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# Investigation of *Hammada scoparia* antidiabetic activity and toxicity in rat

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## Abstract

To investigate *Hammada scoparia*claimed antidiabetic effect, and to search for potential nontoxic active principles.

Aqueous and methanol extracts were prepared from *H. scoparia* aerial parts, and their phytochemical constitution was defined. Then they were subjected to three procedures to check their antidiabetic effect. The first (acute effect on fasting glycemia) and the second (oral glucose tolerance test, OGTT) werecarried out in normal rats, while the third procedure (7 days treatment) was carried out in streptozotocin-diabetic rats. Methanol extract was also subjected to further studies; sub-chronic oral toxicity test (21days), effect on postprandial glycemia in normal rats and effect on intra-tissue glycogen concentrations. Finally methanol extract was fractionated on chromatography column; each fraction was thoroughly analysis using phytochemical tests and subjected to OGTT in normal rats.

Using the three procedures, only the methanol extract (containing flavonoids, alkaloidsand saponins) showed an antidiabetic effect similar to glibenclamide effect, and this was only when hyperglycemia was induced by oral glucose charge. On the other hand methanol extract had no toxicity in rats;it hadno effect on postprandial glycemia or on intra-tissue glycogen concentrations. Four fractions were obtained using chromatography column, FA (containing flavonoids), FB (alkaloids, saponins), FC (alkaloids), and FD (saponins). Unlike the antihyperglycemic effect observed with methanol extract in normal rats, none of these fractions were effective.

*Hammada scoparia*could be a promising source of new antidiabetic agents, further studies arenecessary to find its active principles and to understand its mechanism of action.

**Keywords:** *Hammada scoparia*, alkaloids, flavonoids, saponins, experimental diabetes,toxicity.

## Introduction

Diabetes mellitus is a major health problem that affects a lot of people in the world [1].Its prevalence in adult Algerian population was estimated to be 4.6% in 2000, by the year 2025, it is expected to rise to 6.3% [2].Diabetes mellitus is a metabolic disorder characterized by an elevated blood glucose level resulting from insulin resistance, insulin secretion defects or both, and it is associated with various complications [3, 4].For decreasing hyperglycemia to normal values,many therapies are available, and despite the progress made to understand their mechanism of action, researchers still having a great challenge to achieve a well-managed treatment [5], plants could have a part in possible solutions. In fact many reviews have discussed the anti-diabetic effect of plants used in traditional medicine to treat type 2 diabetes mellitus[6, 7, 8], experimental and clinical data support their efficacy as anti-diabetic agents [9].The development of an approved anti-diabetic drug,the biguanide metformin from the French lilac (*Galega officinalis*) is an evident proof [10].*Hammada scoparia* (POMEL) ILJIN = *Arthrophytum scoparium* (POMEL) ILJIN = *Haloxylon articulatum* subsp. scoparium (POMEL) BATT. = *Haloxylon scoparium* POMEL (Chenopodiaceae) is a small and highly-branched halophytic shrub which turns dark on drying. It grows in desert and semi desert areas of Algeria and other Mediterranean countries and the Near East. *H. Scoparia* has been reported to have hepatoprotective,antioxidant[11],anti-cancer,antimalarial[12]and molluscicidal activities [13].Recently melanogenesis inhibitionby*H. scoparia* was also proven [14].Phytochemical constitution of *H. scoparia* wasalso well studied; alkaloid[15, 16] and flavonoid [16, 17]structures were identified. *H.scoparia* was reported to be used in folk medicine for diabetes mellitus treatment [18] but no experiment has been conducted. In the present study, we investigate the antidiabetic activity of *H.scoparia* in normal and streptozotocin induced diabetic rats.

## Materials and methods

### Plant material

*Hammada scoparia* aerial parts were collected in January 2010 from Ain Sefra, a desert area in south west of Algeria. The plant was botanically identified and a voucher specimen (N° LVE748) was deposited in the Laboratory of Vegetal Ecology, Es Senia

Oran University (Algeria). The plant material was washed and air-dried at room temperature, and ground into a fine powder.

