**In vivo** antidiabetic evaluation of nanoparticles encompass dual bioflavonoid

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**Article History:**

Received on: 11 May 2018
Revised on: 15 Jun 2018
Accepted on: 27 Jun 2018
Published on: 09 Jul 2018

**Volume:** 3 **Issue:** 1

**Keywords:**

Diabetes Mellitus, worldwide, hyperglycemic, medicine, bioflavonoid 7& nanoparticles

**ABSTRACT**

Out of all the illness, Diabetes Mellitus (DM) is that the leading cause for morbidity and mortality worldwide. The overall international incidence of Diabetes mellitus is a rise from 171 million in 2000 to 366 million in 2030 as a result of various genetic level, fashionable life vogue factors like fatness, nutrition physical inactivity, ageing & stress. Hence hyperglycemic circumstance of diabetes patient is untreatable; however, the blood sugar level is controlled by daily administration of a dose of medicinal drug and medicine. At this juncture the isolates of the plant are used, within the gift study to use and make sure the possible medicinal drug and inhibitor activity mistreatment numerous in vivo models. Throughout this study, the associate initiative has been taken to check the activity of novelized preparation in addition to a pure drug to determine the efficiency of preparation. The work additionally designed to comprise a comparative illustration of activity of pure bioflavonoid, single loaded isolate nanoparticles and dual loaded nanoparticles. Therefore the primary aim of the analysis is to arrange Apigenin (Ap NP), Hesperidin (Hs NP) associated Apigenin -Hesperidin (Ap-Hs NP) twin loaded flavonol compound nanoparticles to beat the restrictions of Apigenin and Hesperidin synergistically augment of the therapeutic worth. Efficacy antidiabetic study was observed slightly decrease blood glucose level in diabetic rats treated with pure compound and with Ap and Hs loaded polymeric nanoparticles as compared to diabetic control rats.

**INTRODUCTION**

Diabetes is a metabolic disorder in which the metabolism of carbohydrates, proteins and fats are affected. In Diabetes, imbalance in glucose homeostasis is seen, which is of two types which include lack of enough insulin production or the lack of response to the secreted insulin by the body cells [1]. Excess urine formation, increase in the frequency of urination, either reduced or increased thirst and hunger are some of the common symptoms of Diabetes mellitus. There is a gradual increase in the number of diabetic patients since the last few decades [2]. In 1980’s the global prevalence of Diabetes of age group over 18 years was found to be 4.7% and it raised to 8.5% in 2014 and mortality is due to elevated blood glucose level before 70 years of age. When compared to synthetic compounds for treating human diseases, the usage of herbal products became more fascinated mainly because of two
reasons, one is safety profile, and the other one is the affordable price [3, 4]. The bioactive compounds are responsible for the pharmacological activity which has same/more therapeutic index. The bio-active compound, which is responsible for antidiabetic action, is seen in a large number of plants among which the bioactive compounds of 400 plants exhibited type-antidiabetic potential. In the last decade, 49% of all approved drugs are plant-derived drugs having antidiabetic potential [5, 6]. Nanomaterials have been studied widely because of its extensive application in nanomedicine for diagnosis, monitoring, treating and preventing disease. Properties of nanomaterials such as the large surface area to volume ratio and dominance of quantum effects at the nanoscale which are lacking in bulk materials. These two effects are responsible for the enhanced properties like strength, reactivity, strength, optical, magnetic and electrical characteristics and in vivo behaviour of nanomaterials. Some drugs are not efficiently absorbed and distributed in the body because of poor bioavailability which results in decreased systemic concentration for exhibiting the pharmacological action. Because of this, a nanoparticle drug delivery system can be used to change the pharmacokinetic properties of the drugs, which will reflect on pharmacodynamic activity in therapeutic application. Nano formulations are advantageous when compared to conventional formulations which include enhanced solubility and bioavailability, sustained drug release, targeted drug delivery, reduced doses and minimum possible side effects. Different polymer-based and lipid-based nanoparticulate systems have been developed for facilitating the delivery of therapeutic agents and various imaging agents which includes nanoparticles, nanogels, polymersomes, dendrimers, liposomes, polymeric micelles and solid lipid nanoparticles [7, 8]. Our present study focuses on synthesis and characterization of biocompatible and biodegradable polymeric nanoformulations which include both nanoparticles and nanocapsules, and evaluation of antidiabetic potential of bioactive compounds by comparing with metformin taking it as standard. In this study, we also investigated the antidiabetic potential of the combined nanoformulation of two bioactive compounds. When submicron-sized polymeric colloidal particles in which therapeutic agent is embedded or encapsulated within the polymeric matrix or conjugated or absorbed onto the surface, it is termed as polymeric nanoformulation. Morphological features such as nanospheres or nanocapsules will be decided by the method following for the preparation of nanoparticles. Polymeric nanoformulations have been widely used for encapsulation of either hydrophilic or hydrophobic bioactive compounds to protect them from degradation, enhancing the efficacy, drug loading, bioavailability and controlled release feature to the drug which can be attained by prolonging the residence time between the therapeutic molecule and biomembrane [9, 10].

