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Amelioration of Metformin-induced Hypothyroidism by *Withania somnifera* and *Bauhinia purpurea* Extracts in Type 2 Diabetic Mice

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An investigation was carried out to reveal the possible ameliorative role of two plant extracts on an antidiabetic drug-induced hypothyroidism in Type 2 diabetic animals. Dexamethasone (1.0 mg/kg, i.m.) administration caused hyperglycemia with a parallel increase in renal lipid peroxidation (LPO), relative risk ratio (RR), and the concentrations of serum insulin; total cholesterol (TC); low-density lipoprotein cholesterol (LDL-C); very low-density lipoprotein cholesterol (VLDL-C) and triglycerides (TG). It decreased serum triiodothyronine (T_3), thyroxine (T_4) and high-density lipoprotein cholesterol (HDL-C) levels as well as renal superoxide dismutase (SOD); catalase (CAT) and reduced glutathione (GSH) content. Administration with metformin (150 mg/kg, orally) to diabetic animals further reduced circulating T_4 level and caused severe hypothyroidism. It also reduced renal LPO, RR, serum concentrations of insulin; glucose and LDL-C with a parallel increase in cellular antioxidants. While oral administration with either *Withania somnifera* (1.4 g/kg) or *Bauhinia purpurea* (2.5 mg/kg) extract along with dexamethasone and metformin elevated the concentrations of circulating T_3 and T_4 to euthyroid level. The plant extracts also corrected RR ratio and serum concentration of lipids. The findings of the present study, for the first time, reveal that the evaluated plant extracts have a potential to ameliorate metformin-induced hypothyroidism in Type 2 diabetic subjects.

Keywords: dexamethasone; lipid peroxidation; metformin; mice; thyroid hormones; Type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by disturbed carbohydrate, fat and protein metabolism leading to inappropriate hyperglycemia, either due to low levels of the hormone insulin, or to the abnormal resistance to its fucntions (Ganong, 2005; Ma *et al.*, 2007; Mlinar *et al.*, 2007). The World Health Organization recognizes three main forms of diabetes mellitus i.e. Type 1, Type 2, and gestational diabetes. Out of these, Type 2 diabetes mellitus comprises about 90–95% of the diabetic population, in which target cells fail to respond to normal level of circulating insulin (Roith *et al.*, 2000; Buckingham, 2006; Mlinar *et al.*, 2007).

The etiology of Type 2 diabetes in which beta cells of the pancreas are very often functional includes genetic and other factors such as food intake; reduced physical activity; aging, smoking, administration of drugs and hormonal imbalance (Buckingham, 2006; Mlinar *et al.*, 2007). Among the hormones, adrenocorticoids are well known for their diabetogenic effects and are responsible for most of the steroid diabetes (Roith *et al.*, 2000; Jatwa and Kar, 2006a; Jatwa *et al.*, 2007). For the treatment of Type 2 diabetes mellitus, several oral hypoglycemic agents including thiazolidinediones, sulphonylureas, alpha-glucosidase inhibitors, and biguanides are commonly in use. Metformin is a member of biguanides group of oral hypoglycemic drugs, and has been used for the treatment of Type 2 diabetes mellitus for many years across the globe (Vigerrsky *et al.*, 2006). However, some recent clinical reports have indicated that metformin therapy may result in negative alteration in thyroid metabolism and lead to drug-induced hypothyroidism (Vigerrsky *et al.*, 2006; Isidro *et al.*, 2007).

Herbal drugs are gradually becoming more popular and acceptable, as they are believed to be safe (Kar and Panda, 2005; Hamza *et al.*, 2008). In recent years, ample work has been done on phytomedicine. In general, there is a paucity of scientific literature on the regulation of allopathic drug-induced hormonal imbalance and oxidative stress by herbal extracts. To the best of our knowledge, to date there is no report available on the ameliorating role of plant extracts on antihyperglycemic drug-induced thyroid dysfunction, despite the fact that several plant extracts have been investigated for the regulation of altered thyroid metabolism and diabetes mellitus (Panda and Kar, 1999; Kar and Panda, 2005; Babu *et al.*, 2007).

