

## Original Article

# Amelioration of Some Manifestations of Metabolic Syndrome in Rats by Allopurinol Irrespective of Lowering Serum Uric Acid Level: Role of Adiponectin

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## A B S T R A C T

**Introduction:** Some studies suggest that increased serum uric acid may simply be a consequence of oxidative stress or hyperinsulinemia present in subjects with metabolic syndrome, while others postulate that uric acid could have a contributory causal role. Although lowering uric acid in fructose fed rats can improve insulin sensitivity and features of metabolic syndrome, the mechanisms involved in these beneficial effects remain unclear. The strong association between serum uric acid and the metabolic syndrome led some authors to postulate a correlation between uric acid level and adipose tissue. Down regulation of adiponectin, an adipokine released from adipose tissue, is associated with obesity-linked diseases including coronary artery disease and type 2 diabetes. Clinical observations demonstrated that hypo adiponectinemia is closely related to endothelial dysfunction in blood vessels. The aim of the present work was to investigate whether the beneficial effects of allopurinol in metabolic syndrome are secondary to reduction of serum uric acid or to other mechanisms such as elevation of adiponectin level.

**Materials and Methods:** A model of metabolic syndrome in male Wistar rats was induced by feeding animals high fat diet and single intraperitoneal injection of low dose streptozotocin. 3 weeks later, rats that developed metabolic syndrome received a vehicle or glibenclamide 0.5 mg/kg/day (as a standard therapy of type 2 diabetes mellitus). Allopurinol was administered in combination with glibenclamide in two different doses, 50 mg/Kg and 100 mg/Kg/day. Treatment was continued for 3 weeks. Metabolic syndrome parameters, adiponectin level, vascular reactivity in addition to intima/media ratio of aorta and liver histopathology were investigated.

**Results:** Allopurinol induced dose dependent improvement in insulin sensitivity and serum glucose but uric acid reduction was dose independent. Allopurinol showed dose dependent improvement in endothelial function, intima/media ratio of aorta with remarkable improvement in features of hepatic steatosis. Both low and high dose of allopurinol induced elevation of adiponectin compared to untreated group with no significant difference between both doses.

**Conclusion:** Allopurinol could improve some parameters of metabolic syndrome in a rat model of metabolic syndrome irrespective to lowering uric acid level. The effects of allopurinol may be related to elevation of the lowered level of adiponectin associated with obesity linked diseases

**Key Words:** allopurinol, adiponectin, metabolic syndrome, rats.

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## 1. INTRODUCTION

Uric acid is associated with metabolic syndrome and its components, obesity, dyslipidemia, hypertension, insulin resistance, and endothelial dysfunction, which represents a predominant early feature of components of metabolic syndrome and cardiovascular diseases (De oliveira and Burini, 2012). Some studies suggest that increased uric acid may simply be a consequence of the presence of oxidative stress or hyperinsulinemia present in subjects with metabolic syndrome. Others postulate that uric acid could have a contributory causal role (Dawson and Walters, 2006). Although lowering uric

acid in fructose fed rats can improve insulin resistance and features of metabolic syndrome, it is not clear whether this beneficial effect is due to improvement in endothelial function or to direct actions of uric acid on adipocytes (Lanaspa et al., 2011). Adipose tissue is not only an important site for fat metabolism, but it is also considered as a major endocrine gland that secretes adipokines. Adiponectin, an adipokine, has a role in fatty acid metabolism, antiatherogenesis and insulin sensitization (Haluzik et al., 2004). Therefore, it is an important tool for obtaining a better

understanding of the link between obesity, metabolic and cardiovascular disorders (**Pischon and Rimm 2006**). Adiponectin levels are inversely correlated with insulin resistance and anthropometrical indices in obese subjects (**Kolahi and Mahdi, 2012**). The role of adiponectin in cardiovascular diseases was investigated by **Selthofer-Relatic and coworkers (2011)**. They postulated that low adiponectin level was associated with a reduction in the protective effect on left ventricular wall thickness in overweight hypertensive adult patients. In addition, adiponectin exerts a protective role in myocardial ischemia reperfusion injury in streptozotocin-induced diabetes in rats (**Wang et al., 2011b**). Adiponectin downregulation is associated with obesity-linked diseases, including coronary artery disease and type 2 diabetes. Furthermore, clinical observations demonstrated that hypoadiponectinemia is closely related to endothelial dysfunction in peripheral arteries (**Tan et al., 2004**).

Type 2 diabetes mellitus, obesity and dyslipidemia often coexists with non-alcoholic fatty liver diseases (NAFLD). Hyperlipidemia and insulin resistance, in particular, importantly contribute to the initiation and progression of NAFLD (**Chitturi et al., 2002**). **Ashour and his group (2010)** stated that patients with hepatitis C virus of the genotype-4, who suffer from steatosis, had a lower adiponectin level, which was inversely correlated with insulin resistance.

