 25 (Duncan et al., 1995).

Glucose Tolerance Test (GTT) & Insulin Response Test (IRT): Method of Shrwaikar et al. was used for the OGTT. On the days of the test, a subset (n=6) of animals was fasted for 12 h followed by an intraperitoneal administration of glucose (3g/kg).Blood glucose levels were determined in blood samples from the tail vein at 0 (prior to glucose administration), 30, 60, 90 and 120 min after glucose administration. For Insulin response test, another subset (n=6) of animals was injected intraperitoneally 0.2 IU of Insulin (Human Insulin, Lantus, Aventis Pharma GmBH, Germany) and blood glucose was determined at different time points as described

above. The results were expressed as integrated area under the curve (AUC) for glucose, that was calculated by the trapezoid rule [AUC = C1+ C2/2X (t_1- t_2)] and changes in glucose concentrations during OGTT were expressed as $AUC_{glucose}$ (mg/dl per min).

Plasma Lipid Profile (as given in Chapter 1).

Measurement of blood pressure and vascular reactivity:

Another subset of rats was used for measurement of blood pressure and vascular reactivity in control and experimental groups as described by Balaraman *et al.* (2007). After completion of treatment schedule, rats from each group were anesthetized using 1.2 g/kg (i.p.) of urethane. Tracheotomy was performed to facilitate breathing. The left common carotid artery and left femoral vein were cannulated with polyethylene tubing filled with heparinised saline (500 IU/ml) to prevent clotting. The hemodynamic parameters like systolic, diastolic and mean arterial blood pressures (SBP, DBP and MABP respectively) were measured in the left common carotid artery using pre-calibrated pressure transducer SS13L and Biopac MP-30 data acquisition system (BIOPAC Systems, Inc. CA, USA). After 30 min of equilibration, vascular reactivity to intravenous (i.v.) injection (via femoral vein) of adrenaline (1µg/kg), phenylephrine (1µg/kg), isoprenaline (0.1mmol/kg), and acetylcholine (0.1mmol/kg) were recorded. Rats received a maintenance i.v. infusion of 0.9% sodium chloride (1ml/h) throughout the experimental duration. All the data were analyzed using Biopac Student Lab Pro software (Version 3.6.7).

Statistical analysis : Statistical evaluation of the data was done by one way ANOVA followed by Bonferroni's multiple comparison test .The results are expressed as mean ± S.E.M using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California USA.

Results:

Metabolic and glycemic status (Table 2, 3; Fig. 1, 2, 3) : Table 2 shows the mean body weight changes observed in control and experimental groups at baseline (Week 1) till the end of study period (Week 6). Weight gain, food consumption and fluid intake were found to be similar ($p>0.05$) in all the groups. Fructose fed rats developed hyperglycemia (+37.5%) and hyperinsulinemia (+51.7%) at the end of 6 week period (Table 3, Fig. 1, 2). OI supplemented group (Fru + OI 250 and Fru + OI 500) exhibited significant decrease in plasma glucose (159.25 ± 4.65 Vs 132.98 ± 2.47 and 159.25 ± 4.65 Vs 115.54 ± 3.77) and insulin titers (456.36 ± 19.99 Vs 325.65 ± 12.36 and 456.36 ± 19.99 Vs 240.36 ± 20.32), which were comparable to metformin treated rats. Analysis of fasting insulin resistance index (Table 3, Fig. 3) indicated that, Fru + OI 500 could significantly prevent insulin resistance and a three fold decrease was observed, which was comparable with metformin treated rats.

Glucose tolerance and insulin response tests (Fig. 4, 5, 6): Analysis of glucose tolerance pattern and AUC of glycemia during 120 min test period in control and experimental animals revealed that, fructose fed rats developed glucose intolerance (Fig. 4, Fig. 5).The rate of glucose disposal was more or less stable during 90-120 min period in fructose fed rats and the AUC of glucose during OGTT rose to 40% as compared to control rats. OI extract (Fru + OI 250 and Fru + OI 500) supplemented rats showed lower glucose elevation and faster glucose disposal rates thereby displaying significant improvement ($P<0.001$) in glucose tolerance pattern. During insulin response tests, fructose fed rats showed constant decrease in plasma glucose

levels throughout the 120 min period (Fig. 6) while, OI extract (Fru + OI 250 and Fru + OI 500) groups displayed improved recovery rates attributable to improved insulin sensitivity.

Lipid profile (Table 4; Fig. 7-12): The plasma levels of cholesterol (Fig. 7), triglycerides (Fig. 8), LDL-C (Fig. 10), VLDL-C (Fig. 11) and FFA (Fig. 12) increased significantly ($p<0.001$) while, HDL levels (Fig. 9), decreased significantly (($p<0.001$) in fructose fed rats (Table 4). OI extract (Fru + OI 250, Fru + OI 500) supplementation decreased significantly ($p<0.001$) cholesterol (23.4%, 29.3%), triglycerides (45.8%, 55.5%), LDL-C (42.5%, 51.4%), VLDL-C (46.1%, 55.2%) and FFA (35%, 40.7%) while increased HDL-C levels (37.5%, 40.82%).