In the present study, an attempt has been made to work out the possible amelioration of metformin-induced hypothyroidism in dexamethasone-induced Type 2 diabetic mice (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007) by simultaneous therapy of *Withania somnifera* root or *Bauhinia purpurea* bark extracts which are known as pro-thyroid agents (Panda and Kar, 1999). As it is well documented that in diabetes mellitus renal tissues are

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mostly affected (Lannello *et al.*, 2005; Jatwa and Kar, 2006a; Jatwa *et al.*, 2007), we concentrated on the alterations in renal lipid peroxidation (LPO) and in the activities of superoxide dismutase (SOD) and catalase (CAT) as well as in reduced glutathione (GSH) content. We also investigated alterations in the serum concentrations of glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) as well as relative risk ratio (RR). The RR was calculated by dividing the value of TC with HDL-C. As women are known to be more prone to thyroid abnormalities (Bulow *et al.*, 2006), for the present investigation only female animals were considered.

MATERIALS AND METHODS

Drug and chemicals. The test drugs metformin (Diabose(R), Micro Labs Ltd, Bangalore, India) and dexamethasone (Decdan(R), Merind Co., New Delhi, India) were purchased from a registered local medical store. Pyrogallol, hydrogen peroxide, diethylene triamine penta acetic acid, sodium dodecyl sulfate, ethylene diamine tetra acetic acid (EDTA) and sulfuric acid were obtained from E. Merck Ltd (Mumbai, India), whereas 2-thiobarbituric acid (TBA), Ellman reagent and m-phosphoric acid were from Sigma Chemicals Co. Ltd (St Louis, MO, USA). Radioimmuoassay (RIA) kits, for the estimation of serum T_3; T_4 and insulin were procured from Bhabha Atomic Research Centre (BARC) (Mumbai, India). All other chemicals were of reagent grade and purchased from Loba Chemie (Mumbai, India).

Experimental animals. Healthy colony-bred adult Swiss albino female mice weighing 30 ± 2 g, were considered for the study. These were maintained in polypropylene cages under constant temperature (23 ± 2 °C) and photo schedule (14 h light: 10 h dark). They were provided with commercial rodent feed supplied by Gold Mohur feeds Ltd (New Delhi, India) *ad libitum* and had free access to drinking water throughout the experimental period. Standard ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments in Animals (CPCSEA), Ministry of Environment and Forests, Government of India and Departmental Ethical Committee for Handling and Maintenance for Experimental Animals were followed.

Plant extracts. Ethanolic extract of *W. somnifera* root powder (herb: extract 5:1 w/w) and aqueous alcoholic extract of *B. purpurea* bark powder (herb: extract 8:1 w/w) were donated by AMSAR Private Ltd (Indore, India). The voucher specimens, WS-RJ-07 and BP-RJ-07, for *W. somnifera* and *B. purpurea*, respectively were deposited in departmental herbarium.

Experimental design. *Experiment-1: Metformin-induced thyroid dysfunction: a preliminary study*. Twenty-one healthy mice were divided into three groups of seven each. Group 1 animals receiving the vehicle, normal saline (0.1 ml/animal/day, i.m.) served as control, while Groups 2 and 3 received pre-standardized dose of dexamethasone (1.0 mg/kg/day, i.m. for 7 days) to induce Type 2 diabetes mellitus (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007). From the eighth day onwards, animals in Groups 1 and 2 continued to receive equivalent amounts of vehicle or dexamethasone respectively along with distilled water (0.1 ml/animal/day, p.o). Group 3 animals received equivalent amount of dexamethasone as well as metformin (150 mg/kg/day; p.o.) for 15 days (Yoon *et al.*, 2007). Drug/vehicle administration was done by gastric intubation between 10 am and 11 am to avoid possible circadian variation.