Increased reactive oxygen species (ROS) levels in metabolic syndrome have toxic effects on cells and tissues through increased oxidation of carbohydrates, lipids, and proteins. This has a major role in the development and progression of cardiovascular diseases (**Lassègue and Griending 2010**). Thus, attenuation of ROS could have a positive implication on adiponectin level and endothelial dysfunction associated with metabolic syndrome. Xanthine oxidoreductase enzyme (XOR), which is responsible for the formation of uric acid, can also be responsible for the formation of ROS associated with endothelial dysfunction (**McNally et al., 2003**). Allopurinol, an XOR inhibitor, can reduce serum uric acid level and attenuate oxidative stress induced by XOR. There is a debate about whether the amelioration of the manifestations of the metabolic syndrome by allopurinol is due to reduction of uric acid or to its antioxidant effect. The aim of the present work was to investigate whether the beneficial effects of allopurinol in metabolic syndrome are secondary to reduction of serum uric acid or to other mechanisms such as elevation of adiponectin level.

## 2. MATERIALS AND METHODS

**2.1. Drugs and chemicals:** Allopurinol, urethane, L-phenylephrine hydrochloride and acetylcholine were purchased from Sigma Chemicals (USA).

Glibenclamide tablets (Daonil® 5 mg tablet) were crushed and freshly suspended in water with a few drops of Arabic gum, before administration.

**2.2. Animals:** Thirty adult male Wistar rats weighing 200-220 grams were used in this study. They were housed in standard laboratory conditions under a 12 h light/dark cycle and controlled temperature of  $22 \pm 3^\circ\text{C}$ , with free access to food and water. The studies were conducted in accordance with the laws and regulations of governing authorities and approved by the Institutional Animal Care and Use Committee at the Faculty of medicine, Ain-Shams University.

**2.3. Model of metabolic syndrome:** A model of metabolic syndrome in male Wistar rats was induced by feeding animals a high fat diet (HFD) composed of 58% fat, 25% protein and 17% carbohydrate, as a percentage of total caloric intake, for a total period of 6 weeks. Two weeks later, animals were administered a single intraperitoneal (i.p.) injection of low dose streptozotocin (STZ; 35 mg/kg; **Srinivasan et al., 2005**). One week later, non-fasting blood glucose level was determined using Glucocard 01-ARKRAY. Rats with blood glucose level  $\geq 200$  mg/dl were considered diabetic.

**2.4. Experimental groups and design:** Rats were divided into five groups, six rats each. These were a normal control group (Normal) and four groups with metabolic syndrome, one of which were left untreated (MS). The other three groups received glibenclamide 0.5 mg/kg as a standard therapy for type 2 diabetes mellitus (**Lal et al., 2011**). Allopurinol was co-administered with glibenclamide in two groups. One group received 50 mg/kg (**Augustin et al., 1994**) and the other received 100 mg/kg (**Giray et al., 2001**). Rats were treated with the designated drugs for 3 weeks following the development of metabolic syndrome. HFD continued until the end of the study.

## 2.5. Outcome Measures

### 2.5.1. Measurement of body weight

### 2.5.2. Measurement of systolic blood pressure (SBP)

At the end of 6<sup>th</sup> week, SBP was measured by using indirect tail cuff plethysmography (ADI instrument, Australia). The inflated cuff pressure was computed using power lab/85p (ML 785 software program). SBP for each rat was calculated as the mean of at least 3 readings.

### 2.5.3. Metabolic parameters

By the end of the study, blood samples were collected from fasting animals under urethane anesthesia. Samples were collected from the ophthalmic venous plexus through retro-orbital approach. Blood was allowed to clot and then

centrifuged at 5000 rpm for 5 min. Serum was separated and samples were stored at -80°C until assayed. **Fasting serum glucose and serum uric acid levels** were measured using Synchron cx5 autoanalyzer (Beckman, USA). **Serum triglyceride and total cholesterol levels** were determined by enzymatic colorimetric methods using TRIGLYCERIDES GPO-PAP Detection Kit (Greiner Diagnostic GmbH, Germany) and CHOLESTEROL CHOD-PAP Detection Kit (Greiner Diagnostic GmbH, Germany). methods are modified from those originally described by **Fossati and Prencipe (1982)** and **Allain et al. (1974)**. **Serum high density lipoprotein (HDL) levels** were determined according to **Fruchart (1982)** and **serum low density lipoprotein (LDL) cholesterol** was calculated using the Friedewald formula (**Friedewald et al., 1972**).

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{Triglyceride}/5)$$

**Serum insulin levels** were determined using insulin ELISA kit for rat (ALPCO diagnostics, Windham, NH, USA) and **serum adiponectin levels** were determined using adiponectin ELISA kit for rat (Chemicon international, Inc., USA).

#### 2.5.4. Calculation of quantitative insulin sensitivity check index (QUICKI)

Quantitative insulin sensitivity check index was calculated according to **Katz and colleagues (2000)** using the following equation:

$$\text{QUICKI} = 1/(\log \text{insulin} + \log \text{glycemia in mg/dL})$$

Rats are considered as insulin resistant, when QUICKI  $\leq 0.33$  (**McAuley, 2001**).

