AASCIT Communications
Volume 2, Issue 6
January 26, 2016 online
ISSN: 2375-3803

American Association for Science and Technology

Effect of Ethanolic Extract of *Hibiscus sabdariffa* and 2, 4-Dinitrophenylhydrazine on Kidney Function Parameters of Rats

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Keywords

Hibiscus sabdariffa, Ethanolic Extract, 2, 4-dinitriphenylhydrazine, Kidney Function Parameters, Antioxidant Property

The aim of this study was to evaluate the effects of ethanolic extract of *H. sabdariffa* and 2, 4-dinitrophenylhydrazine on the kidney function parameters of rats. 20 rats were evenly distributed into 4 groups and treatment lasted for a week. For the first six (6) days, the animals in group one (1) and group four (4) were treated with water alone while groups two (2) and three (3) rats received ethanolic extract of *H. sabdariffa* alone. At the end of the 6th day, the animals were fasted overnight and on the 7th day, each animal in groups 3 and 4 received 28 mg per kg body weight of 2, 4-dinitrophenylhydrazine (2, 4-DNPH) alone and after 7 hours they were sacrificed under diethyl ether anesthesia and their serum and kidney samples taken and prepared for biochemical assays to determine their urea, sodium, potassium, bicarbonates, chloride, creatinine and malondialdehyde levels. The high levels of MDA and creatinine observed in the serum of the rats treated with 2, 4-dinitrophenylhydrazine alone relative to their respective controls show the ability of 2, 4-dinitrophenylhydrazine to provoke cytotoxicity in the kidney. It can also be established from this study that the ethanolic extract of *H. sabdariffa* has anti-oxidant property which was shown in the significant reduction in the level of MDA and creatinine in the serum of rats treated with the ethanolic extract *H. sabdariffa* prior to the treatment with 2, 4-dinitrophenylhydrazine when compared to the rats treated with 2, 4-dinitrophenylhydrazine alone.

Introduction

Hibiscus sabdariffa Linn (Roselle) belongs to the family of *Malvaceae*, which is native to old World tropics, probably in the East Indies; now cultivated throughout the tropics (Duke and Atchley, 1984; Duke, 1985). Roselle is used in jams, jellies, sauces and wines. The young leaves and tender stem are eaten raw in salads and chutney. They are also added to curries and some Malaysian dishes as seasoning. The seeds are somewhat bitter but, in Africa, they are ground into meal for human food due to their high protein content. They have also been roasted to use as a substitute for coffee (Morton, 1987). The vegetable is widely grown and commonly used as port herb or soup in the northern part of Nigeria. In Nigeria, especially in the northern part, the extract of the red calyces is consumed as a beverage known as zobo. In northern Nigeria, roselle seeds are fermented in the presence of some spices to prepare a food known as Mungza Ntusa (Balami, 1998). Its seeds also contain a substantial amount of oil that resembles cotton seed oil (Mohammed *et al*, 2007; Mohd-Esa *et al.,* 2010). Studies by Muhammad and Shakib (1995) have shown that roselle can prevent cancer, lower blood pressure and improve the digestive system in humans. Its calyx extract has also been used as an effective treatment for patients with kidney stones due to its uricosuric effect (Prasongwatana *et al*., 2008). In addition to having the above mentioned activities, roselle extract has been reported to possess antioxidant properties. For example, it protects against low density lipoprotein (LDL)-oxidation and has hypolipidemic effects *in vivo* (Hirunpanich *et al.,* 2006). In some instances, it is also used to preserve food. Some studies have reported that *Hibiscus sabdariffa* is effective for decreasing the levels of total lipids, cholesterol and triacylglycerol, suggesting the possibility that *Hibiscus sabdariffa* functions as hypolipidemic agent (Hirunpanich *et al.,* 2006). Our previous reports had supported the claim that the calyx extracts of *H. sabdariffa* possess potent antioxidant principles (Ologundudu *et al*., 2006a, b; Ologundudu *et al*., 2009a, b; Ologundudu *et al*., 2010; Olusola *et al*., 2012a, b; Olusola, 2014.