Experiment-2: Effects of plant extract on drug-induced thyroid dysfunction. In this experiment 35 healthy mice were divided into 5 groups of 7 each. Group 1 animals receiving the vehicle, normal saline served as control, while Groups 2, 3, 4 and 5 received dexamethasone for 7 days as done in previous experiment. From the eighth day onwards, animals in Groups 1 and 2 continued to receive equivalent amount of vehicle and dexamethasone, respectively along with distilled water, while Group 3 received metformin along with dexamethasone for 15 days. Animals in Groups 4 and 5 received equivalent amount of dexamethasone and metformin along with either *W. somnifera* root (1.4 g/kg, p.o.) or *B. purpurea* bark (2.5 mg/kg, p.o.) extract, respectively for same duration (Panda and Kar, 1999).

Preparation of serum and tissue samples. On the day of termination (23rd day), the final body weight of each animal was recorded and over-night-fasted animals were killed by cervical dislocation after exposure to mild ether anesthesia. Blood from each animal was collected and serum was isolated for the estimation of different biochemical and hormonal parameters including concentrations of T_3, T_4, insulin, glucose, TC, HDL-C, LDL-C, VLDL-C and TG. After exsanguinations, the kidneys were removed quickly, freed from blood clots and washed thoroughly with phosphate buffered saline (0.1 M, pH 7.4), weighed and processed for the estimations of LPO, SOD, CAT, GSH and protein.

Hormone assays of T_3 and T_4. Total circulating T_3 and T_4 were estimated by RIA in serum samples following the protocols provided in the kits as routinely followed in our laboratory (Jatwa and Kar, 2006a, 2006b; Jatwa *et al.*, 2007; Jatwa and Kar, 2008). In brief, RIA was performed using tris hydroxy-methyl amino methane buffer (0.14 M, containing 0.1% gelatin; pH 8.6). The antisera, specific hormone standards, radio labeled hormones (I^{125} Insulin, I^{125} T_4 and I^{125} T_3) and the control sera were reconstituted with assay buffer/double distilled water. The reaction mixture comprised of standard/sample, buffer, radio-labeled hormone and the respective antibody. The reaction mixture was then incubated at 37 °C (30 min. for T_4 and 45 min. for T_3). Incubation was terminated by the addition of polyethylene glycol. Tubes were then centrifuged at 2000 × g for 20 min. After decanting the supernatant, traces of liquid were removed with the help of filter paper wicks without disturbing the precipitate. Finally the tubes were subjected to radioactivity counting for one minute (CPM) using an I^{125} gamma counter. A set of quality control sera was also used with each assay.

Radioimmunoassay of insulin. Total serum insulin was also estimated following the protocol provided in RIA

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kit, as routinely followed in our laboratory (Jatwa and Kar, 2006b; Jatwa *et al.*, 2007; Jatwa and Kar, 2008). In brief, the tubes containing 200 µl of assay buffer with 100 µl of serum sample/standard was mixed and then 100 µl of primary antibodies (antiporcine guinea pig IgG) were added and the mixture was incubated at 4 °C for overnight. After incubation, 100 µl of I^{125}-labeled insulin hormone was added. After 3 h of incubation at room temperature, 100 µl of secondary antibodies (antiguinea pig-rabbit IgG) were added followed by the addition of 1 ml polyethylene glycol. After gentle mixing, tubes were incubated at room temperature for 20 min and then centrifuged at 1500 × g for 20 min at room temperature. After decanting the supernatant, traces of liquid were removed with the help of filter paper wicks without disturbing the precipitate. Finally the tubes were subjected to radioactivity counting for 1 min (CPM) using an I^{125} gamma counter. A set of quality control sera was also used with each assay.