#### 2.5.5. Isolated Rat Aortic Rings Preparation

Rats were anaesthetized by intraperitoneal injection of 1.2 g/kg urethane (**Michael, 1996**). The chest was rapidly opened and the descending thoracic aorta was separated from surrounding connective tissue for endothelial and histological studies. Rings about 4-5mm width were prepared and mounted between 2 parallel hooks made of stainless steel wire in a 15 ml organ bath. An initial resting tension of 4 grams was set and the rings were then allowed to equilibrate for 1-2 hours during which the preload was continuously adjusted to be 4 grams, the temperature was maintained at 37°C (**Furchgott and Zawadzki, 1980**). Isometric responses were measured with a force transducer (K30, Hugo Sacks Electronics, Freiburg, Germany) connected to a bridge coupler type 570 and the trace was displayed on a two-channel recorder (Lineacorder, HSE, WR 3310). Following equilibration, rings were sensitized by being repeatedly contracted with 100  $\mu\text{M}$  of phenylephrine until two reproducible contractions were obtained, then a cumulative dose-response curve was constructed by cumulative addition of phenylephrine

(0.1-100  $\mu\text{M}$ ) to the bath. The mean effective concentration 50 ( $\text{EC}_{50}$ ) and the maximal contractile responses ( $\text{E}_{\text{max}}$ ) to phenylephrine were determined for each curve. **Acetylcholine-induced endothelial relaxation** was estimated, when a plateau tension is achieved in response to a submaximal concentration of phenylephrine contraction. Rings were relaxed by exposure to stepwise increase in acetylcholine concentration (0.6 to 100  $\mu\text{M}$ /bath). Percent relaxation of the aortic rings were determined.

#### 2.5.6. Histopathological studies

After animals were sacrificed, thoracic aorta and liver were isolated and fixed in 10% formalin. 5  $\mu\text{m}$  sections were prepared, stained with Hematoxylin-Eosin and examined under light microscopy. For Aorta, slides were analyzed with a color image analysis system (Video Pro 32; Leading Edge Pty Ltd) and the width of the intima and the media were measured to calculate the intima/media ratio (**Ruengsakulrach et al., 1999**).

**2.6. Statistical Analysis:** Statistical analysis was carried out using Graph pad prism, software program, version 5.0 (2007). Inc., CA, USA. All values are expressed as means  $\pm$  SD. For all parameters, statistical differences among 2 groups was determined using student's t-test and differences among all groups were analyzed using one way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. P values  $< 0.05$  were considered statistically significant.

In the experiments of phenylephrine induced contraction, all doses were transformed into log values and the contractile responses for each preparation were expressed as a percentage of the maximum response achieved by each ring separately, which is considered in this case, the 100% response of that particular ring. The next step was to plot the log concentration against the responses expressed as percentages, in a linear regression curve, then  $\text{EC}_{50}$  was determined.

### 3. RESULTS

#### 3.1. Induction of the metabolic syndrome model

HFD for 6 weeks together with a single dose of STZ resulted in significant changes in metabolic parameters, including body weight, SBP, lipid profile, fasting serum glucose and serum uric acid levels (**Table 1**). Serum insulin was significantly elevated from  $2.45 \pm 0.18$  to  $6.42 \pm 0.71$   $\mu\text{U/ml}$  and serum adiponectin was significant reduced by 43% (**Table 2**).

### 3.2. Effect of allopurinol (50 and 100 mg/kg) co-administered with glibenclamide on metabolic parameters

Allopurinol 100 mg/kg co-administered with glibenclamide significantly increased HDL level compared to both normal control group and the lower dose of allopurinol (Fig. 1). Allopurinol 50 mg/kg co-administered with glibenclamide significantly decreased serum uric acid level by 63.8 % compared to untreated group. In contrast, the higher dose of allopurinol significantly elevated serum uric acid level compared to the lower dose of allopurinol (Table 3).

### 3.3. Effects of allopurinol (50 and 100 mg/kg) co-administered with glibenclamide on serum adiponectin and insulin levels

Allopurinol significantly elevated serum adiponectin levels compared to both the untreated and the glibenclamide treated groups. There was no significant difference between the two allopurinol doses (Fig. 2a). As for serum insulin levels, the higher dose of allopurinol (100 mg/kg) showed a significant reduction of serum insulin compared to both the untreated and glibenclamide treated groups. Again, there was no significant difference between the two allopurinol doses (Fig. 2b).

### 3.4. Effect of allopurinol (50 and 100 mg/kg) co-administered with glibenclamide on calculated insulin sensitivity check index (QUICKI):

The calculated index for insulin sensitivity was  $\leq 0.33$  in untreated metabolic syndrome and glibenclamide treated subgroups, denoting insulin resistance. Co-administration of allopurinol was associated with significant improvement in insulin

sensitivity compared to the untreated group. The higher dose of allopurinol showed a significantly higher improvement than the lower dose (Fig. 2c).

### 3.5. Effects of allopurinol (50 and 100 mg/kg) co-administered with glibenclamide on vascular reactivity of isolated aortic rings:

Metabolic syndrome resulted in a significant increase in  $E_{max}$  (Fig. 3a) and a significant reduction in acetylcholine-induced relaxation (Fig. 3c). Both doses of allopurinol significantly increased  $EC_{50}$  and acetylcholine-induced relaxation. The higher dose showed a significantly higher effect compared to the lower dose in both parameters (Fig. 3b&c).

### 3.6. Histological studies

#### 3.6.1. The effects of allopurinol (50 and 100 mg/kg) co-administered with glibenclamide on intima/media ratio of aorta

Metabolic syndrome induced a significant increase in intima/media ratio. Treatment with glibenclamide significantly reduced this increase in ratio. Co-administration of allopurinol with glibenclamide induced a further significant reduction in intima/media ratio that was dose independent (Fig. 4&5).

