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Sanay Leaves Treatment in Constipation Rats Restores Defence System in Red Blood Cells

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Summary

The defensive enzymes like glutathione preoxidase, glutathione reductase and catalase along with the defensive metabolites like reduced glutathione and oxidised glutathione were measured in red blood cells of acute constipated rats also along with sanay treated constipated rats. It was observed a decrease in GPx activity in both sexes of rats i.e., males and females. The GR activity in constipated rats was shown to be increased by more than GPx activity. The catalase activity has been observed to be increased insignificantly in acute condition. However, the GSH levels were found to be decreased in acute constipated rats but GSSG levels enhanced in acute condition. The sanay leaves treatment to acute condition rats restored the changed enzymes activities and metabolites levels.

The liver function test carried out by the two enzymes namely GOT & GPT which increased in constipated rats. It shows that the enhanced GOT & GPT activities caused dysfunction of liver and invite kinds of liver diseases. The creatinine levels in red blood cells showed insignificant changes in both acute constipated rats and its treated condition with sanay leaves. It may be concluded that sanay treatment restores the low defense system found in constipated rats as well as the liver function which is slightly changed during constipation.

Introduction

Acute constipation is a slowing of intestinal transit with a decrease in bowel movements and the appearance of dehydration. The person feels difficulty in defecating or may not be able to defecate all. Constipation is presently found in most people who have a different life-style and makes life miserable to feel of bloated, headachy, and irritable condition. Each year in the U.S., chronic constipation leads to around 2.5 million doctor visits and medication costs of many hundreds of millions of dollars.

Acute constipation includes female sex, older age (McCrea et. al., 2009), inactivity, low caloric intake, low-fiber diet (Wald et. al.,1989; Rao S., 2007), taking a large number of medications (Tally et. al., 2003) etc., The incidence of constipa¬tion is three times higher in women and are twice as likely as men to schedule physician visits for constipation (Sonnenberg and Koch, 1989; Gleason et. al., 1972; Johanson et. al., 1989).

Constipation alleviates a few metabolic disorders like gases formation, indigestion, less appetite, liver dysfunction, laziness, less immune system, etc. The reduced glutathione plays a major role in the regulation of the intracellular redox state of vascular cells by providing reducing equivalents for many biochemical pathways (Combs, G. 2004; Copeland and Driscoll, 1999). In the absence of adequate GPx activity or glutathione levels, hydrogen peroxide and lipid peroxides are not detoxified and may be converted to OH-radicals and lipid peroxyl radicals respectively. The GPx/glutathione system is thought to be a major defense in low-level oxidative stress.

GSH protects the cell caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals (Mullineaux and Creissen, 1997). The GSH:GSSG ratio is therefore an important bio-indicator of cellular health, with a higher ratio signifying less oxidative stress in the organism. The present paper described about the effect of sanay leaves extract preparation in hot water so that only extract has been used in treating acute constipation with determining the defence of red blood cells, liver dysfunction and kidney health in experimental constipated rats. Considering these facts the blood parameters like the defensive enzymes GPx, GR and catalase which are key of enzymes in RBCs may defend against enhance oxidative stress during constipation. The liver dysfunction has been characterized and tested by the levels of GOT and GPT and the kidney health are determined by the levels of creatinine.

Methodology

Development of acute constipation

Wistar rats with a mean weight 140±10gm. were obtained from the experimental animal house of Jamia Millia Islamia (JMI) in the laboratory of Prof. S A Husain, Department of Bioscience. Rats were grouped into three categories: (a) Control (C) (b) Acute Constipation (AC). (C) Acute constipation rats treated with sanay extract (AC + S).

Constipation was developed in experimental rats by giving oral administration of 1ml of loperamide with a dose of 3mg/kg body weight for three days (Hughes S et.al., 1984). The stools were tested and found to be hard with less quantity and mostly devoid of water. The fourth day after loperamide administration, the day first was counted for constipation duration.

The experimental animals were kept for one month for acute constipation out of twenty experimental rats ten rats were kept untreated and ten rats treated with sanay leaves and 5 rats for control.

The constipation rats were daily monitored like observing the stool quality and its amount, abdomen enlargement and activeness of the animal. The constipation rats were not given any type of fibrous food. The feeds were made of rice pellets and leafy vegetables. The treated rats were given standard food pellets and other vegetables.