Blood pressure and vascular reactivity (Fig. 13-17): Fructose treated rats displayed increase in mean arterial blood pressure at the end of 6 week period (Fig. 13) OI extract (Fru + OI 250 and Fru + OI 500) supplemented rats showed significant decrease in arterial blood pressure (162.0 ± 3.86 Vs 142.3 ± 2.7 and 162.0 ± 3.86 Vs 135.4 ± 2.8) and was comparable to metformin treated rats. However, changes in heart beats were similar ($p>0.05$) in all groups (Figure 4). Presser responses to phenylephrine (Fig. 14) and adrenaline (Fig. 15) increased significantly ($p<0.001$) at end of 6 week period and decreased significantly ($p<0.001$) when challenged with isoprenaline (Fig. 17) or acetylcholine in fructose fed rats (Fig. 16). Fru + OI 500 treated group showed significant decrease ($p<0.001$) in vasoconstrictor responses when challenged with phenylephrine and adrenaline and surprisingly, they were

found to be even better than metformin group. Also, Fru + OI 250 and Fru + OI 500 groups displayed significant ($p<0.001$) increase in vascular responses to acetylcholine and isoprenaline.

Table 1. Composition of diet (g/100gm)

Ingredients	Control diet	High fructose diet
Corn Starch	**60.0**	**-----**
Fructose	**-----**	**60.0**
Casein	**20.0**	**20.0**
Methionine	**0.7**	**0.7**
Groundnut Oil	**5.0**	**5.0**
Wheat bran	**10.6**	**10.6**
Salt Mixture*	**3.5**	**3.5**
Vitamin mixture**	**0.2**	**0.2**

* The composition of mineral mix (g/kg) – $MgSO_4$ $7H_2O$, 30.5; NaCl, 65.2; KCl, 105.7; KH_2PO_4, 200.2; $MgCO_3$, 3.65; $Mg(OH)_2$ $3H_2O$, 38.8; Fe $C_6H_5O_7$ $5H_2O$, 40.0; $CaCO_3$, 512.4; KI, 0.8; NaF, 0.9; $CuSO_4$ 5H2O, 1.4; $MnSO_4$, 0.4 and $CONH_3$, 0.05.

** One kilogram of vitamin mix contained thiamine mono nitrate, 3 g; riboflavin, 3g;pyridoxine HCl, 3.5 g; nicotinamide, 15 g; d-calcium pantothenate, 8 g; folic acid, 1 g; d-biotin, 0.1 g; cyanocobalamin, 5 mg; vitamin A acetate, 0.6 g; a-tocopherol acetate, 25 g and choline chloride, 10 g.

Table 2. Changes in body weight and, food and fluid intake.

	Con	Fru	Fru + OI 250	Fru+ OI 500	Fru + Met
Body weight Initial (gm)	245.4±6.4	251.8±6.0	247.8±8.4	251.4±6.4	244.8±6.10
Body weight Final (gm)	275.4±4.5	279.8±8.4	280.4±6.4	283.9±8.10	278.4±6.10
Weight gain (gm)	30.0±1.64	28.0±1.72	32.6±1.46	32.5±1.81	33.6±1.38
Food Intake (gm/day/animal)	22.4±2.4	21.5±2.1	19.37±1.5	22.4±2.5	20.4±1.8
Fluid Intake (ml/day/animal)	28.5±2.10	35.6±3.20	33.24±2.40	32.80±1.8	30.42±2.40

All values are expressed as SEM of 6 animals in each group.

Table 3. Changes in glucose, insulin and FIRI levels.

	Plasma glucose mg/dl	Plasma insulin pmol/L	FIRI
Con	100.59 ± 3.28	220.36 ± 23.32	866.96 ± 56.36
Fru	159.25 ± 4.65$^{\blacklozenge}$	456.36 ± 19.99$^{\blacklozenge}$	2854.25 ± 69.99$^{\blacklozenge}$
Fru + OI 250	132.98 ± 2.47$^{\blacklozenge\Omega}$	325.65 ± 12.36$^{\bullet\,\delta}$	1632.00 ± 70.23$^{\blacklozenge\,\Omega}$
Fru + OI 500	115.54 ± 3.77$^{\Omega}$	240.36 ± 20.32 $^{\Omega}$	1109 ± 55.56 $^{\Omega}$
Fru + Met	121.11 ± 3.72$^{\blacksquare\,\Omega}$	225.36 ± 23.33 $^{\Omega}$	1084.97 ±59.69 $^{\Omega}$

All values are expressed as SEM of 6 animals in each group.