Phenylhydrazine and its derivatives 2, 4-dinitrophenylhydrazine are toxic agents. Their toxic action has been attributed to their ability to undergo auto oxidation. This increased oxidant potential enables them to oxidize enzymes, membrane proteins and phospholipids (Jain and Hochstein, 1980; Clemens *et al*., 1984; Maduka *et al.,* 2003; Ologundudu and Obi, 2005; Ologundudu *et al.,* 2006a, b; Ologundudu et al*.,* 2009a, b). The work done so far on this research area has focused largely on tissues like the blood, liver and brain with resultant dearth of information on the kidney parameters of the animal models.

Thus, this work was carried out to evaluate the effect of ethanolic extract of *H. sabdariffa* and 2, 4-dinitrophenylhydrazine on the kidney function parameters of rats.

Methods

Preparation of the Ethanolic Extract

Dried flowers of *Hibiscus sabdariffa* were purchased at the market in Ikare Akoko, Ondo State, Nigeria. Detached calyces from the *Hibiscus* flower were washed and dried until constant weight. After drying, the calyces were macerated using a laboratory blender to reduce the calyces to powder. 25 g of the blended *Hibiscus sabdariffa* calyces was measured using an analytical balance and transferred into the thimble of a soxhlet apparatus for extraction using 200 ml of ethanol as the extracting solvent at 65°C. The extraction process lasted for 12 hours, and the extract was then concentrated using a rotary evaporator and thereafter freeze-dried. The dried extract obtained was weighed and then resuspended in 100 ml of distilled water and kept in the refrigerator until required.

Treatment of Animals and Toxicant Administration

Twenty pathogen-free rats with initial mean weight between 50-80g obtained from the Biochemistry Department of University of Ibadan, Oyo State, Nigeria, were used for this study. The rats were acclimatized on guinea grower feed for a month. The rats were weighed after one month and then evenly distributed into 4 groups of 5 animals each in galvanized cages. The animals had access to tap water and grower's mash *ad libitum* throughout the experiment.

Group I (control): received 2.5 ml/kg body weight distilled water twice daily by gavage for 7 days. Group II: animals received 100 mg/kg body weight of the ethanolic extract of *H. sabdariffa* for 7 days.

Group III: animals received 100 mg/kg body weight of the ethanolic extract of *H. sabdariffa* for 7 days + 28 mg/kg body weight of 2, 4-dinitrophenylhydrazine at the end of the 7th day after overnight fasting.

Group IV: treated with 28 mg/kg body weight of 2, 4-dinitrophenylhydrazine after overnight fasting.

Collection and Preparation of Blood and Kidney Samples

At the end of the treatment, the animals were anaesthetized inside di-ethylether chamber and the thoracic regions were opened to expose the heart. The blood samples were obtained through cardiac puncture into EDTA bottles. Plasma samples were subsequently obtained by centrifugation at 3000 rpm for 10 minutes and stored in the laboratory refrigerator at 4°C until they were needed.

The kidneys were also quickly excised and homogenized in ice cold normal saline (1:4 wt/vol) and centrifuged at 3500 rpm for 15 minutes. The supernatants were transferred to labelled bottles and also stored at 4°C.

Biochemical Assay Protocol

Lipid peroxidation was determined spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method as described by Varshney and Kale (1990), and was expressed in terms of malondialdehyde (MDA) formed per mg protein. Measurement of absorbance was done at 523 nm. Urea, creatinine, electrolytes (sodium, potassium, chloride and bicarbonate) levels were determined using automated machine, Reflotron plus manufactured by Roche.

Statistical Analysis

The data obtained were subjected to standard statistical analysis of variance (ANOVA) using the SAS software (SAS Inst. Inc. 1999). Treatment means were compared using the Duncan procedure of the same software. The significance level was set at $P<0.05$.

Results

Figure 1 shows the effect of ethanolic extract of *Hibiscus sabdariffa and* 2, 4-dinitrophenylhydrazine on the level of urea in the kidneys and serum of rats. The results show no difference in the level of urea both in the kidneys and serum of rats in any of the treatments relative to control.