MATERIALS & METHODS

Wistar rats of 150-200 gms body weight were used in our study. Animals were procured from Institutional Animal house of Dr Samsun immune clinical Research Laboratory, Coimbatore. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27°C and humidity 60-65% with 12:12 light: dark cycles. Food was provided in the form of dry pellets and water ad libitum). All the experiments were conducted between 9.00 and 15.00 hours. The animals were allowed to get acclimatized to the laboratory conditions for seven days before the commencement of the experiment. Food was withdrawn 18 hours before the beginning of the experiment. All experiments involving animals comply with the ethical standards of animal handling and approved by the Institutional Animal Ethics Committee (Regd.No: 882/04/ABC/CPCSEA/27). The study included eight groups containing six healthy adult Wistar rats (150-200 gms) in polypropylene cages layered with husk and maintained in controlled room temperature at (22±3°C) and light (12 hours light/dark cycle). Animals were provided free access to water and standard pellet diet. Rats were chosen "as per laboratory animals" (NIH 1985), and the study was conducted in agreement with the board for the function of manage and supervision on experiments on animals (CPCSEA). The animals were randomly chosen and made a mark to consent to identify the rats.

Hence then, kept in their cages for at least five days before dosing for accommodation to the laboratory. Diabetes was induced to the animal using Streptozotocin. Streptozotocin was mixed with 0.1 Molarity trisodium citrate buffer (pH - 4.5) and injected intra-peritoneally at the dose of 40-50 mg/kg body weight. However, the animals were allowed to drink a 5% glucose solution overnight to avoid hypoglycaemia. After 48hrs in Streptozotocin injection, the animals are having fasting blood glucose level more than 300 mg/dl were fixed as diabetic rats and utilized. All the experimental rats fasted overnight, and the blood was taken from retro-orbital sinus on 0 days. Animals in Group 1 (Normal control) received distilled water given orally for 14 days. Animals in Group 2 (Diabetic control) received distilled water given orally for 14 days. Animals in
Group 3 (Standard), received standard drug Glibenclamide orally at the dose of 5mg/kg body weight once daily for 14 days. Animals in Group 4 received pure Apigenin orally at the dose of 50 mg/kg of body weight once daily for 14 days. Animals in Group 5 received pure Hesperidin orally at the dose of 50 mg/kg of body weight once daily for 14 days. Animals in Group 6 received Apigenin loaded polymeric nanoformulation orally at the dose equivalent to 50 mg/kg of body weight once daily for 14 days. Animals in Group 7 received Hesperidin loaded polymeric nanoformulation orally at the dose equivalent to 50 mg/kg of body weight once daily for 14 days. Animals in Group 8 received Apigenin -Hesperidin loaded dual polymeric nanoformulation orally at the dose equivalent to 50 mg/kg of body weight once daily for 14 days. All the experimental rats fasted overnight and blood collected by retro-orbital sinus on 7th day and 14th day to measure glucose level by Glucometer using strips. A significant change in body weight observed during the period of experimental animals [11, 12] .