$p>0.05$ = non-significant, $p<0.05$=●/δ, $p<0.01$=■/β, $p<0.001$= ♦/Ω

●, ■, ♦ = Other experimental groups were compared with Control

δ, β, Ω = Other experimental groups were compared with Fructose

Table 4. Changes in lipid profile levels

Lipid Profile	Con	Fru	Fru + OI 250	Fru + OI 500	Fru + Met
Cholesterol	42.6±2.3	68.3±3.8 $^{\blacklozenge}$	52.3±1.76 $^{\Omega}$	48.3±1.8 $^{\Omega}$	38.6±2.8 $^{\blacklozenge\Omega}$
Triglycerides	53.6±3.61	142.3±5.1 $^{\blacklozenge}$	76.61±3.2 $^{\blacksquare\Omega}$	63.81±3.1 $^{\Omega}$	73.8±3.2 $^{\Omega}$
HDL-C	23.87±0.71	11.64±0.68 $^{\blacklozenge}$	18.64±0.81 $^{\blacklozenge\Omega}$	19.67±0.92 $^{\blacksquare\Omega}$	18.54±0.51 $^{\blacksquare\Omega}$
LDL-C	29.5±0.48	85.12±2.17 $^{\blacklozenge}$	48.98±1.27 $^{\blacklozenge\Omega}$	41.39±1.48 $^{\blacklozenge\Omega}$	34.76±1.81 $^{\blacklozenge\Omega}$
VLDL-C	10.77±0.62	28.46±0.88 $^{\blacklozenge}$	15.32±0.38 $^{\blacksquare\Omega}$	12.76±0.48 $^{\Omega}$	14.7±1.08 $^{\Omega}$
Free fatty acid	27.62±1.87	68.31±2.8 $^{\blacklozenge}$	44.38±2.1 $^{\blacklozenge\Omega}$	40.62±2.8 $^{\blacklozenge\Omega}$	36.7±2.4 $^{\Omega}$

All values are expressed as SEM of 6 animals in each group.

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TC = total cholesterol, HDL = high density lipoprotein, VLDL = very low density lipoprotein, LDL = low density lipoprotein, TG = triglycerides

Fig. 1. Changes in Plasma Glucose level in control and experimental animals

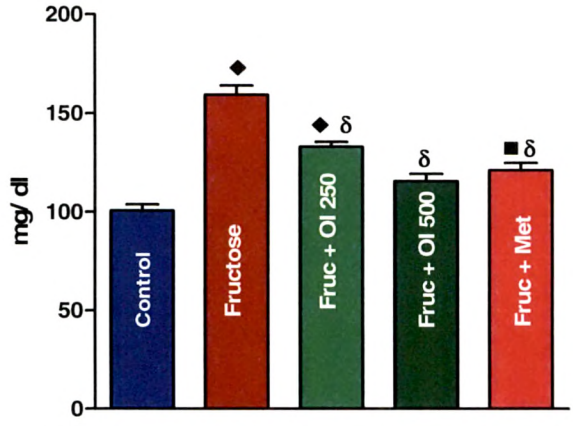

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Fig. 2. Changes in Plasma Insulin level in control and experimental animals

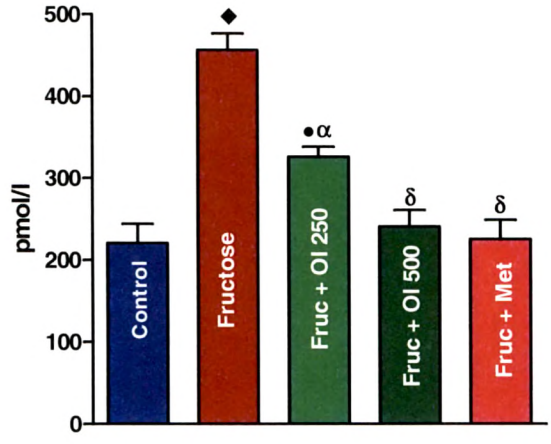

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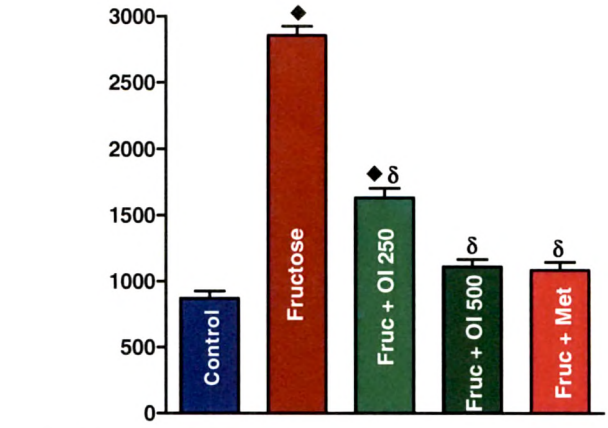
Fig. 3. FIRI values in control and experimental animals



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α = p <0.05, β = p <0.01, δ = p <0.001: OI treated experimental groups were compared with Fructose

Figure 4. Changes in glucose tolerance pattern in control and experimental groups