#### 3.6.2. The effects of allopurinol co-administered with glibenclamide on liver histology

The percent of macrovesicular steatosis was reduced in allopurinol treated groups with more reduction by the higher dose (Fig. 6).

**Table (1): Effect of high fat diet (HFD) for 6 weeks plus single intraperitoneal streptozotocin (STZ) injection (35mg/kg) on metabolic parameters**

Parameters Groups.....	BW (g)	SBP (mmHg)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)	FSG (mg/dl)	Serum UA (mg/dl)
Normal control	212.5 ± 9.35	112 ± 2.53	69.17 ± 2.48	29.39 ± 2.35	43.5 ± 2.89	51.5 ± 1.187	59.83 ± 3.19	1.8 ± 0.09
Metabolic Syndrome	321.7 ± 25.03	162.7 ± 2.06	97 ± 6.72	22.49 ± 2.14	59.67 ± 3.14	141.8 ± 5.38	209.8 ± 6.99	2.32 ± 0.15
Percent change	+ 51.4	+45.2*	+40.2*	+23.48*	+37.18*	+175.3*	+250.6*	+28.9*

Data are presented as mean ± SD, n = 6 rats/group. \*indicates  $P < 0.05$ , unpaired Student's t-test. BW, Body weight; SBP, systolic blood pressure; TC, total cholesterol; HDL, high density lipoprotein; LDL, Low density lipoprotein; TG, triglycerides; FSG, fasting serum glucose; UA, uric acid; MS, metabolic syndrome.

**Table (2): Effect of high fat diet (HFD) plus single intraperitoneal streptozotocin (STZ) injection (35mg/kg) on serum adiponectin and serum insulin level**

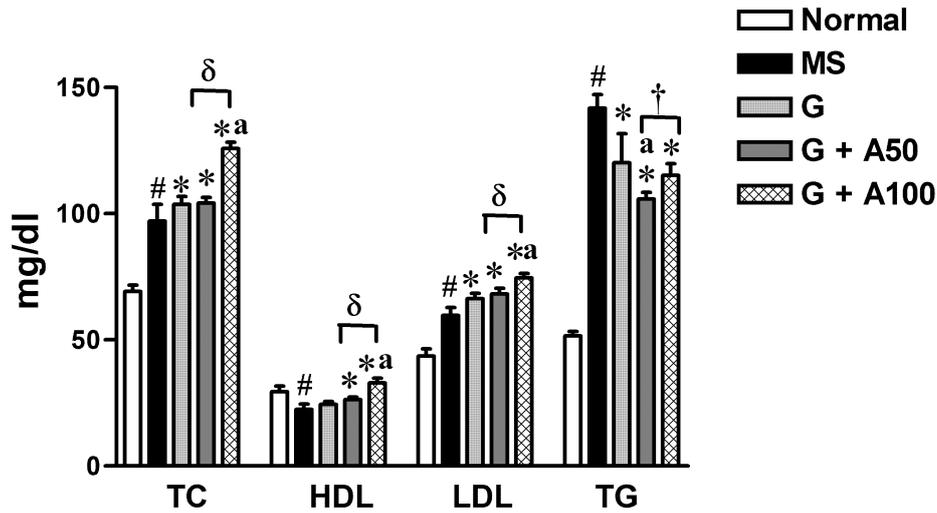
Groups Parameters	Serum adiponectin (ng/ml)	Serum insulin ( $\mu$ U/ml)
Normal control	3.95 $\pm$ 0.18	2.45 $\pm$ 0.18
Metabolic syndrome	2.25 $\pm$ 0.25	6.42 $\pm$ 0.71
Percent change	-43*	+162*

Data are presented as mean  $\pm$  SD, n = 6 rats/group. \*indicates P < 0.05, unpaired Student's t-test.

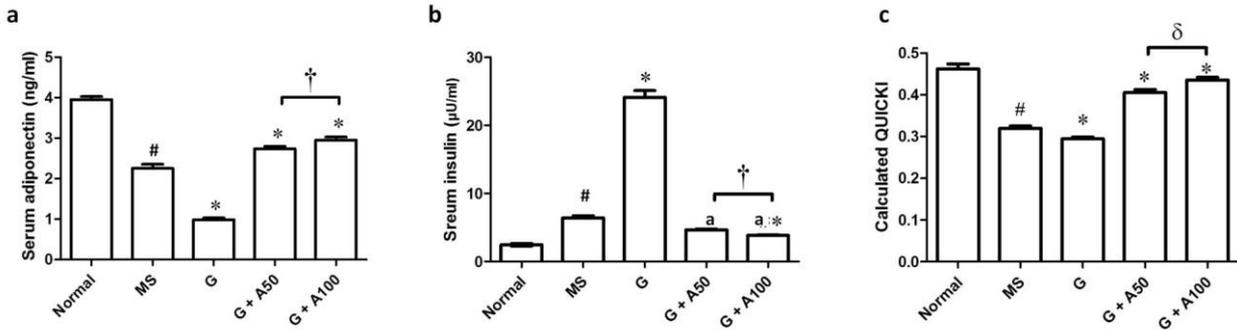
**Table (3): Effect of allopurinol (50 and 100 mg/kg) co-administered with glibenclamide on some metabolic parameters in rats with metabolic syndrome**