RESULTS AND DISCUSSION

A significant decrease in the body of diabetic control rats was observed when compared with the normal control group. Diabetic rat treated with pure compound did not influence any substantial changes in body weight in comparison with the diabetic control group. Diabetic rat treated with Ap and Hs loaded polymeric nanoparticles mild changes in body weight in comparison with the diabetic control group. Diabetic rat treated with Ap-Hs(Ap-Hs NP) dual loaded polymeric nanoparticles showed tremendous changes in body weight in comparison with the diabetic control group. Blood glucose level significantly increases in diabetic rats [13, 14] . Efficacy study was observed slightly decrease blood glucose level in diabetic rats treated with pure compound and with Ap and Hs loaded polymeric nanoparticles as compared to diabetic control rats. In the efficacy studies significantly decrease in blood glucose level was observed in diabetic rats treated with Ap-Hs(Ap-Hs NP) dual loaded polymeric nanoparticles contrast to diabetic organize the group.

Blood glucose levels were also found to be significantly decreased in the diabetic rats treated with Ap-Hs(Ap-Hs NP) dual loaded polymeric nanoparticles, and these levels were approximately equal to the Glibenclamide (standard) treated group. These results demonstrate that Ap-Hs(Ap-Hs NP) dual loaded polymeric nanoparticles offers an efficacious oral therapy with reduced dose and dosing inci-
Table 1: Oral glucose tolerance test at different time intervals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>0 min</th>
<th>0.5hr</th>
<th>1 hr</th>
<th>1.5hr</th>
<th>2 hr</th>
<th>2.5hr</th>
<th>3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Carboxymethyl Cellulose(CMC)</td>
<td>0.5%</td>
<td>68.67±</td>
<td>140.8±</td>
<td>169.5±</td>
<td>165.2±</td>
<td>156.5±</td>
<td>148.7±</td>
<td>135.8±</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td></td>
<td>68.33±</td>
<td>102.7±</td>
<td>104.8±</td>
<td>99.83±</td>
<td>95.83±</td>
<td>91.67±</td>
<td>83.67±</td>
</tr>
<tr>
<td>Ap pure</td>
<td>5</td>
<td>68.83±</td>
<td>109.0±</td>
<td>119.3±</td>
<td>113.0±</td>
<td>103.7±</td>
<td>99.83±</td>
<td>94.67±</td>
</tr>
<tr>
<td>Ap nano</td>
<td>5</td>
<td>68.67±</td>
<td>121.3±</td>
<td>129.5±</td>
<td>127.3±</td>
<td>119.8±</td>
<td>113.7±</td>
<td>106.3±</td>
</tr>
<tr>
<td>Hs Pure</td>
<td>5</td>
<td>68.33±</td>
<td>103.5±</td>
<td>109.3±</td>
<td>105.7±</td>
<td>99.33±</td>
<td>93.67±</td>
<td>86.33±</td>
</tr>
<tr>
<td>Hs nano</td>
<td>5</td>
<td>68.17±</td>
<td>117.0±</td>
<td>112.2±</td>
<td>108.5±</td>
<td>103.3±</td>
<td>99.83±</td>
<td>96.50±</td>
</tr>
<tr>
<td>Ap - Hs Nano</td>
<td>5+5</td>
<td>68.00±</td>
<td>99.3±</td>
<td>98.17±</td>
<td>95.33±</td>
<td>92.17±</td>
<td>86.83±</td>
<td>81.50±</td>
</tr>
</tbody>
</table>