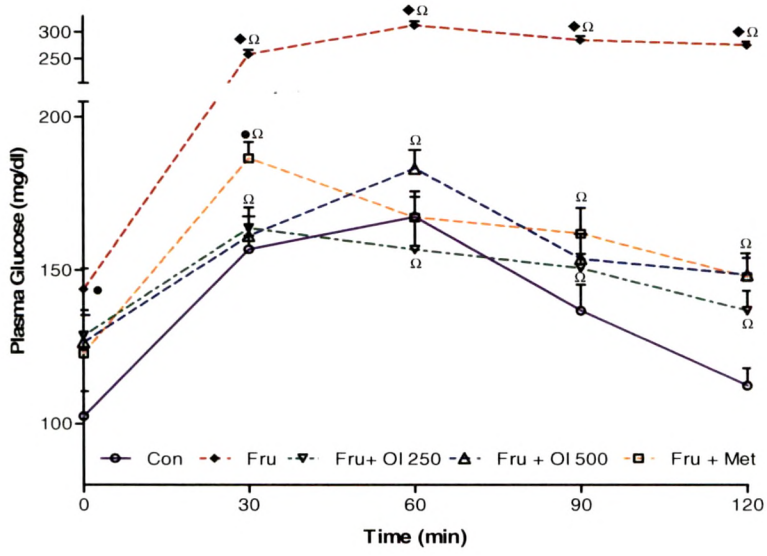

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Figure 5. Changes in $AUC_{glucose}$ pattern of control and experimental animals

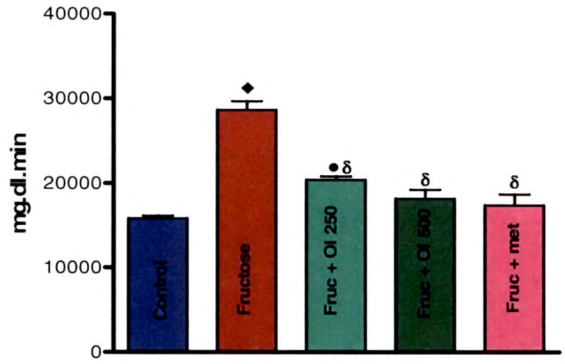

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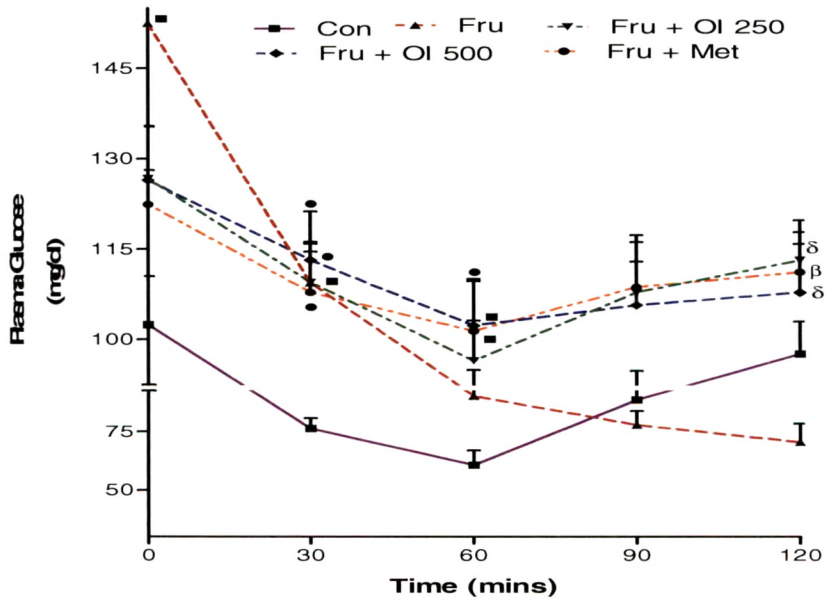
Figure 6. Changes in glucose levels control and experimental animals when challenged with insulin



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δ= , p<0.05, β= p<0.01, Ω = p<0.001: OI treated experimental groups compared to Fructose

Fig. 7. Plasma Cholesterol level in control and experimental animals

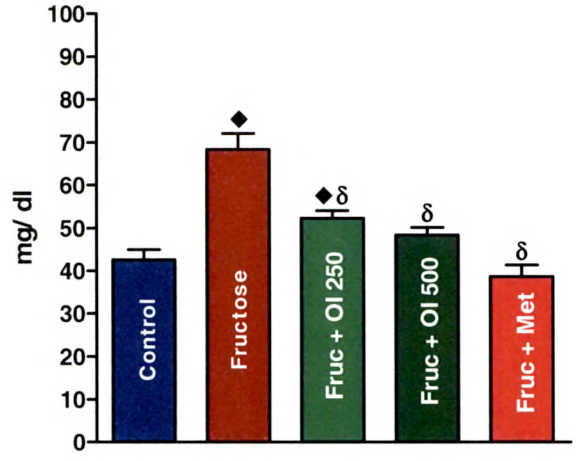

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Fig. 8. Plasma Triglyceride level in control and experimental animals