## Preparation of extracts of *H. Scoparia* aerial parts

The aqueous extract was prepared by heating30 g of the ground plant into water, under reflux for 1 hour; the cooled extract was then filtrated under vacuum and dried, a brownish viscous residue was obtained, the yield expressed to dry plant was 27.9%(w/w).The methanol extract was also prepared. Using soxhlet apparatus, 30 g of the ground plant were successively extracted by three solvents with graded polarity, hexane (for 24h), dichloromethane (16h) and methanol (24h). The alcohol solvent was removed by rotary vacuum evaporator under reduced pressure and low temperature; to produce a brown semisolid residue at yield of 17% (w/w).

## Fractionation of methanol extract of *H. scoparia* aerial parts

The methanol extract was fractionated on an open chromatography column loaded by silica gel (Kieselgel 60, 70-230, mesh). Elution was done by mixtures of methanol and dichloromethane. Equal volumes were obtained in a set of tubes, then the content of each tube was checked on using thin layer chromatography (solvent system; methanol- dichloromethane, v: v), the tubes containing the same components (spots) were collected into four fractions(A-D). Each fraction was then evaporated to dryness in rotary evaporator. Yields expressed to dry plant were 0.19% (w/w) for Fraction A,3.87% (w/w) for fraction B, 3.02%(w/w) for fraction C and 1.42 %(w/w) for fraction D.

## Phytochemical screening

Aqueous and methanol extracts and fractions A-D were subjected to phytochemical tests either in tube or using thin layer chromatography, in order to detect the presence of *H. Scoparia* known secondary metabolites flavonoids [19], alkaloids[20] and saponins[21].

## Experimental animals

Healthy adult Wistar rats of both sexes, weighing about 160 to 300 g were used for the experiments. Female rats (body weight <200g) were suggested for toxicity study. The animals were maintained on 12hours light/dark cycle and appropriate experimental conditions with free access to standard commercial food and clean drinking water. Ethical conditions governing the conducts of experiments with life animals as stipulated by Festing, [22] were strictly observed.

## Induction of diabetes

Diabetes was produced in 16 hours fasted-rats by intraperitoneal injection of 60mg /kg streptozotocin (sigma) dissolved just prior to use in citrate buffer (0.01 mol/L, pH=4.5). After 72h, a diabetic state became evident in all injected animals, it characterized by elevated fasting blood glucose levels above 16.65 mmol/L, polyuria and glycosuria.

## Evaluation of antidiabetic effect of aqueous and methanol extracts of *H. scoparia* aerial parts

In order to investigate the claimed plant antidiabetic effect, two extracts and three procedures have been used. In the first procedure (acute effect on fasting glycemia), fifteen normal rats were randomly divided into three groups (n=5), after an overnight fast, 0.8% tween-80 suspension (10ml/kg),aqueous extract (500mg/kg) and methanol extract(300mg/kg)were given orally to group 1(control group), 2 and3 (treated groups) respectively. Glycemia was recorded at, 0 min (prior drugs administration) and at 60,120,180 and 240min. In the second procedure (OGTT), twenty normal rats were randomly divided into four groups(n=5), 0.8% tween-80 suspension (10ml/kg) aqueous extract (500mg/kg), methanol extract (300mg/kg) and glibenclamide (0.1mg/kg),were given orally 30 min prior glucose solution feeding (2g/kg),to group 1(control group),2, 3(treated groups) and 4(positive control group) respectively. Glycemia was recorded at 0 min (prior drug administration), and over a period of 150 min, at 30 min intervals. In the third procedure, twenty-one diabetic rats are randomly dived into three groups(n=7),0.8% tween-80 suspension (10ml/kg), aqueous extract(200mg/kg) and methanol extract(300mg/kg) were given daily over a period of 7 days to group 1(control group), 2 and 3(treated groups) respectively. Fasting glycemia was recorded at day one and seven, body weight, food and water intake were also considered.