Biochemical estimations of LPO and GSH. For the evaluation of LPO, the kidney tissues were homogenized in 10% (w/v) ice-cold phosphate buffered saline (0.1 M, pH 7.4), centrifuged at 2000 × g for 30 min and the supernatant was used for the assay. In brief, LPO was determined by the reaction of 2-thiobarbuturic acid with malondialdehyde (MDA), one of the major products formed by peroxidation of lipids, in acidic medium (Ohkawa *et al.*, 1979; Jatwa and Kar, 2006a; Jatwa *et al.*, 2007; Jatwa and Kar, 2008). Tissue glutathione content (reduced sulfhydryl groups) was measured by taking the absorbance of the product formed by the reaction of Ellman's reagent with GSH at 412 nm (Extinction coefficient, $\varepsilon = 1.36 \times 10^4$) as earlier followed in our laboratory (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007; Jatwa and Kar, 2008).

Estimations of SOD and CAT activities and protein content. The endogenous SOD activity was determined using the pyrogallol autoxidation inhibition assay; as routinely done in our laboratory (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007; Jatwa and Kar, 2008). The rate of autoxidation was determined by recording the increase in the absorption at 420 nm while renal CAT activity was estimated by considering the amount of hydrogen peroxide decomposed following our routine protocol (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007; Jatwa and Kar, 2008). Tissue protein estimation was done by the routine method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Estimations of serum glucose and lipids. For the estimation of fasting serum glucose concentration; a spectrophotometric method was followed, where 4-aminoantipyrine and phenol react with glucose to yield a red colored complex (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007; Jatwa and Kar, 2008), while estimations of serum TC, HDL-C and TG levels were done using spectrometric methods, as earlier done (Jatwa and Kar, 2006b; Jatwa and Kar, 2007). Low-density lipoprotein cholesterol, VLDL-C and RR were calculated using the formula: TC-(HDL-C+VLDL-C), TG/5, and TC/HDL-C respectively (Jatwa and Kar, 2006b).

Statistical analysis. Data are expressed as mean ± SEM. For statistical evaluation of the data Student's *t*-test was used. A '*p*' value 0.05 or less was considered as the level of statistical significance.

RESULTS

Experiment-1 (Metformin-induced thyroid dysfunction). A significant increase in the concentrations of serum-fasting glucose and insulin ($P < 0.001$ for both) was observed in dexamethasone-induced diabetic animals, while serum T_3 and T_4 levels ($P < 0.01$ and $P < 0.001$, respectively) decreased. However, metformin administration along with dexamethasone for 15 days further reduced serum T_4, insulin and glucose levels ($P < 0.01$ for first two and $P < 0.001$ for last one) (Table 1).

Experiment-2. As depicted in Table 2, administration of dexamethasone to mice significantly increased the serum concentrations of fasting glucose, TC, TG, VLDL-C and LDL-C and RR ($P < 0.001$ for all); but decreased HDL-C ($P < 0.001$). Dexamethasone administration also increased the LPO ($P < 0.01$) and decreased CAT, GSH and SOD activities ($P < 0.001$ for first two and $P < 0.01$ for last one, Table 3) in renal tissues. It also decreased the serum concentrations of T_3 and T_4 ($P < 0.001$ for both, Fig. 1). On one hand, metformin administration along with dexamethasone to mice reduced concentrations of serum LDL-C, fasting glucose, insulin ($P < 0.01$ for first one and $P < 0.001$ for rest two) as well as renal LPO and RR ($P < 0.01$ for both). On the other hand, it increased the concentration of serum HDL-C ($P < 0.001$) as well as renal SOD; CAT and GSH content ($P < 0.001$ for first two and $P < 0.01$ for last one). Interestingly, metformin administration to Type 2 diabetic animals further reduced the serum concentration of T_4 ($P < 0.001$, Fig. 1).