Table 2: Streptozotocin-Induced Diabetes Mellitus on Prepared nanoparticles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Carboxymethyl Cellulose(CMC)</td>
<td>0.5%</td>
<td>79.83±</td>
<td>300.3±</td>
<td>282.5±</td>
<td>268.2±</td>
<td>298.0±</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>2</td>
<td>72.33±</td>
<td>270.5±</td>
<td>127.0±</td>
<td>114.2±</td>
<td>118.0±</td>
</tr>
<tr>
<td>Ap pure</td>
<td>5</td>
<td>83.17±</td>
<td>290.5±</td>
<td>142.3±</td>
<td>128.7±</td>
<td>121.0±</td>
</tr>
<tr>
<td>Hs pure</td>
<td>5</td>
<td>86.17±</td>
<td>294.5±</td>
<td>233.0±</td>
<td>172.2±</td>
<td>198.7±</td>
</tr>
<tr>
<td>Ap nano</td>
<td>5</td>
<td>76.50±</td>
<td>272.5±</td>
<td>131.2±</td>
<td>119.3±</td>
<td>112.2±</td>
</tr>
<tr>
<td>Hs nano</td>
<td>5</td>
<td>82.83±</td>
<td>286.3±</td>
<td>186.2±</td>
<td>159.0±</td>
<td>179.5±</td>
</tr>
<tr>
<td>Ap - Hs Nano</td>
<td>5+5</td>
<td>69.50±</td>
<td>263.5±</td>
<td>126.0±</td>
<td>116.3±</td>
<td>114.2±</td>
</tr>
</tbody>
</table>
Table 3: Hypoglycemic Test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>0 hr</th>
<th>0.5 hr</th>
<th>1 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control carboxymethyl cellulose</td>
<td>0.5%</td>
<td>68.50±</td>
<td>68.33±</td>
<td>72.33±</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>2</td>
<td>68.33±</td>
<td>50.33±</td>
<td>27.50±</td>
</tr>
<tr>
<td>Ap pure</td>
<td>5</td>
<td>68.17±</td>
<td>60.17±</td>
<td>44.00±</td>
</tr>
<tr>
<td>Hs pure</td>
<td>5</td>
<td>68.33±</td>
<td>68.17±</td>
<td>55.50±</td>
</tr>
<tr>
<td>Ap nano</td>
<td>5</td>
<td>68.00±</td>
<td>54.00±</td>
<td>33.83±</td>
</tr>
<tr>
<td>Hs nano</td>
<td>5</td>
<td>67.83±</td>
<td>62.83±</td>
<td>44.50±</td>
</tr>
<tr>
<td>Ap - Hs nano</td>
<td>5+5</td>
<td>67.50±</td>
<td>49.00±</td>
<td>26.17±</td>
</tr>
</tbody>
</table>

Standard error (n= each group consist of 6 animals)(p<0.05)*, (p<0.001)**

Figure 4: Oral glucose tolerance test after 2 hour
Figure 5: Oral glucose tolerance test after 1.5 hour
Figure 6: Oral glucose tolerance test after 2 hour
Figure 7: Oral glucose tolerance test after 2.5 hour
Figure 8: Oral glucose tolerance test after 3 hour

Figure 9: Streptozocin induced DM - 3rd day

Figure 10: Streptozocin induced DM - 7th day

Figure 11: Streptozocin induced DM - 14th day

Figure 12: Streptozocin induced DM - 21th day

dence for management of Diabetes and patient compliance. A significant decrease in the body of diabetic control rats was observed when compared with the healthy control group. Diabetic rat treated with pure compound did not influence any substantial changes in body weight in comparison with the diabetic control group [15]. Diabetic rat treated with Ap and Hs loaded polymeric nanoparticles showed tremendous changes in body weight in contrast with the diabetic control group [16-18]. Blood glucose level significantly increases in diabetic rats. Efficacy study was observed slightly decrease blood glucose level in diabetic rats treated with pure compound and with Ap and Hs loaded polymeric nanoparti-
icles as compared to diabetic control rats. In the efficacy studies significantly decrease in blood glucose level was observed in diabetic rats treated with Ap-Hs(Ap-Hs NP) dual loaded polymeric nanoparticles contrast to diabetic organize the group. Glucose levels were also found to be significantly decreased in the diabetic rats treated with Ap-Hs(Ap-Hs NP) dual loaded polymeric nanoparticles, and these levels were approximately equal to the Glibenclamide (standard) treated group. These results demonstrate that Ap-Hs(Ap-Hs NP) dual loaded polymeric nanoparticles offers an efficacious oral therapy with abridged does incidence for management of Diabetes and is hence patient compliant [19, 20].

CONCLUSION

By utilizing nanotechnology, the nanoparticle-based medication conveyance frameworks are being researched for a long time to beat the constraints of traditional medications. As a finish of this basic research paper, these progressed nanoparticles tranquilize conveyance frameworks have been seen as possibly valuable for novel diabetic medications. Sooner rather than later, this nanoparticles based insulin transportation could displace the regular subcutaneous insulin infusions. Nanoparticles conveyance frameworks are intended to get controlled or delayed medication transportation and to improve bioavailability just as strong. Likewise, nanoparticles can also show a few favorable circumstances like constraining vacillations of medications inside the remedial range, diminishing reactions identified with traditional dose structure, shielding drugs from debasement, diminishing dosing recurrence, improving patient consistence, accommodation and help the better nature of living of DM patients.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

FUNDING SUPPORT

None

ACKNOWLEDGEMENTS

The authors are thankful to all who have extended their constant support for the completion of the work.

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