Groups Parameters	Normal control	Untreated MS	G 0.5 mg/kg	G 0.5 mg/kg + A 50 mg/kg	G 0.5 mg/kg + A 100 mg/kg
Body weight (g)	212.5 $\pm$ 9.35	321.7 $\pm$ 25.03	247.5 $\pm$ 32.52	187 $\pm$ 18.55	196.3 $\pm$ 5.46
Percentage change to	Normal control	+51.4*	+16.5	-34.9*	-7.6
	MS		-23.1*	-41.8*	-38.9*
	G (0.5 mg/kg)			-24.4*	-27.3*
	G + A (50 mg/kg)				+4.9
Systolic Blood Pressure (mmHg)	112 $\pm$ 2.54	162.7 $\pm$ 2.06	169.2 $\pm$ 4.44	162.2 $\pm$ 4.11	151.7 $\pm$ 2.34
Percentage change to	Normal control	+45.2*	+50.9*	+44.7*	+35.1*
	MS		+3.9*	-0.03	-6.7 *
	G (0.5 mg/kg)			-4.1	-10.3*
	G + A (50 mg/kg)				-6.4*
Fasting serum glucose (mg/dl)	59.83 $\pm$ 3.19	209.8 $\pm$ 6.99	102.5 $\pm$ 4.51	62.7 $\pm$ 3.01	51.5 $\pm$ 2.43
Percentage change to	Normal control	+250.6*	+71.2*	+4.7	-13.9 *
	MS		-51.1*	-70.2*	-75.5*
	G (0.5 mg/kg)			-38.9*	-49.8*
	G + A (50 mg/kg)				-17.9*
Serum uric acid (mg/dl)	1.8 $\pm$ 0.089	2.32 $\pm$ 0.15	2.4 $\pm$ 0.14	0.95 $\pm$ 0.11	1.22 $\pm$ 0.18
Percentage change to	Normal control	+28.9*	+30*	-42.2*	-27.8*
	MS		+1.3	-63.8*	-52.2*
	G (0.5 mg/kg)			-60.4*	-49.2*
	G + A (50 mg/kg)				+27*

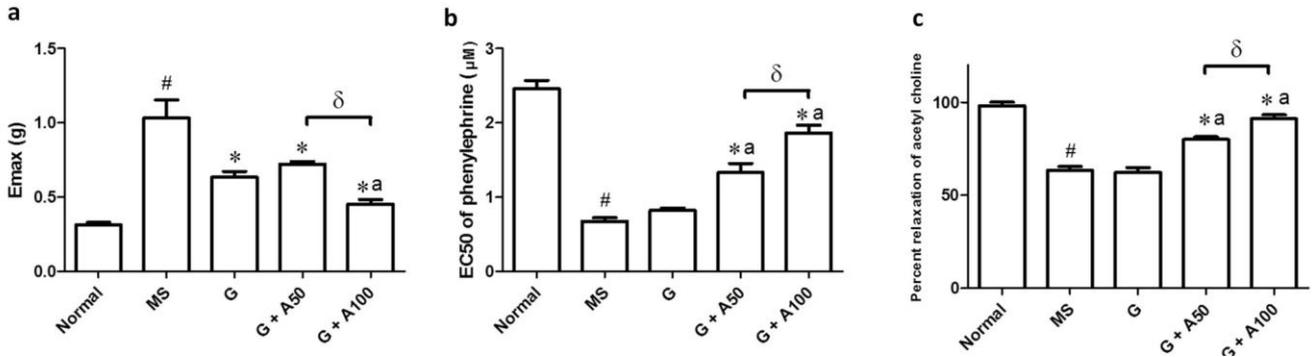
Data are presented as mean  $\pm$  SD, n = 6 rats/group. \*indicates P < 0.05, one way ANOVA, followed by Tukey's multiple comparisons test. MS, metabolic syndrome; G, glibenclamide; A, Allopurinol.



**Fig. 1: Effects of allopurinol co-administered with glibenclamide on lipid profile.** Data are presented as mean  $\pm$  SD, n = 6 rats/group. MS, metabolic syndrome; G, glibenclamide 0.5 mg/kg; G + A 50, glibenclamide 0.5 mg/kg + allopurinol 50 mg/kg; G + A 100, glibenclamide 0.5 mg/kg + allopurinol 100 mg/kg. \*indicates  $p < 0.05$  compared to the metabolic syndrome group, # indicates  $p < 0.05$  compared to the normal control group, <sup>a</sup> indicates  $p < 0.05$  compared to the glibenclamide group,  $\delta$  indicates  $p < 0.05$  compared to the low dose allopurinol group, † indicates insignificant difference; one way ANOVA, followed by Tukey's multiple comparisons test.