Effects of *W. somnifera* root extract. *W. somnifera* root extract administration along with dexamethasone and

Table 1. Effects of Metformin (150 mg/kg/day, p.o.) administration for 14 days on the concentrations of serum triiodothyronine (T_3); thyroxine (T_4); insulin and fasting glucose in Dexamethasone-treated (1 mg/kg, i.m.) female mice

	Control	Dexamethasone	Dexamethasone + Metformin
T_3 (ng/ml)	1.32 ± 0.06	1.01** ± 0.04	0.91*** ± 0.03
T_4 (ng/ml)	44.66 ± 2.02	33.32*** ± 1.60	27.00$^{\$\$,***}$ ± 1.00
Insulin (U/ml)	8.03 ± 0.30	13.16*** ± 0.71	8.91$^{\$\$}$ ± 0.51
Glucose (mg/dl)	109.92 ± 6.32	154.73*** ± 7.12	98.24$^{\$\$\$}$ ± 5.12

Data are mean ± SEM ($n = 7$); ***, $P < 0.001$; **, $P < 0.01$ as compared to the respective control values and $^{\$\$\$}$; $P < 0.001$ and $^{\$\$}$, $P < 0.01$ as compared to the Dexamethasone-treated values.

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Table 2. Effects of either *W. somnifera* (WS) root or *B. purpurea* (BP) bark extracts administration along with Metformin (Met, 150 mg/kg/day, p.o.) for 14 days on the concentrations of serum insulin, fasting glucose, total cholesterol (TC), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C) and triglycerides (TG) as well as on relative risk ratio (RR) in Dexamethasone-induced (Dexa, 1 mg/kg/day, i.m.) Type 2 diabetic female mice

	Control	Dexa	Dexa + Met	Dexa + Met + WS	Dexa + Met + BP
Insulin (U/ml)	11.12 ± 0.61	16.62*** ± 0.70	10.5$^{\$\$\$}$ ± 0.62	12.5$^{\$\$\$}$ ± 0.63	10.83$^{\$\$\$}$ ± 0.62
Glucose (mg/dl)	95.59 ± 5.25	143.11*** ± 7.81	89.11$^{\$\$\$}$ ± 5.23	98.82$^{\$\$\$}$ ± 4.78	101.59$^{\$\$\$}$ ± 4.65
TC (mg/dl)	115.45 ± 6.01	201.81*** ± 10.01	179.99*** ± 8.20	125.90$^{\$\$\$,@@@}$ ± 5.81	105.14$^{\$\$\$,@@@}$ ± 6.01
HDL-C (mg/dl)	40.01 ± 2.40	26.68*** ± 1.90	44.5$^{\$\$\$}$ ± 2.42	49.59$^{\$\$\$}$ ± 3.01	47.97$^{\$\$\$}$ ± 3.12
LDL-C (mg/dl)	59.50 ± 3.11	154.56*** ± 8.81	114.08***,$^{\$\$}$ ± 5.13	61.62$^{\$\$\$,@@@}$ ± 3.14	41.04**,$^{\$\$\$,@@@}$ ± 3.53
VLDL-C (mg/dl)	15.47 ± 0.70	23.04*** ± 1.50	20.69*** ± 0.8	14.99$^{\$\$\$,@@@}$ ± 0.61	16.03$^{\$\$\$,@@}$ ± 0.81
TG (mg/dl)	77.38 ± 3.41	117.25*** ± 5.13	103.47*** ± 4.32	74.99$^{\$\$\$,@@@}$ ± 4.50	79.68$^{\$\$\$,@@}$ ± 4.01
RR	2.89 ± 0.16	7.55*** ± 0.51	4.01***,$^{\$\$}$ ± 0.32	2.54 $^{\$\$\$,@@}$ ± 0.14	2.20**,$^{\$\$\$,@@@}$ ± 0.14

Data are mean ± SEM (n = 7); ***, $P < 0.001$ and **, $P < 0.01$ as compared to the respective control values. $^{\$\$\$}$, $P < 0.001$ and $^{\$\$}$, $P < 0.01$ as compared to the respective Dexa-treated values; $^{@@@}$, $P < 0.001$ and $^{@@}$, $P < 0.01$ as compared to the respective values of Dexa + Met-treated group.