Sennea leaves (Cassia angustifolia) extract preparation

3-4gm. of senna leaves were added to 100 ml of boiling for one minute. It was then cooled down and filtered. This filtered water (supernatant) is ready for treatment to constipated rats to drink from water pipe.

Treatment to constipated rats

Sanay leaves extract was given to experimental constipated rats in a clean bottle especially during night time because rats are nocturnal animals. During the day time, the normal water was given to rats. Sanay leaves extract was given to constipated rats on alternate day for one month of treatments.

Blood collection from rats

The blood was collected from Wistar rat's caudal tail commonly used because through sampling minimum pain or invasiveness. The tail is first of all washed with warm water so that the vein is visible. The needle of the syringe is punched in the vein and then piston of the syringe was slowly lifted so that the blood is sucked from the vein. Only 2ml of blood was collected from one rat in a syringe washed with anticoagulant i.e. Na_2 EDTA. The blood is then transferred to a centrifuged tube having 2ml of normal saline.

Isolation of Red blood cells from blood

The blood sample was centrifuged in Sorvall centrifuged machine at 3000 rpm 5 minute for the separation of RBCs. These cells were washed three times with cold phosphate buffer saline (Gupta and Baquer, 1998).

Preparation of RBC hemolysate

The hemolysate was prepared by forceful lysis with phosphate buffer (5 mM, pH 8.0). The hemolysate was used for the assay of enzymes and metabolites. The forceful lysis was done by taking 1 ml of RBC in 50ml of test tube with 39ml of phosphate buffer was taken in a syringe. It becomes 1:40 hemolysate.

Blood Hemoglobin Measurement

The blood is diluted in a solution containing potassium ferricyanide and potassium cyanide. Potassium ferricyanide oxidizes the iron in heme to the ferric state to form methemoglobin, which is converted to hemiglobincyanide (HiCN) by potassium cyanide.

Measurement of enzymes activities
Glutathione peroxidase (E.C 1.11.1.9)

The activity of glutathione peroxidase was assayed by the method of Beutler (1976). All the ingredients in the final concentration were namely 0.1M Pot. Phosphate buffer pH 7.0,1mM GSH, 4mM EDTA, 3U glutathione reductase, 4mM sodium azide, 0.2mM NADPH and 50µl of 1:40 erythrocytes hemolysate were taken in 1ml cuvette. The blank without GSH and the assay cuvette were incubated at 37°C for 10 minute in a water bath. The reaction was started by the addition of 0.1mM tert-butyl hydroperoxide (tBHP) and the decrease in absorbance was followed at 340nm in a Shimadzu 260 spectrophotometer (Gupta, et.al., 2004).

Additional blanks were also taken in which all ingredients were present except GSH and blanks without tBHP were also used. Additional blanks were carried to substract this value from the assay for interference of other non specific peroxidases.

Glutathione Reductase (EC 1.6.4.2)

The activity of glutathione reductase was assayed at 25°C by the modified method of Erden and Bor (1984). 3µ1 of packed erythrocytes was added to the assay mixture containing the following in the final concentration of : 4.1 mM Tris-HCl buffer pH 7.5, 15mM $MgCl_2$, 5.7mM EDTA, 60mM KCl, 0.017% Saponin, 2.6mM GSSG and 0.2mM NADPH in 1.0 ml final volume. The decrease in absorbance was measured at 340nm after 10 minute of incubation against blanks containing all ingredients except GSSG (Gupta et.al., 2004).

Catalase (EC 1.11.1.6)

The catalase activity was measured by the method of Beutler (1986), within half an hour of 1:2000 hemolysate preparation. 2ml of assay mixture contained the following in the final concentration of 0.1ml Tris-HCL/EDTA, 0.1ml, pH 6.5; 1ml 10mM H_2O_2 and 0.8ml distilled water. The mixture was incubated at 37°C for 10 minute. 0.1ml of 1:2000 hemolysate was added to start the reaction and the change in absorbance was read at 230nm.