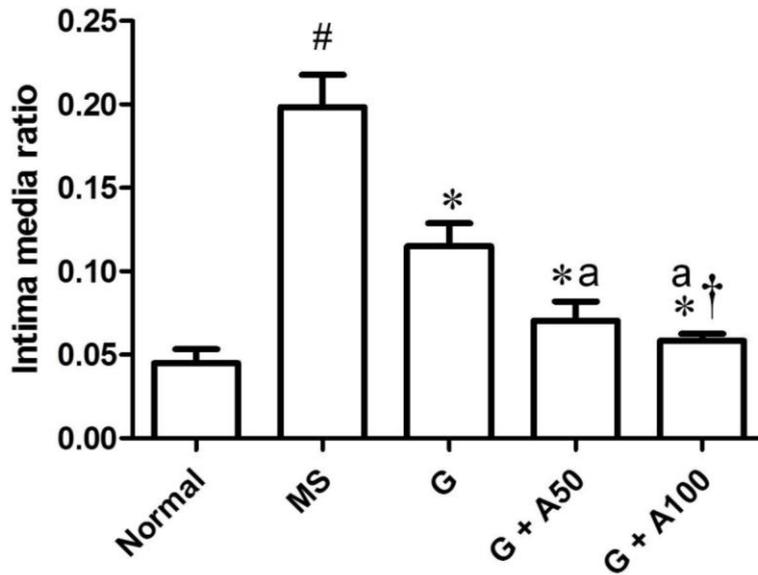


**Fig. 2: Effects of allopurinol co-administered with glibenclamide on serum adiponectin, serum insulin level and calculated insulin sensitivity check index (QUICKI).** Data are presented as mean  $\pm$  SD, n = 6 rats/group. MS, metabolic syndrome; G, glibenclamide 0.5 mg/kg; G + A 50, glibenclamide 0.5 mg/kg + allopurinol 50 mg/kg; G + A 100, glibenclamide 0.5 mg/kg + allopurinol 100 mg/kg. # indicates  $p < 0.05$  compared to the normal control group, \*indicates  $p < 0.05$  compared to the metabolic syndrome group, <sup>a</sup> indicates  $p < 0.05$  compared to the glibenclamide group,  $\delta$  indicates  $p < 0.05$  compared to the low dose allopurinol group, † indicates  $p > 0.05$  compared to the low dose allopurinol group; one way ANOVA, followed by Tukey's multiple comparisons test.



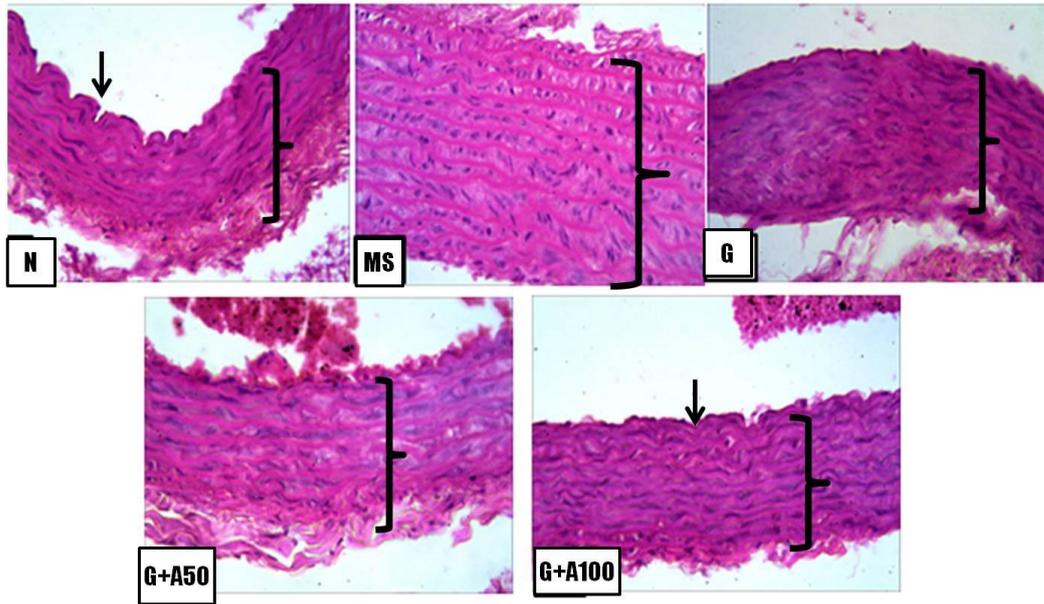
**Fig. 3: Effects of allopurinol co-administered with glibenclamide on vascular reactivity of isolated aortic rings.**

(a) shows maximal contractile responses ( $E_{max}$ ) to phenylephrine, (b) shows the mean effective concentration 50 ( $EC_{50}$ ) and (c) shows percent of relaxation of the aortic rings in response to acetylcholine. Data are presented as mean  $\pm$  SD,  $n = 6$  rats/group. MS, metabolic syndrome; G, glibenclamide 0.5 mg/kg; G + A 50, glibenclamide 0.5 mg/kg + allopurinol 50 mg/kg; G + A 100, glibenclamide 0.5 mg/kg + allopurinol 100 mg/kg. # indicates  $p < 0.05$  compared to the normal control group, \* indicates  $p < 0.05$  compared to the metabolic syndrome group, <sup>a</sup> indicates  $p < 0.05$  compared to the glibenclamide group,  $\delta$  indicates  $p < 0.05$  compared to the low dose allopurinol group; one way ANOVA, followed by Tukey's multiple comparisons test.