Table 3. Effects of either *W. somnifera* (WS) root or *B. purpurea* (BP) bark extracts administration along with Metformin (Met, 150 mg/kg/day, p.o.) for 14 days on renal lipid peroxidation (LPO), superoxide dismutase (SOD), catalase CAT and on reduced glutathione content (GSH) in Dexamethasone-induced (Dexa, 1 mg/kg/day, i.m.) Type 2 diabetic female mice

	Control	Dexa	Dexa + Met	Dexa + Met + WS	Dexa + Met + BP
LPO (nM MDA formed/mg protein/hr)	1.01 ± 0.06	1.53** ± 0.14	1.08$^{\$\$}$ ± 0.09	0.83$^{\$\$\$,@}$ ± 0.04	0.96$^{\$\$\$}$ ± 0.05
SOD (U/mg protein)	4.12 ± 0.22	3.08** ± 0.21	4.69$^{\$\$\$}$ ± 0.30	6.89***,$^{\$\$\$,@@@}$ ± 0.40	6.14***,$^{\$\$\$,@}$ ± 0.41
CAT (μM H_2O_2 decomposed/min/mg protein)	32.58 ± 2.01	21.58*** ± 1.50	33.50$^{\$\$\$}$ ± 2.10	47.12***,$^{\$\$\$, @@}$ ± 3.01	46.57***,$^{\$\$\$,@@}$ ± 3.14
GSH (μM GSH/mg protein)	8.65 ± 0.38	6.04*** ± 0.31	7.85$^{\$\$}$ ± 0.29	8.89$^{\$\$\$,@}$ ± 0.33	8.71$^{\$\$\$}$ ± 0.31

Data are mean ± SEM (n = 7); ***, $P < 0.001$; **, $P < 0.01$ as compared to the respective control values and $^{\$\$\$}$, $P < 0.001$ and $^{\$\$}$, $P < 0.01$ as compared to the respective Dexa-treated values. $^{@@@}$, $P < 0.001$; $^{@@}$, $P < 0.01$ and $^{@}$, $P < 0.05$ as compared to the respective values of Dexa + Met-treated group.

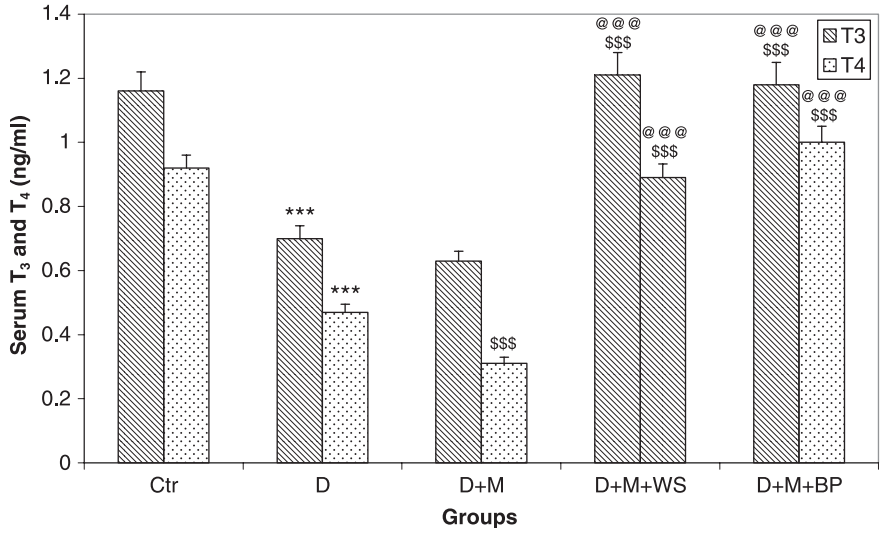

Figure 1. Changes in the concentrations of serum T_3 and T_4 (×100) following the administration of *Withania somnifera* (WS) and *Bauhinia purpurea* (BP) extracts in Metformin-(M)-induced thyroid dysfunction in dexamethasone-induced Type 2 diabetic female mice. Each vertical bar represents the mean ± SEM (n = 7). ***, $P < 0.001$ as compared to the respective control values and $^{\$\$\$}$, $P < 0.001$ as compared to the dexamthasone treated values. $^{@@@}$, $P < 0.001$ as compared to dexamethasone + Metformin-treated group.