Glutamate Oxaloacetate Transaminase (GOT) (EC 2.6.1.1)

Glutamate Oxaloacetate Transaminase (GOT) were measured by the method of Bergmeyer and Bernt (1974) for AsAT assay the reaction mixture in 1.5 ml in a final concentration contained potassium phosphate buffer 80 mM, pH 7.4, L-aspartate 66 mM, pH 7.4, malate dehydrogenase (dialysed) one unit; 2mM NADH and 10 ml of 1:40 diluted hemolysate. The reaction was started by adding 2-oxoglutarate 12 mM, pH 7.4 and the change in absorbance was measured at 340 nm.

Glutamate Pyruvate Transminase (EC 2.6.1.2)

AlaAT was measured by the method of Bergmeyer and Bernt (1974) by taking the following in the final concentration of assay mixture: potassium phosphate buffer 80 mM, pH 7.4; L-alanine 200 mM, pH 7.4 lactate dehydrogenase (LDH) dialysed 2 unit; NADH 2mM and 50 ml of 1:40 diluted hemolysate. The reaction was started by adding 2- oxoglutarate 0.18 mM, pH 7.0 and the change in absorbance was measured at 340 nm.

Unit of the Enzyme Activity

One unit of enzyme activity was defined as one µ mole of NADPH oxidized per unit time per gm of hemoglobin.

Measurment of metabolites by spectrophotometer
Reduced gluthione (GSH)

Reduced gluthione (GSH) was measured by the method of Griffith (Griffith 1980). The reaction mixture contained the following in a final concentration of: 0.20 mm NADPH, 0.6 mm 5,5¢ - dithiobis- (2-nitrobenzoic acid, 0.5U glutathione reductase in 125 mm sodium phosphate buffer (pH 7.5), 6.3mm EDTA and appropriate sample volume. The rate of reduction of 5,5-dithiobis- (2-nitrobenzoic acid) was measured at 412nm.

Oxidised Gluthione (GSSG)

GSSG level was assayed by the method of Beutler (Beutler 1986). 0.01ml of packed red cells was mixed with 20 µ1 of 0.25mM N-ethyl maleimide. This was precipitated with 30% TCA and the supernatant was extracted three times with ice-cold diethyl ether. The remaining diethyl ether was remover by passing a stream of air. The assay was performed in one ml of cuvettes containing DTNB buffer ($Na2HPO_4$, 0.1M, pH 7.2; EDTA,10mM;BSA, 0.04% and DTNB, 0.2mM); HCl, 1mM; NADPH 2mM and glutathione reductase, 3 U/ml. the calculation was performed by making a standard curve of GSSG (10nM - 100nM).

Measurement of serum creatinine by kits purchased from Ank Cares Pvt. Ltd., ahmedabad, Gujrat, India.

Results

General Parameters changes after sanay leaves extracts treatments.

In the beginning of experiments, particulary one month of constipation it was observed that there is a body weight loss in the sanay leaves extract treatment to constipated rats. It was also observed that the stools were hard and less in amount which normalized in sanay treated condition. All these changes have been presented in Table 1.

		Acute constipated Rats (AC)	*Treated with Sanay leaves extract*
Body Weight		176 ± 14	172 ± 12
Stomach size (in inch)		Increase	Normal
Abdomen Swelling		Belly bulged	Normal
Stool Quality	Amount	Less and Hard	Normal
	Shape and size	Hard and oval size	Normal

Table 1: Physical parameters of acute constipations rats and treated with sanay leaves extract.

Changes of Enzymes Activities in Red Blood Cells and Sanay leaves treatments.

Glutathione Peroxidase

It was observed that the glutathione peroxidase (GPx) activity in red blood cells of control was less in female in comparison to male rats. In acute constipation, the glutathione peroxidase activity was decreased by 12% in the case of male rats and 8% in female rats respectively. The sanay leaves extracts treatments to acute constipated rats normalized of the enzyme activity in both male and female rats. All these changes have been presented in Table 2.