## Further studies on methanol extract of *H. scoparia* aerial parts

## Sub- chronic toxicity test of methanol extract

Fourteen normal rats are randomly divided into two groups(n=7), 0.8% tween-80 suspension (10ml/kg)and methanol extract (300 mg/kg) were given daily and orally to group 1(control group) and 2(treated group) respectively over a period of 21 days, body weight was considered. At the end of the experiment all the surviving rats were terminally anaesthetized, the abdomen was opened, and the rats were subjected to macroscopic examination. Blood collection was performed from the aorta abdominalis for determination of urea, creatinine, serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities, using commercial diagnostics kits.

## Effect of methanol extract on postprandial glycemia, and on intrahepatic and intramuscular glycogen concentrations

Sixteen non-fasted normal rats were randomly divided into two groups (n=8), 0.8% tween-80 suspension (10mL/kg) and methanol extract (300mg/kg)were given orally to group 1(control group) and 2(treated group) respectively. Postprandial glycemia was recorded immediately before, and 1,2,3,5 and 24hoursafterextract administration. At 24 h the rats are terminally anaesthetized, the abdomen was opened, and approximately 1 gram of liver and skeletal muscle was immediately removed for intrahepatic and intramuscular glycogen estimation[23]. A chemical method was used to glycogen determination[24];the amount of glucose released was estimated by arsenomolybdate method of Nelson[25].

## Evaluation of antihyperglycemic effect of methanol fractions

Methanol extract and its four fractions (A-D) were suggested to oral glucose tolerance test. Thirty normal rats were randomly divided into 6groups(n=5), 0.8%tween-80 suspension (10ml/kg), FA (50mg/kg), FB(280mg/kg), FC(190mg/kg), FD(190mg/kg) and methanol extract(300mg/kg),) were given orally 30 min prior glucose solution administration (2g/kg)to group 1(control group), 2,3,4,5 and 6 (treated groups) respectively. Glycemia was recorded at 0 min (prior glucose administration), and over a period of 150 min, at 30 min intervals.

## Measurement of blood glucose levels

In all of these experiments, blood glucose samples were collected from the tail and glycemia was measured using a glucometer (Accucheck-active).

## Data analysis

When two groups were compared, Student's t-test was used and when more than two groups were compared, one way ANOVA followed by Dunnett' test were used. All data are expressed as mean ± SEM. Differences between groups were considered significant at $p<0.05$.

# Results

## Phytochemical screening

Phytochemical tests revealed the absence of flavonoids from the aqueous extract and their presence in methanol extract, while alkaloids and saponins are present in both extracts. Fractionation of methanol extract gave three more or less enriched fractions; FA with flavonoids, FC with alkaloids and FD with saponins, although alkaloids and saponins still regrouped in FB (Table 1).

**Table 1. Phytochemical screening of *Hammada scoparia* extracts, and fractions (FA-FD) obtained from methanol extract fractionation on chromatography.**

| Secondary metabolites | Aqueous extract | Methanol extract | FA | FB | FC | FD |
|---|---|---|---|---|---|---|
| Flavonoids | - | + | + | - | - | - |
| Alkaloids | + | + | - | + | + | - |
| Saponins | + | + | - | + | - | + |

(+) Presence; (-) Absence. Fractions (A-D) were obtained from methanol extract fractionation on chromatography column

## Evaluation of antidiabetic effect of aqueous and methanol extracts

Aqueous and methanol extracts given orally to normal rats at dose levels of 500mg/kg and 300 mg/kg respectively, showed no effect on fasting glycemia over 240min examination period, when compared with vehicle effect (Figure1).