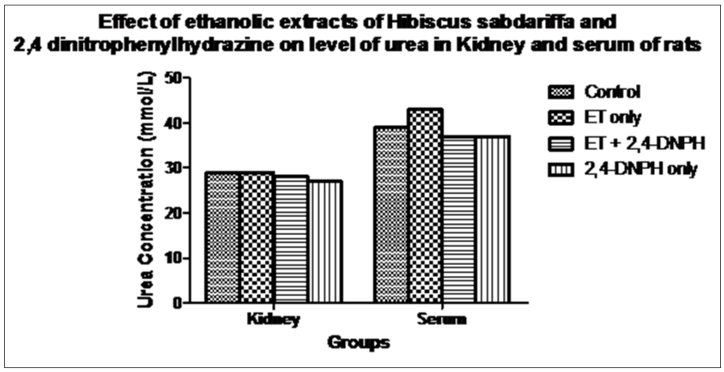

Figure 1. *Effect of ethanolic extract of Hibiscus sabdariffa and 2, 4- dinitrophenylhydrazine on level of urea in kidney and serum of rats. Key: Ethanolic extract (ET), 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH).*

The effect of ethanolic extract of *Hibiscus sabdariffa and* 2, 4-dinitrophenylhydrazine on sodium ion level in kidney and serum of rat is shown in Figure 2. The group treated with 2, 4-DNPH alone did not show any difference in the level of sodium ion when compared with control in the kidney of rats but this same group showed an increase in the level of sodium ion when compared with control in the serum of rats. The group that took the extract of *H. sabdariffa* showed a significant reduction in the level of sodium ion when compared with control in both kidney and serum of rats. Furthermore, the group that was pretreated with the extract before treatment with 2, 4-DNPH did not show any difference in the level of sodium ion in both the kidney and serum of rats when compared with control group. This same group however, showed a reduction in the serum in the level of sodium ion when compared with the group treated with 2, 4-DNPH alone but did not show any difference in the level of sodium ion in the kidney when compared with 2, 4-DNPH alone.

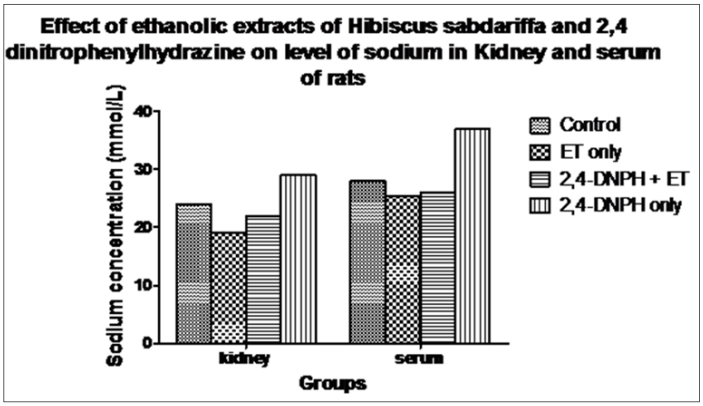

Figure 2. *Effect of ethanolic extract of Hibiscus sabdariffa and 2, 4-dinitrophenylhydrazine on level of sodium in kidney and serum of rats. Key: Ethanolic extract (ET), 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH).*

Figure 3 shows the effect of ethanolic extract of *Hibiscus sabdariffa and* 2, 4-dinitrophenylhydrazine on the potassium level in the kidney and serum of rats. Relative to control, treatment with DNPH alone showed a reduction in the level of potassium in both serum and the kidney of rats whereas treatment with the extract alone and prior treatment with the extract before DNPH administration did not cause any alteration in potassium level of the tissues.

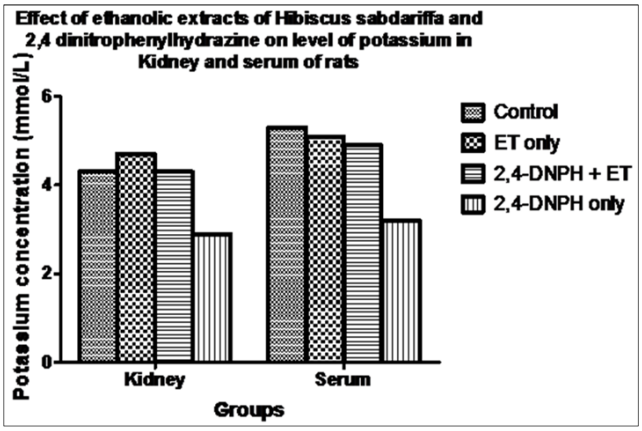

Figure 3. *Effect of ethanolic extracts of Hibiscus sabdariffa and 2, 4-dinitrophenylhydrazine on level of potassium in kidney and serum of rats. Key: Ethanolic extract (ET), 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH).*