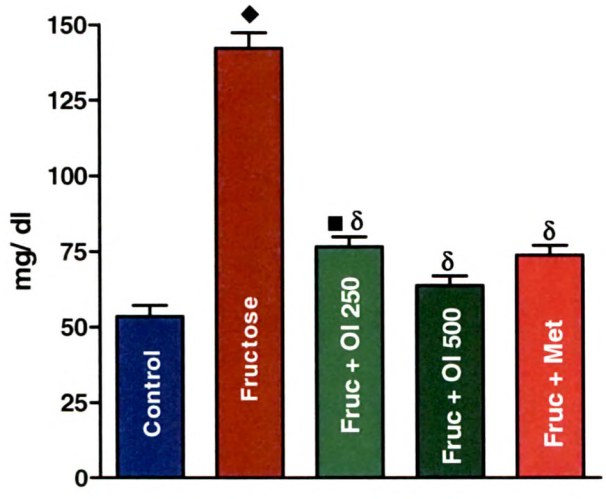

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Fig. 9. Plasma HDL-C level in control and experimental animals

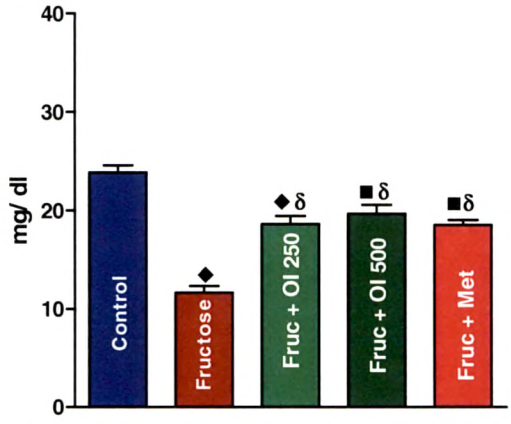

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Fig. 10. Plasma LDL-C level in control and experimental animals

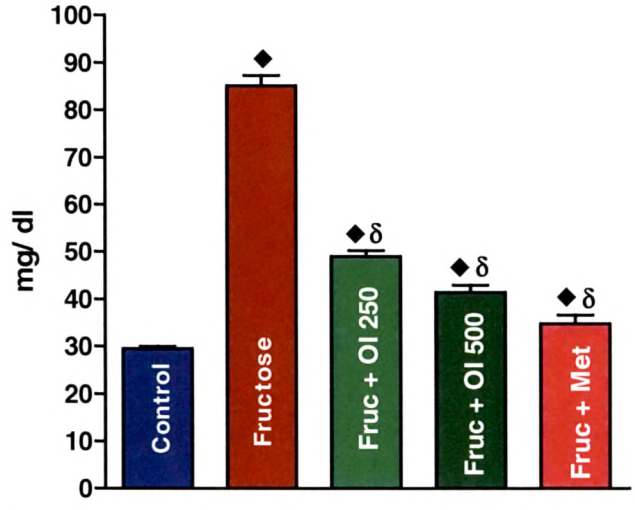

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Fig. 11. Plasma VLDL-C level in control and experimental animals

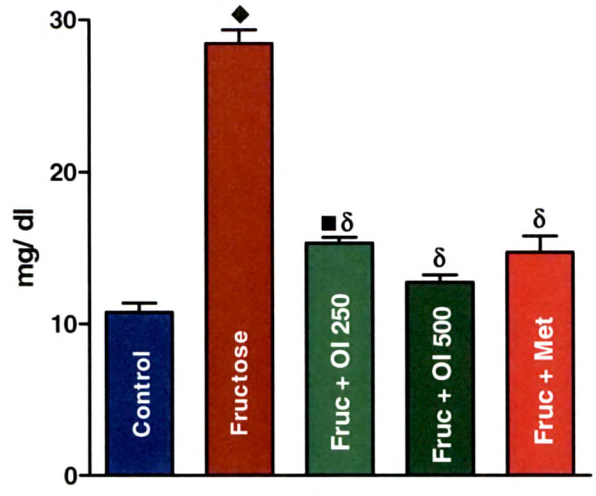

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Fig.12. Plasma Free Fatty Acid level in control and experimental animals

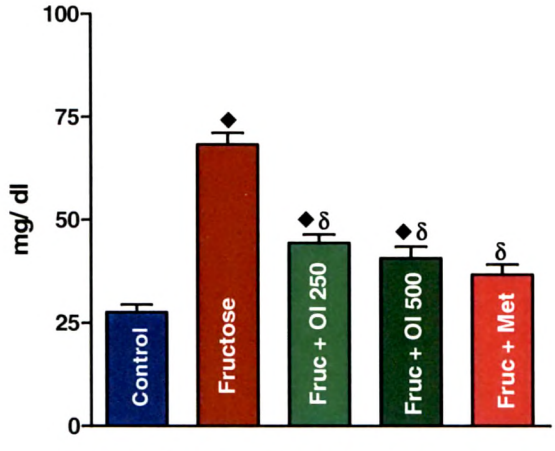

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Fig. 13. Changes in Mean Arterial Blood Pressure in control and experimental animals