metformin increased the concentrations of serum T_3 and T_4 ($P < 0.001$ for both, Fig. 1) to normal level, as no significant differences were observed when the comparisons were made between the values of control and this (dexamethasone + metformin + *W. somnifera*) group. A significant decrease in the serum levels of TC, TG, LDL-C, and VLDL-C ($P < 0.001$ for all, Table 2) were also noticed in this group as compared to dexamethasone + metformin group. *W. somnifera* extract also reduced renal LPO and RR ($P < 0.05$ and $P < 0.01$, respectively), and increased the activity of SOD ($P < 0.001$); CAT and GSH ($P < 0.01$ and $P < 0.05$,

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respectively), as compared to the respective values of dexamethasone + metformin group (Table 3).

Effects of *B. purpurea* bark extract. While *B. purpurea* bark extract administration along with dexamethasone and metformin increased the concentrations of serum T_3 and T_4 ($P < 0.001$ for both, Fig. 1) to nearly normal level, a significant decrease in the serum levels of TC, LDL-C, VLDL-C and TG ($P < 0.001$ for first two and $P < 0.01$ for rest two) was observed (Table 2). Administration of *B. purpurea* extract also reduced RR ($P < 0.001$), and increased the activity of renal SOD and CAT ($P < 0.05$ and $P < 0.01$, respectively), as compared to dexamethasone + metformin group (Table 3).

DISCUSSION

Following dexamethasone administration, an increase in fasting serum glucose and insulin concentration exhibiting Type 2 diabetic conditions was observed, similar to the earlier reports (Severino *et al.*, 2002; Jatwa and Kar, 2006a; Jatwa *et al.*, 2007). This is quite expected, as glucocorticoids, secreted from the adrenal gland, positively influence the glucose metabolism (Roith *et al.*, 2000). In fact, when the gland remains hyperactive for a longer period, it always increases serum glucose level (Roith *et al.*, 2000). When metformin was administered in addition to dexamethasone for 15 days, the diabetic condition was reversed, as evidenced by decreased fasting glucose and insulin concentration.

Observations made on the changes in thyroid hormones also indicated a hypothyroid condition following dexamethasone administration as reported earlier by our laboratory (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007) and by other workers (Severino *et al.*, 2002). Surprisingly, simultaneous administration with metformin and dexamethasone to diabetic subjects further reduced the level of circulating T_4 and resulted in hypothyroidism. To the best of our knowledge, for the first time we report the metformin-induced thyroid dysfunction in an animal model of Type 2 diabetes mellitus.

Drug-induced disorders, in the form of adverse drug events or drug interactions are common in all health care environments (Lazarou *et al.*, 1998; Ma *et al.*, 2007). Unfortunately, significant morbidity and mortality are often the consequence of these reactions. Several studies have reported that an average of 10% of all hospital admissions may be attributable to drug-induced disorders (Holland and Degruy, 1997; Lazarou *et al.*, 1998). However, on adverse drug reactions with particular reference to thyroid function limited literature is available (Meier and Burger, 2000; Ma *et al.*, 2007). Recent reports suggest that chronic medication with metformin to Type 2 diabetic patients may also result in hypothyroidism (Vigerrsky *et al.*, 2006; Isidro *et al.*, 2007). In the present study, the test drug was found to also reduce the level of circulating T_4, suggesting that metformin inhibits thyroid function at glandular level, the only source of T_4 synthesis.