The effect of ethanolic extract of *Hibiscus sabdariffa* and 2, 4-dinitrophenylhydrazine on the bicarbonate level in kidney and serum of rat is shown in Figure 4. The group treated with 2, 4-DNPH alone showed an increase in the level of HCO_3^- when compared with control in both kidney and serum while the group treated with the extract alone and the group pre-treated with the extract before DNPH intoxication showed no difference in the level of HCO_3^- when compared with control in both serum and kidney of rats.

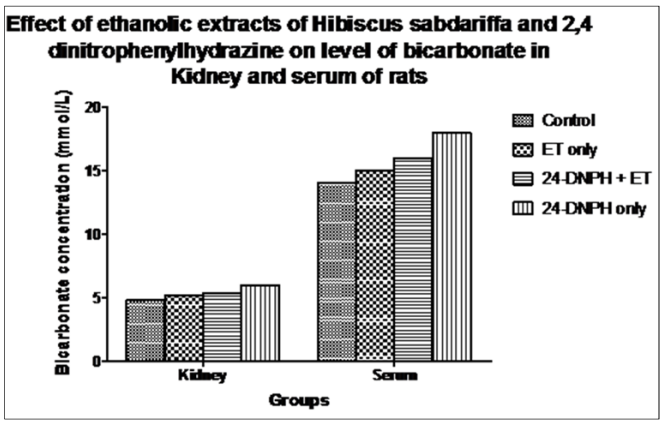

Figure 4. *Effect of ethanolic extract of Hibiscus sabdariffa and 2, 4-dinitrophenylhydrazine on level of Bicarbonate on kidney and serum of rats. Key: Ethanolic extract (ET), 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH).*

The effect of ethanolic extract of *Hibiscus sabdariffa* and 2, 4-dinitrophenylhydrazine on the chloride level in kidney and serum of rat is shown in Figure 5. Treatments in all groups showed no difference relative to controls in both tissues.

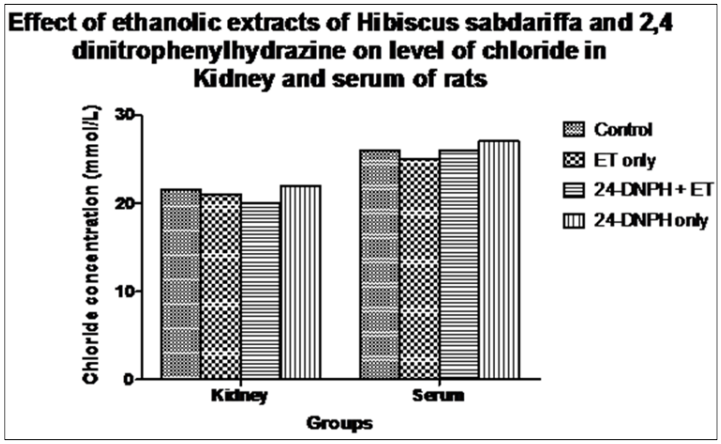

Figure 5. *Effect of ethanolic extract of Hibiscus sabdariffa and 2, 4-dinitrophenylhydrazine on level of chloride on kidney and serum of rats. Key: Ethanolic extract (ET), 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH).*

The effect of ethanolic extract of *Hibiscus sabdariffa* and 2, 4-dinitrophenylhydrazine on the creatinine level in kidney and serum of rat is shown in Figure 6. Relative to the controls, the different treatments showed significant reduction in the level of creatinine in the kidney. In the serum, the group treated with the extract alone and the one pretreated with the extract prior to DNPH intoxication showed no alteration while treatment with DNPH alone produced elevation in creatinine level.