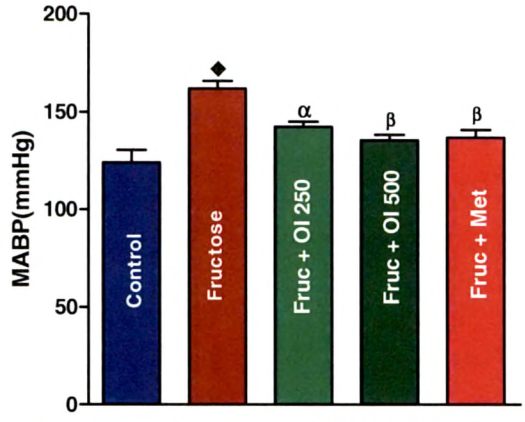

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Fig. 14. Changes in Vascular Response with Phenylepinephrine in control and experimental animals

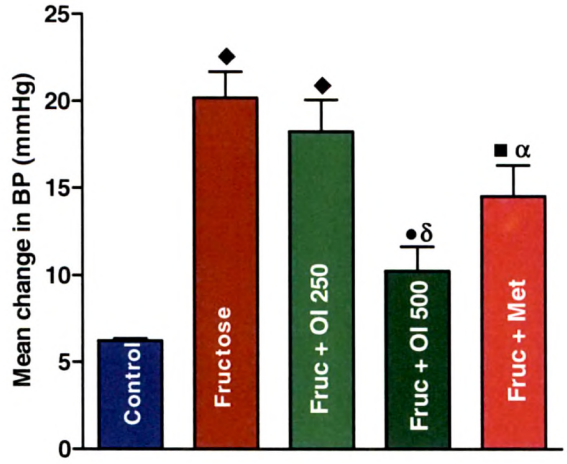

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Fig. 15. Changes in Vascular Response with Adrenaline in control and experimental animals

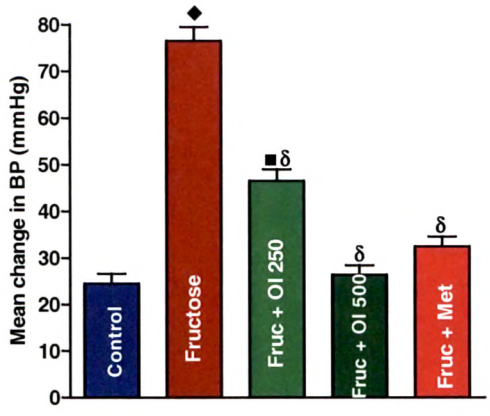

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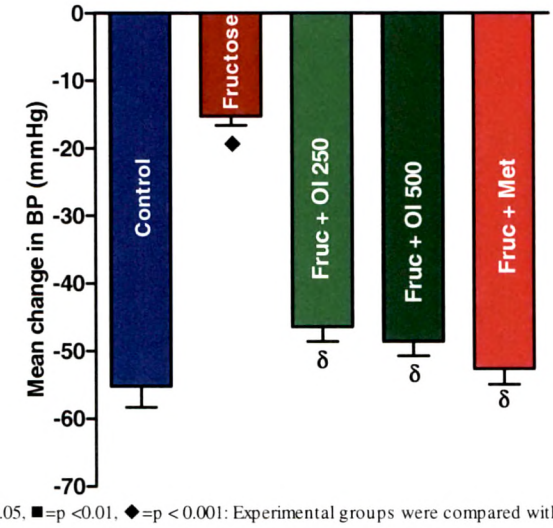
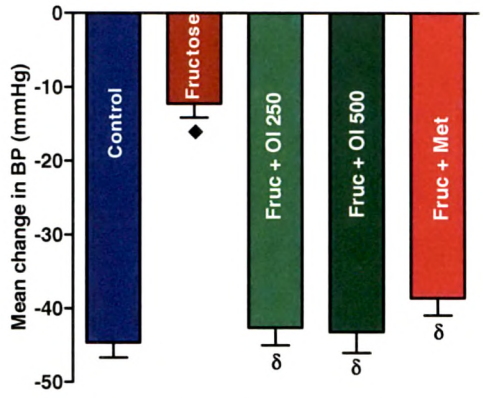
Fig. 16. Changes in Vascular Response with Acetylcholine in control and experimental animals



•= p < 0.05, ■=p <0.01, ◆=p < 0.001: Experimental groups were compared with Control

α = p <0.05, β = p <0.01, δ = p <0.001: OI treated experimental groups were compared with Fructose

Fig. 17. Changes in Vascular Response with isoprenaline in control and experimental animals



• = p < 0.05, ■=p <0.01, ◆=p < 0.001: Experimental groups were compared with Control

α = p <0.05, β = p <0.01, δ = p <0.001: OI treated experimental groups were compared with Fructose

Discussion:

Development of metabolic symptoms such as dyslipidemia, hyperglycemia, hyperinsulinemia and insulin resistance (Reaven *et al.,* 1989; Zavaroni *et al.,* 1980; Hwang *et al.,* 1987; Erlich and Rosenthal, 1996) and hypertension (Bunnag *et al.,* 1997; Heise *et al.,* 1998; Fang and Huang, 1998; Dimo *et al.,* 2001a, b) have been adequately demonstrated in high fructose diet fed rats. The metabolic lesions leading to essential hypertension suggesting a possible link between the two has also been reported (Madar *et al.*, 1997; Rosen *et al.,* 1997). In the present study, the efficacy of aqueous extract of OI leaves has been assessed in terms of above manifestations in high fructose fed *Charles foster* rats. The results in general tend to show a potent efficacy of OI 500 mg to completely prevent all manifestations to a degree equivalent to or even better than that of metformin. The observations made on fructose fed rats essentially confirm the significant expression of various manifestations like hyperglycemia, hyperinsulinemia, insulin resistance, glucose intolerance, dyslipidemia and hypertension.