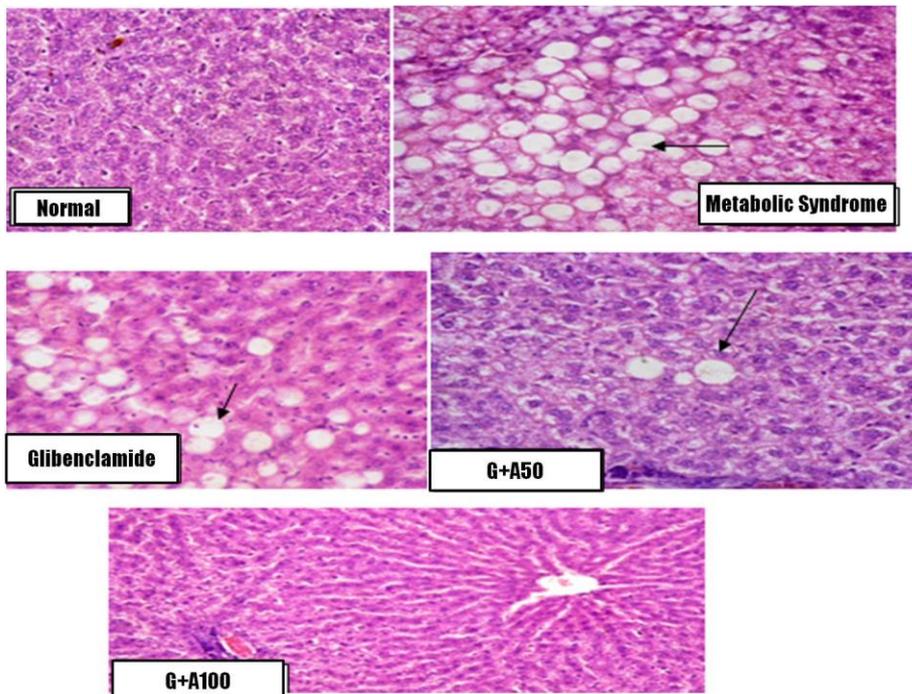
**Fig. 4: Effects of allopurinol co-administered with glibenclamide on intima/media ratio of aorta.**

Data are presented as mean  $\pm$  SEM,  $n = 6$  rats/group. MS, metabolic syndrome; G, glibenclamide 0.5 mg/kg; G + A 50, glibenclamide 0.5 mg/kg + allopurinol 50 mg/kg; G + A 100, glibenclamide 0.5 mg/kg + allopurinol 100 mg/kg. # indicates  $p < 0.05$  and  $\dagger$  indicates  $p > 0.05$  compared to the normal control group, \* indicates  $p < 0.05$  compared to the metabolic syndrome group, <sup>a</sup> indicates  $p < 0.05$  compared to the glibenclamide group; one way ANOVA, followed by Tukey's multiple comparisons test.



**Fig. 5: Histological pictures of aortae isolated from different animals**

N, normal control group, appearance and thickness of the aorta with normal endothelial corrugation. MS, metabolic syndrome group, loss of corrugation of the endothelium with apparent increase in the intima/media thickness. G, glibenclamide treated group (0.5 mg/kg), reduction in thickening of wall of aorta. G+A 50, group treated with glibenclamide (0.5 mg/kg) + allopurinol (50 mg/kg), showing reduction in thickening of wall of aorta with mild effect on endothelial corrugation. G+A 100, group treated with glibenclamide (0.5 mg/kg) + allopurinol (100 mg/kg), marked reduction in thickening of wall of aorta and moderate corrugation of endothelium. Arrows point to endothelial corrugation and braces indicate thickness of the wall of the aorta.



**Fig. 6: Histological pictures of liver sections**

Normal: control group showing normal appearance of the hepatic tissue. Metabolic syndrome group, showing marked macrovesicular steatosis. Glibenclamide treated group (0.5 mg/kg), showing reduction of macrovesicular steatosis. G+A50, group treated with glibenclamide (0.5 mg/kg) + allopurinol (50 mg/kg), showing further reduction of macrovesicular steatosis. G+A100, group treated with glibenclamide (0.5 mg/kg) + allopurinol (100 mg/kg), showing marked reduction in macrovesicular steatosis. Arrows point to macrovesicular steatosis.

#### 4. DISCUSSION

It is not clear whether the beneficial effects of allopurinol in metabolic syndrome are due to reduction of circulating uric acid level, or to other factors. In the present work, an attempt was made to determine whether these beneficial effects are correlated to the degree of lowering of serum uric acid or it is related to its effect on serum adiponectin level.

In the present study, co-administration of allopurinol with glibenclamide showed a significant dose dependent improvement in blood glucose, insulin sensitivity index and vascular effects. These vascular effects were associated with a dose dependent improvement of both the intima/media ratio of aorta and hepatic histopathology. In contrast, the effect on serum uric acid level was not related to the doses of allopurinol.