Enzymes	Male			Female		
	Control	Acute Const.	AC + Sanay	Control	Acute Const.	AC + Sanay
GPX	108.8±11.7	95.6±9.1[a]	106.2±14.4	105.3±14.2	96.1±10.5[b]	103.6±12.5
GR	1.36±0.32	1.56±0.36[a]	1.31±0.18	1.27±0.41	1.45±0.2[b]	1.26±0.21
GOT	106.4±11.2	116.3±12.1[b]	108.3±17.8	108.3±15.4	119.4±18.6[b]	109.1±13.6
GPT	58.3±6.4	64.3±3.8[b]	60.2±3.3	55.7±8.8	66.7±3.4[b]	58.4±3.4
Catalase	6.2±0.46	6.4±0.5	6.2±0.2	6.2±0.46	6.4±0.5	6.2±0.2

The enzyme activity is expressed as μ mol/min/g Hb. Values are Mean ± SEM of 18 exprimental rats. Fisher's 'p' values are shown as a. p<0.001 b. p<0.05

Table 2: The levels of defensive enzymes (GPx, GR & CAT), GOT & GPT in RBCs of acute constipated rats and its treatment with sanay leaves extracts.

Enzymes	Male			Female		
	Control	Acute Const.	AC + Sanay	Control	Acute Const.	AC + Sanay
GSH	7.8±0.6	6.5±0.4[b]	7.3±0.5	6.7±0.7	6.2±0.6[b]	6.4±0.6
GSSG	0.18±0.01	0.21±0.03[b]	0.19±0.03	0.21±0.03	0.24±0.02[b]	0.22±0.06
Creatinine	1.32±0.04	1.34±0.05	1.32±0.06	1.31±0.06	1.33±0.06	1.32±0.08

The values of GSH expressed as um/gm Hb for GSSG and GSH; Creatinine as ml/minute. Values are p of Mean±SEM of 18 experimental rats (9 males & 9 females). Fisher's p values are shown as a. p<0.001 b. p<0.05

Table 3: Levels of GSH, GSSG and Creatinine in acute constipation of male and female rats and its treatment with sanay leaves extract.

Glutathione Reductase

The glutathione reductase activity in red blood cells of control was same in both male and female rats. In acute constipation male and female rats the activity of glutathione reductase increased by 14% in RBCs of constipated rats.

Glutamate Oxaloacetate Transaminase (GOT)

The activity of GOT was measured in red blood cells of acute constipation condition of rats for 3 months duration. It was found that the enzyme activity was observed to be increased by 10% approximately in both male and female acute constipation rats red blood cells which was reversed to normal after sanay leaves extract treatments.

Glutamate Pyruvate Transaminase (GPT)

Glutamate pyruvate transaminase (GPT) activity was measured in red blood cells of acute constipated rats, the GPT activity was increased by 19% in the case of female rats and 10% in male rats respectively. The sanay leaves extracts treatments to acute constipated rats reversed the enzyme activity to normal in both male and female rats.

Catalase

The catalase (CAT) activity was found to show an insignificant change of the enzyme activity and its treatment with sanay leaves extracts.

Changes of Metabolites Level in Red Blood Cells and Sanay leaves treatments.
Reduced Glutathione (GSH)

In acute constipation, the GSH level decreased by 16% in the case of male rats and 7% in female rats respectively. The sanay leaves extracts treatments to acute constipated rats reversed the GSH levels to normal in both male and female rats.

Oxidised Glutathione (GSSG)

In acute constipation, the GSSG levels were increased by 16% in the case of male rats and 14% in female rats respectively. The sanay leaves extracts treatments to acute constipated rats normalized the GSSG levels in both male and female rats.

Creatinine

In acute constipation, the creatinine levels were shown insignificant increase in the case of both male and female rats. The sanay leaves extracts treatments to acute constipated rats did not show any change in creatinine levels.

Discussion

Constipation has been cosmopolitan in nature all over the world. It is mainly due to changes in the lifestyle and food habits. In the field of constipation, a little work has been documented till date for its definition, causes, symptoms, and treatments. The holding of stool in the large intestine for a longer time shows toxicity in the body by involving toxic gases like sulphur dioxide, ammonia, NO_2, etc. The consequences of held up of stools in the large intestine has been little documented so far.