Both free fatty acid and triglyceride contents, are significantly high in the plasma of fructose fed rats. High plasma free fatty acid level is known to contribute to insulin resistance by inhibiting insulin signaling and also by suppressing pancreatic insulin secretion (Arner, 2003; Bays *et al.,* 2004). Elevation in plasma free fatty acid level in obesity and type 2 diabetes has been accredited to non-esterification in adipose tissue and the consequent escape from adipose tissue to plasma (Riemens *et al.*, 2000). The high plasma triglyceride level can be related with increased hepatic synthesis using the freely available free fatty acid and the resultant

hypertriglycerdemia in turn can contribute to insulin resistance (Steiner, 1994; Grundy, 1999).

OI extract (OI 500) was able to prevent the doubling plasma free fatty acid level and the near four time increase in triglyceride level induced by fructose diet and, the effect was in fact even better than that of metformin. Concurrently, OI 500 was also able to prevent completely the fructose induced hypercholesterolemia by maintaining near normal levels of HDL, LDL and VLDL. These effects on total cholesterol and various fractions of cholesterol were in fact even better than the effects of Metformin.

Overall, OI extract had potent ability to prevent hyperlipidemia characteristic of fructose rich diet. It is suggested that, high fructose diet induced hypertriglycerdemia could be due to increased hepatic secretion of VLDL triglycerides by decreased removal of triglyceride rich lipoprotein from circulation (Zavaroni *et al.,* 1980; Lee *et al.,* 1994). Apparently, OI extract is able to prevent these metabolic alterations and consequently the manifestations of hypertriglycerdemia in fructose induced hypertensive rats. Taken together with the control of plasma cholesterol and total lipid levels, OI extract seems to be a very potent antihyperlipedemic agent. Similar hypotriglycerdemic effects have been shown by other investigators with green tea extract administered to fructose fed hypotriglycerdemic rats and hamsters (Yang *et al.,* 2001; Wu *et al.,* 2004; Li *et al.,* 2005).

Apart from the observed dyslipidemia, fructose rich diet also contributes to hyperglycemia, insulin resistance and hyperinsulinemia. The reports on the effects of dietary fructose on plasma glucose level are highly variable with no change (Hwang *et al.*, 1987; Dimo *et al.*, 2002; Navarro-Cid *et al.*, 1996; Sambandam *et al.*, 1997); transient elevation (Riemens *et al.*, 2000) to a significant elevation (Srividhya and Anuradha, 2002; Rosen *et al.*, 1997; Balakrishnan and Anuradha, 1997; Anuradha and Balakrishnan, 1999; Anurag and Anuradha, 1999; AnithaNandhini *et al.*, 2005). Hyperglycemia seen in association with hyperinsulinemia is suggestive of impaired insulin action in fructose fed rats. Enzymological and glucose uptake studies tend to show a gluconeogenic state in the liver and poor insulin stimulated glucose oxidation in liver, muscle and adipose tissue in fructose fed rats (AnithaNandhini *et al.*, 2005). It is therefore inferable that, the observed hyperglycemia in the present study could be a consequence of fructose diet induced metabolic alterations leading to gluconeogenesis and poor glucose oxidation. The variations observed with reference to development of hyperglycemia or maintenance of normoglycemia in fructose fed rats may be related with essentially a strain dependent response. Whereas, the *Wistar* strain seems to show the greatest variation, the *Charles foster* strain used in our studies seems to show consistent hyperglycemia on fructose feeding. Irrespective of glycemic status, all studies are unequivocal in reporting insulin resistance as in the present study. The strain difference is also marked by increased glucose clearance from blood seen in the present study in fructose fed rats upon exogenous insulin challenge. Apparently, six weeks of fructose feeding though induces hyperglycemia and hyperinsulinemia, does not seem to develop marked insulin resistance as, further