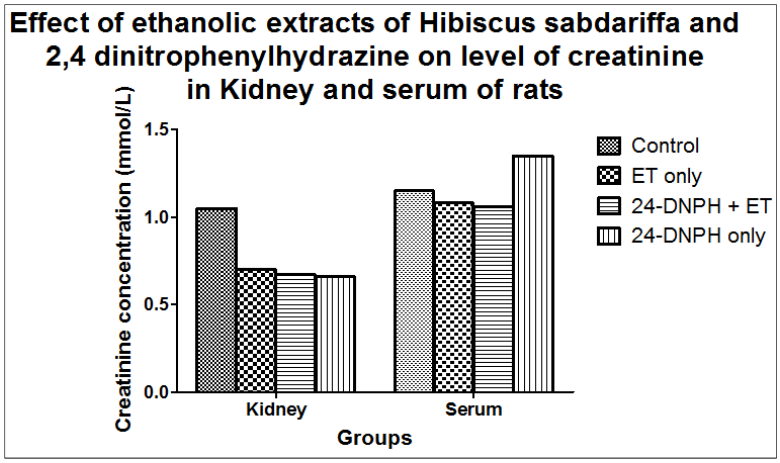

Figure 6. *Effect of ethanolic extract of Hibiscus sabdariffa and 2, 4-dinitrophenylhydrazine on level of creatinine on kidney and serum of rats. Key: Ethanolic extract (ET), 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH).*

The effect of ethanolic extract of *Hibiscus sabdariffa* and 2, 4-dinitrophenylhydrazine on the malondialdehyde level in kidney and serum of rat is shown in Figure 7. The group treated with 2, 4-DNPH alone showed an increase in the level of MDA when compared with control in serum while the group treated with extract alone and the one pre-treated with the extract prior to DNPH treatment showed no significant difference in the level of MDA relative to the control in the serum of rats.

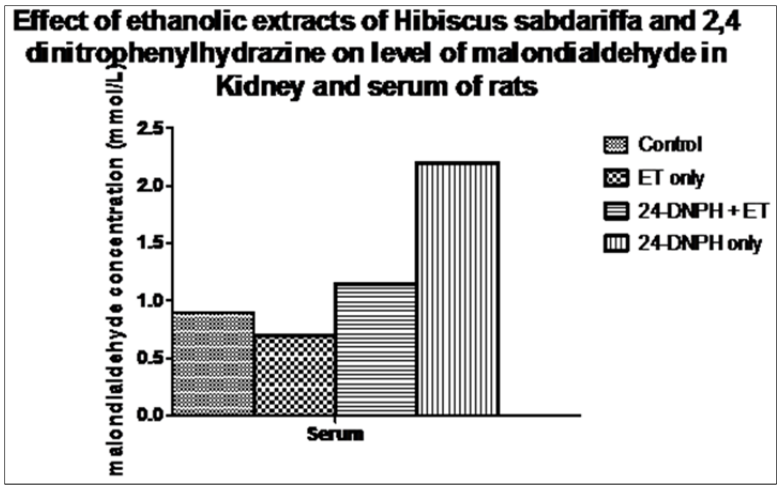

Figure 7. *Effect of ethanolic extract of Hibiscus sabdariffa and 2, 4-dinitrophenylhydrazine on level of malondialdehyde on kidney and serum of rats. Key: Ethanolic extract (ET), 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH).*

Discussion

In recent times, the use of medicinal plants has been on the increase due to their health benefits and ease of availability for food application (Ross, 2003). Urea or carbamide is an organic compound with the chemical formula $CO(NH_2)_2$. The handling of urea by kidneys is a vital part of mammalian metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the counter current exchange system of the nephrons, that allows for reabsorption of water and critical ions from the excreted urine (Walter, 1998). The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood that comes from urea. It is used as a marker of renal function, though it is far less reliable than other markers such as creatinine because blood urea levels are influenced by other factors such as diet and dehydration (Traynor *et al*., 2006). This may be the reason why the different treatments showed no effect on the level of urea in both serum and kidney of rats in this work as shown in Figure 1.

Sodium is found in many foodstuffs. It is necessary for the maintenance of fluid homeostasis. Sodium is also required for nerve and muscle functioning. High levels of sodium can damage the kidneys and increase the chances of high blood pressure. The significantly increased level of sodium in the kidney and serum of rats treated with DNPH alone is an indicator that this substance induces cytotoxic damage in the tissues.