With respect to the changes in different serum lipids, dexamethasone-induced Type 2 diabetes mellitus also enhanced the levels of circulating TC, LDL-C, VLDL-C and TG as observed earlier (Buckingham, 2006; Jatwa *et al.*, 2007). The resultant hyperlipidaemia following dexamethasone administration could be an outcome of the reduced LDL receptors in hepatocytes (Tiwari, 2004), which converts circulating fats to bile. However, administration of metformin to these animals reversed the diabetic condition. Interestingly, it also decreased circulating T_4 concentration, as observed in previous experiment too. The antithyroid nature of the test drug was further supported by the increased levels of other lipids, which are commonly lowered by the thyroid hormones. This observed hyperlipidemic condition following metformin administration in turn could be due to the decrease in the serum level of thyroid hormones, as they are known to be lipolytic in nature (Ganong, 2005).

Consistent with our earlier findings, this time a marked increase in renal LPO with a parallel decrease in tissue antioxidants (SOD, CAT and GSH) was also observed following dexamethasone treatment (Jatwa and Kar, 2006a; Jatwa *et al.*, 2007). However, these alterations were reversed with simultaneous administration of metformin suggesting the beneficial role of the drug with respect to renal functions. The dexamethasone-induced increase in renal LPO could be an outcome of the hyperglycemic condition as enhanced level of circulating glucose provides oxidative environment for basement membrane lipids (Szkudelski, 2001; Tiwari, 2004), and also due to hyperlipidemic condition (Tiwari, 2004).

With respect to the role of herbal extracts, ample literature is available on the regulation of altered glucose and thyroid metabolism, cardiovascular diseases and oxidative stress (Kar and Panda, 2005; Babu *et al.*, 2007; Hamza *et al.*, 2008). However, literature is meager on the ameliorating role of plant extract on drug-induced adverse events including that of oxidative stress (Bagchi *et al.*, 2001; Sivalingam *et al.*, 2008). In literature *W. somnifera* and *B. purpurea* plant extracts have been claimed to increase the level of circulating thyroid hormones and in the regulation of altered glucose metabolism and oxidative stress (Panda and Kar, 1999; Kar and Panda, 2005; Hamza *et al.*, 2008).

Interestingly, when pro-thyroid plant extracts (Panda and Kar, 1999), either of *W. somnifera* or *B. purpurea*, was administered to metformin-treated diabetic animals; they increased the serum level of both the thyroid hormones and ameliorated drug-induced hypothyroidism. This observation is new and suggests that these plant extracts could correct drug-induced thyroid dysfunction in general and metformin-induced hypothyroidism in particular. Consistent with earlier findings, the plant extracts could also reduce oxidative stress to a greater extent (Panda and Kar, 1999; Hamza *et al.*, 2008). However, the observed thyroid stimulatory and antiperoxidative nature of test plant extracts may also be an outcome of the direct action of their active ingredients – Withaferin-A and Bauhiniastatins 1-4 for *W. somnifera* and *B. purpurea*, respectively – at molecular level, as Withaferin-A, an active ingredient of *W. somnifera* has been reported to have an antioxidant effect (Bhattacharya *et al.*, 2000). Although scientific reports on the use of plant extracts in the regulation of drug-induced thyroid dysfunction are few, the observed effects could be similar to some other plant extracts, which have been reported to regulate drug-induced toxicity and oxidative stress (Bagchi, 2001; Sivalingam *et al.*, 2008). The cyto-protective or antiperoxidative role of evaluated plant extracts,

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however, could also be an outcome of increased levels of circulating thyroid hormones (Ganong, 2005; Jatwa and Kar, 2006a, 2006b; Jatwa *et al.*, 2007; Jatwa and Kar, 2008). This fact appears to be more justified, as exogenous administration of T_3 to hepatotoxic animals has been found to ameliorate chemical-induced oxidative stress and altered thyroid metabolism (Maiti and Kar, 1998).

CONCLUSION

W. somnifera and *B. purpurea* plant extracts have been found to ameliorate metformin-induced hypothyroidism in Type 2 diabetic mice without any toxic effect, but with antiperoxidative benefits, as evidenced by a decrease in LPO and an increase in endogenous antioxidants. However, before recommending these plants extracts for clinical use, long-term studies at molecular level will be more beneficial.

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