exogenous administration of insulin induced glucose clearance. It is likely that, though the *Charles foster* strain does develop hyperglycemia and hyperinsulinemia, insulin resistance may need a longer duration of fructose feeding. This is justifiable by the reported greater hyperglycemia and longer duration of fructose feeding in *Wistar* rats (Hariom *et al.,* 2007). Number of factors, principally dyslipidemia marked by hypertriglyceredemia, appears to be the cause for development of insulin resistance. Since, both the doses of OI extract (OI 250 & OI 500) have been equally potent in minimizing the hyperglycemic action of fructose, as potent as metformin, it is inferable that, OI extract is apparently counteracting the possible molecular alterations induced by fructose as cited above. In one of the recent studies, AnithaNandhini *et al.* (2005) have documented the ability of taurine to counter the alterations leading to glycemic dysregulation induced by dietary fructose. Increased plasma free fatty acids and hypertriglycerdemia by way of increased triglyceride formation from free fatty acid recorded in the present study are likely to contribute to insulin resistance by definitive molecular disruptions in the insulin signaling pathway as has been reviewed by Stanhope and Havel (2008). Fructose induced hyperinsulinemia as observed herein is essentially a compensatory overproduction to offset the increasing insulin resistance. The prevalence of insulin resistance in fructose fed rats is clearly indicated by the poor glucose tolerance curve and insulin response as recorded in glucose tolerance and insulin response tests. Simultaneous treatment of fructose fed rats with OI extract brought about near normal glucose tolerance pattern and insulin response when challenged with glucose and insulin respectively. The response curves of both these tests with OI extract are even better

than that seen with metformin. The preventive effect of OI extract on insulin resistance is also well reflected in the noted plasma insulin level. Obviously, the active principles of OI extract are able to resist the metabolic and molecular alterations induced by dietary fructose either directly or indirectly contributing to insulin resistance and hyperinsulinemia. Insulin resistance in fructose fed rats has been attributed to efficient production of adiponectin from adipose tissue (Le and Tappy, 2006) as, such a change has been well established with reference to insulin resistance (Berg *et al.,* 2001; Yamauchi *et al.,* 2001; Kubota *et al.*, 2002).

The fructose model of hypertension is being greatly used by researchers to decipher the mechanics of diet induced hypertension, more so due to modern life style and dietary habits. Despite the definitive development of hypertension, the exact mechanism of fructose induced hypertension is not fully understood. Several mechanisms have been implicated in fructose induced hypertension with quiet a few linking it with hyperinsulinemia and insulin resistance. These include sympathetic activation (Verma *et al.,* 1999), renal abnormalities in handling sodium (Endre *et al.,* 1994; Kageyama *et al.,* 1994), defects in endothelial function (Kamata and Yamashita, 1999; Katakam *et al.,* 1998; Miller *et al.,* 1998; Verma *et al.,* 1997), vascular insulin resistance (Verma *et al.,* 1997; Verma *et al.*, 2000) and increased production and /or activity of vasoactive mediators such as endothelin-1 (Verma *et al.,* 1999; Verma *et al.,* 1996) nitric oxide and thromboxane A_2 (Kamata and Yamashita, 1999; Verma *et al.*, 1997; Galipeau *et al.,* 2001). Sex differences in development of fructose induced hypertension suggest the protective role of estrogen and the potentiating role of testosterone (Galipeau et al., 2002). Recently, 5 HT

(serotonin) mediated mechanism of development of hypertension in fructose fed rats through 5 HT 2B receptors has also been shown (Balaraman *et al.*, 2007). Though there is a link between hyperinsulinemia and insulin resistance with fructose hypertension in males, evidences are also available to suggest a direct development of hypertension independent of hyperinsulinemia and insulin resistance (Hwang *et al.*, 1987; Richey *et al.*, 1998). Apparently, it is inferable from all available inputs that, fructose rich diet induced hypertension is a consequence of a series of factors/ mechanisms interconnected or even independent.

The present observations clearly reveal significantly increased mean arterial blood pressure (MABP) and exaggerated vasopressor responses to adrenaline and phenylepinephrine and, sluggish vasodilator response to acetylcholine and isoprenaline in fructose fed rats. Apparently, both a sympathetic activation as well as parasympathetic inhibition, can be envisaged. Interestingly, our OI extract could prevent essential hypertension developing due to a fructose rich diet and, the FR + OI 500 rats showed normal MABP and complete absence of exaggerated responses to sympathetic agonists and the stunted or sluggish responses to parasympathetic agonists. These observations clearly suggest the ability of OI extract to prevent diet induced hypertension to a degree at par with that of the drug Metformin and, even better with reference to response to parasympathetic agonists. Apart from highlighting the protective role of OI extract against fructose hypertension, the results also confirm the dual effect of fructose on sympathetic and parasympathetic mediation. The presently observed ability of Metformin in preventing fructose induced hypertension finds validity from the observations of direct sympatho-

inhibitory protection and its consequent antihypertensive effect by Verma *et al.* (1996). Since Balaraman *et al.* (2006) have shown 5 HT dependent hypertension in fructose fed rats through greater expression of 5HT-2B receptors and, the ability of extract of fenugreek seeds to prevent the up regulation of 5HT 2B receptors and thereby 5-HT induced hypertension, it is likely that, our OI extract principles are also able to abrogate 5-HT mediated hypertensive changes.

Research attempts are more focused in recent times on phyto-extracts and their active principles on biological outcomes and on understanding of the mechanisms by which they contribute to health promoting activities. This is the most effective alternative mode of therapy and in this context, the present finding on the potent efficacy of OI extract in preventing all manifestations of metabolic syndrome is of great value.

Overall, it can be concluded that, *Oreocnide integrifolia* leaf extract effectively prevents the manifestations of metabolic syndrome in fructose fed insulin resistant - hypertensive rats.