Potassium is a very important mineral for the proper function of all cells, tissues and organs in the human body. Epidemiological studies and studies in animal subjects on high blood pressure and hypertension indicate that diets high in potassium can reduce the risk of hypertension and possibly stroke (by a mechanism independent of blood pressure). Potassium deficiency combined with an inadequate thiamine intake has produced brain damage in children (Folis, 1942; Cruz *et al*., 2012).

Bicarbonate (HCO_3^-) alkaline, a vital component of the pH buffering system of the human body is involved in maintaining acid-base homeostasis. 70-75% of CO_2 in the body is converted into carbonic acid (H_2CO_3), which can quickly turn into bicarbonate. With carbonic acid as the central intermediate species, bicarbonate in conjunction with water, hydrogen ions and carbon dioxide, forms this buffering system. This is maintained at the volatile equilibrium required to provide prompt resistance to drastic pH changes in both the acidic and basic directions. This is especially important for protecting tissues of the central nervous system, where pH changes too far outside of the normal range in either direction could prove disastrous. Bicarbonate also acts to regulate pH in the small intestine. It is released from the pancreas in response to the hormone secretin to neutralise the acidic chime entering the duodenum from the stomach. The observed elevated level of bicarbonate in the group treated with DNPH alone relative to controls in the tissues (Figure 4) indicate that the toxicant could disrupt acid-base homeostasis which may in turn alter metabolic processes especially enzymatic activities. However, treatment with the extract showed no significant effect on the bicarbonate level in the tissues while prior treatment of rats with the extract before DNPH intoxication produced similar effect. It appears that prior treatment of rats with the extract before DNPH treatment significantly mitigated the effect of the DNPH on the bicarbonate level.

Chloride is one of the most important electrolytes in the blood. It helps to keep the amount of fluid inside and outside of the cells in balance. It also helps maintain proper blood volume, blood pressure, and pH of the body fluids. The data presented in this work however, show the treatments had no effect on the chloride levels of rats.

Serum creatinine is an important indicator of renal health because it is an easily measured by-product of muscle metabolism that is excreted unchanged by the kidneys. Creatinine is produced via a biological system involving creatine phosphate and adenosine triphosphate (ATP, the body's immediate energy supply). Creatine is synthesized primarily in the liver from the methylation of glycocyamine (guanidino acetate, synthesized in the kidney from the amino acids arginine and glycine) by S-adenosyl methionine. It is then transported through blood to other organs, muscle, and brain, where, through phosphorylation, it becomes the high-energy compound phosphocreatine (Taylor, 1989). During the reaction, creatine and phosphocreatine are catalysed by creatine kinase, and a spontaneous conversion to creatinine may occur (Allen, 2012). Creatinine is removed from the blood chiefly by the kidneys, primarily by glomerular filtration, but also by proximal tubular secretion. Little or no tubular reabsorption of creatinine occurs. If the filtration in the kidney is deficient, creatinine blood levels rise. The results of this work show the anti-oxidative effect of the ethanolic extract of *H. sabdariffa* against kidney damage induced by 2, 4-dinitriphenylhydrazine.

Malondialdehyde (MDA), one of the major products of lipid peroxidation, has been extensively studied and measured as an index of lipid peroxidation and a marker of oxidative stress (Janero, 1990; Ologundudu *et al.,* 2009a, b). The observed elevation in malondialdehyde level in the serum of rats treated with DNPH alone in this study relative to control can be attributed to ability of 2, 4-dinitrophenylhydrazine to oxidise membrane lipids and proteins which can compromise membrane permeability and integrity leading to cell death (Ologundudu *et al*., 2009a, b; Olusola *et al.,* 2012a, b; Olusola, 2014).

Conclusion

In conclusion, this study showed that 2, 4-dinitrophenylhydrazine exerts some cytotoxic effects on the kidneys. However, the ethanolic extract of *H. sabdariffa* showed ability to ameliorate the oxidative effects of the toxicant. This finding again gives credence to our previous work which showed that the extracts of the plant posses antioxidant principles. However, more work is needed to be able to explain the exact mechanisms involved in 2, 4-ditrophenyhydrazine-induced cytotoxicity and protection offered by extracts of *H. sabdariffa.* ■

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