A longer duration of constipation leads to liver and kidney toxicity as reported by other authors that sanay leaves powder used in experimental animals and humans included laxative compounds and also other compounds. In our experimental design the sanay leaves were boiled so that there is no question of development of microbes and intake of these ingredients. Only filtered extracts of sanay was used. The other products of sanay leaves remained with boiled leaves. This design of treating constipated with sanay leaves observed a least toxicity for a longer duration of time. Sanay leaves have been thought to be effective as a laxative for treating constipation. The side effects of this leaves have been defined as acute hepatoxicity, renal impairment and skin breakdown, etc (Seybold et. al., 2004 ; Spiller et.al. 2003). But as the observations carried out in the treatments, when senna leaves receive moisture and air and broken down into pieces with the mucilaginous property. This may be due to rich contents of carbohydrates and proteins which may invite bacteria and fungi to grow fastly and destroy the leave components as observed in the packaging where sennosides yield decreased (Upadhayay et.al., 2011). Therefore, the powder and tablets forms of Senna may not be beneficial due to these infections. The hot water preparation of Senna leaves in our present study may be beneficial in two ways, firstly hot water destroys bacteria and fungus which infected senna leaves and secondly, the constipated rats do not take other contents of senna leaves which remained in the leaves itself after boiling in water. Senna may be indirectly adding beneficial effects after controlling constipation. In spite of relieving from constipation, senna leaves also corrected the skin infection, liver disorder and hence, increasing the defence system, improving the neurological disorders like legs pain as peripheral neuropathy (Yamada et.al.,2017) and physiological disorders like swelling of legs and cheek.

As presented in Table 1, the quantitative parameters like body weight of constipated rats decreased about by 5%. It may be postulated that a decrease in body weight by sanay leaves treatment may be decreasing the inflammation of the intestine/gut which is due to the effects of constipation. The treatment of constipated rats with sanay leaves led to normalising the stomach size and abdominal swelling throughout the experiments. It may be interpreted that the holding of stool in the intestine is never existed after relieve from constipation.

Also it has been observed that glutathione proxidase activity in red blood cells in male is more than in female rats throughout the experiments. It shows that the defence system is more active in males than females. The causative factors are not known but it may be presumed that hormones may play an important role in males and females separately. The enzyme activity was observed to be normalised after senay treatment. The gastric problems also got corrected completely by sanay leaves extracts.

The GPx activity is decreased, more hydrogen peroxide is present, which leads to direct tissue damage and activation of inflammatory pathways (Yu and Chung, 2006). The GPx activity also decreases with age (Trevisan et.al., 2001; Artur et.al., 1992). Low GPx activity appears to be predictive of cardiovascular events in individuals with suspected coronary disease (Blankenberg et.al., 2003). The GPx provides an effective mechanism against cytosolic injury because it eliminates H2O2 and lipid peroxides by reduction utilizing GSH (Burton and Jauniaux, 2010). The glutathione reductase activity is important protein in disease condition to restore the defence system of the body. In our experimental design it has been observed that this enzyme is more activity in males in comparison to females. If the glutathione reductase is more than it may be inferred that the production of hydrogen peroxide is more. At one hand glutathione peroxidase is compatible to detoxify hydrogen peroxide levels and on the other hand glutathione reductase activity is to produce hydrogen peroxide. In such a situation constipation leads to formation of more GSSG. Both GOT & GPT activities increased in acute constipation. This shows that liver function during constipation was little damaged. The protein break down is more in this condition and it may be presumed that these progressive products like urea and uric acid may be more in this condition. The sanay leaves treatment not only corrected the constipation condition but also stimulates the liver function which is the the main gland in our body.

Glutathione is involved in several defence processes against oxidative damage (Siegers and Younes, 1983; Andreoli et.al., 1986). One of the main mechanisms by which an oxidative stress would lead to cell damage is the peroxidative cleavage of polyunsaturated fatty acids in the phospholipid bilayer of cell membranes (Younes and Siegers, 1984).

It has been observed in our present investigation that an increase in oxidised glutathione levels in red blood cells may either leads to extracellular transported or converted into reduced glutathione (Deponte, M. 2013). Though, it has been observed in our research that excess amount of oxidized glutathione may show injurious to cell structure, integration and function.

Conclusion

The defensive health of RBCs determines the influential effect on good health of liver and kidney where liver perceived a little damaged by enhancing GPT with no effect on kidney as its creatinine levels unchanged. The RBCs received a damage in defence system by decreases in GPx and GSH majorily which can predict the wellnesses of liver and kidney after sanay treatments to constipated rats.

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