The findings observed with low dose allopurinol, here, support the uric acid related hypothesis. **Nakagawa and his group (2006)** elucidated that uric acid inhibits endothelial function in a dose dependent manner. This was manifested by reduction in vasodilatory response of acetylcholine in aortic rings isolated from fructose fed rats. The same authors stated that reduction of uric acid by either allopurinol or benzbromarone (a uricosuric agent) were shown to lower systolic blood pressure, improve insulin sensitivity, and normalize triglyceride levels. Accordingly, they assumed that uric acid may be the cause of metabolic syndrome. Moreover, **Johnson and his associates (2005)** stated that uric acid-mediated vasoconstriction leads to endothelial dysfunction and activation of the renin-angiotensin system in rodents with deterioration of renal function.

The beneficial effects of allopurinol shown in the present study might be related to its antioxidant effects secondary to inhibition of XOR rather than its effect on uric acid. It is possible that elevated uric acid may be associated with higher level of XOR enzyme with the release of ROS. ROS convert nitric oxide to peroxynitrite, which contributes to the deleterious effect on vascular endothelium and platelets (**Cai and Garrison, 2000**). The possibility that the beneficial effects of allopurinol may involve antioxidant mechanisms rather than lowering uric acid is supported by the findings reported by **George and coworkers (2006)**, who stated that probenecid (a uricosuric agent with no effect on xanthine oxidase) lowered serum uric acid levels to a comparable degree with allopurinol, in a group of patients with heart failure but did not lead to improvement in endothelial function.

In the present work, a high dose of allopurinol induced improvement in some metabolic parameters, endothelial dysfunction and liver macrovesicular steatosis, compared to its lower dose. However, these effects were associated with a less reduction in uric acid. A possible explanation for the diminished effect on uric acid may be related to the reduction in formation of oxipurinol, the active form of allopurinol (**Chung et al., 2008**). The higher levels of triglycerides seen in rats treated with the high dose allopurinol may represent another possible explanation, given that synthesis of fatty acids (triglycerides) in the liver is associated with de novo synthesis of purines, which would consequently accelerate uric acid production (**De oliveira and Burini, 2012**).

The lack of correlation between the ability of allopurinol to lower uric acid levels and its beneficial effects on some metabolic parameters and aorta and liver pathology, throws doubts on a causal role of uric acid in metabolic syndrome. In fact, **Szasz and Watts (2010)** reported that uric acid did not affect acetylcholine-induced relaxation in normotensive and DOCA-salt hypertensive rats. The role of uric acid in metabolic syndrome is quite controversial. **Nakayama and associates (2009)** suggested that the elevation of uric acid in fructose fed mice was a result of tubulointerstitial renal injury associated with metabolic syndrome, rather than a cause. An antioxidant effect of uric acid was even postulated by **Kanemitsu and his coworkers (1988)**. They found an increase of brain uric acid in a rat model of cerebral ischemia. In addition, it was stated that uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury, in vivo (**Yu et al., 1998**). So it is argued that elevated serum uric acid in patients with cardiovascular disorders may simply reflect the presence of other risk factors such as hypertension, diabetes, diuretic treatment, impaired renal function, atherosclerosis and increased oxidative stress (**Dawson and Walters, 2006**).

An alternative mechanism for allopurinol's beneficial effects is increasing adiponectin level with subsequent improvement in insulin sensitivity. The strong association between uric acid and metabolic syndrome led some authors to postulate a correlation between uric acid level in serum and adipose tissues. Lowering uric acid by inhibiting XOR in obese mice with metabolic syndrome were reported to improve the proinflammatory endocrine imbalance in the adipose tissue by lowering production of monocyte chemotactic protein (MCP-1) and increasing production of adiponectin (**Baldwin et al., 2011**). Results of the present work are in favor of this

suggestion, since small dose of allopurinol resulted in significant increase in serum adiponectin and insulin sensitivity compared to the untreated metabolic syndrome group. A further significant improvement in insulin sensitivity was noted with the higher dose. This is in agreement with results obtained by **Ping and his group (2011)**, who found positive correlation between adiponectin and lowering serum uric acid by allopurinol in rats. The elevated level of adiponectin may potentiate the antioxidant effect of allopurinol. This was demonstrated by **Li and associates (2007)**, who stated that adiponectin improves endothelial function in hyperlipidemic rats by reducing oxidative/nitrative stress and differential regulation of eNOS/iNOS activity. However, it should be mentioned that **Wang and associates (2011a)** observed that N-Acetyl cysteine, an antioxidant, but not allopurinol, increased cardiac adiponectin concentration and AdipoR2 expression with reduction of myocardial reperfusion injury in diabetic rats.

**Limitation in study:** The effect of allopurinol as regard serum cholesterol level needs further investigations, since the higher dose of allopurinol showed significant elevation of total and LDL-cholesterol despite the significant elevation of HDL-cholesterol. This effect may be due to adiponectin-mediated increase in catabolism of VLDL (**Qiao et al., 2008**). In this regards, the FDA reported 484 cases of elevation of cholesterol level associated with the use of allopurinol. Most of these cases were during the early years of treatment and resolved later on (**ehealthme**).

## 5. CONCLUSION

Allopurinol could improve some metabolic abnormalities and endothelial dysfunction in a rat model of metabolic syndrome, irrespective of lowering serum uric acid level. The results highlight the possible implication of adiponectin in the mechanism involved in the beneficial effects of XOR. The effects of allopurinol on lipid profile require further investigations.

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