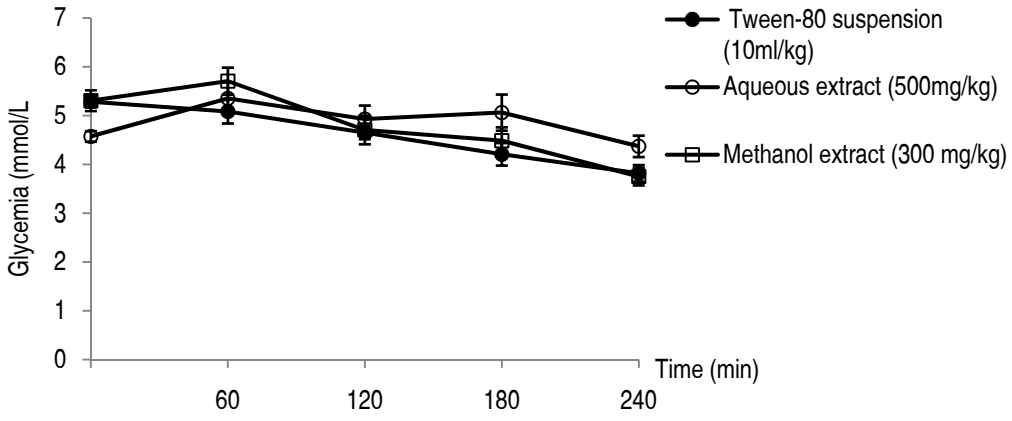


**Figure1. Fasting glycemia recorded in normal rats treated by aqueous and methanol extracts, (n=5).**

When the two extracts were given to normal rats prior glucose feeding (2g/kg),only methanol extract exhibited a significant antihyperglycemic effect by enhancing glucose tolerance at 60min ($p<0.05$), compared with vehicle effect, and this action was similar to glibenclamide effect(Figure 2).The calculated area under curves were 6.04 ± 1.90 mmol/L, 10.12± 3.25 mmol/L, 1.64±1.22 mmol/L($p<0.05$) and 2.78± 1.30 mmol/L($p<0.05$) in control group, aqueous extract treated group, methanol extract treated group and in control positive group respectively.

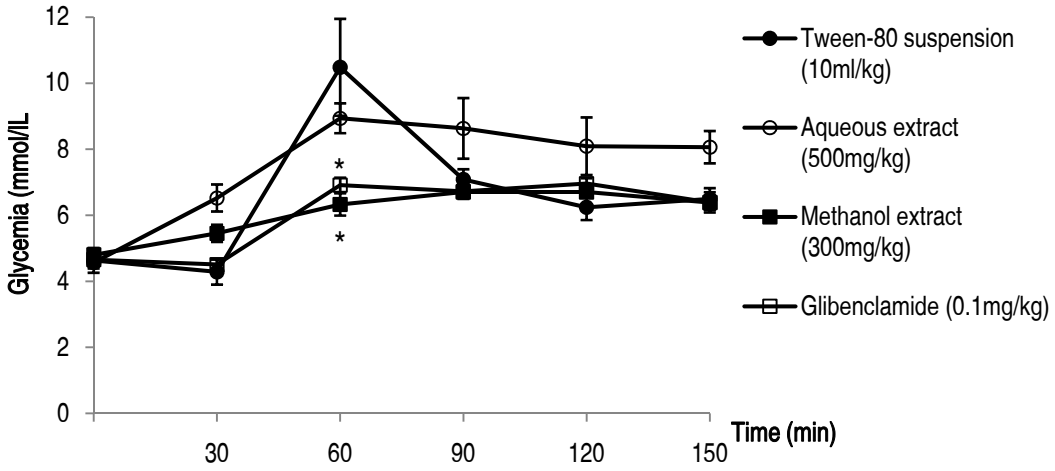


**Figure2. Glycemia recorded in normal rats subjected to OGTT (2g/kg) at 30min,(n=5).**
*: $p < 0.05$. Compared with control group.

Elsewhere aqueous and methanol extracts given daily and orally to streptozotocin diabetic rats at dose levels of 200 mg/kg and 300mg/kg respectively, were without any effect on hyperglycemia, this later averaged at18.02 ±0.79mmol/L (n=21),in the first day and remained at the same magnitude without any significant reduction to become 17.19 ± 1.12 mmol/L(n=21)in the seven day of treatment. This diabetic state was accompanied by a significant reduction in body weight in the two treated groups ($p<0.05$).Furthermore no correction in the amount of food and water consumed were detected(Table 2).

**Table2.Fasting glycemia, body weight, food and water intake recorded in diabetic rats.**

| Daily treatment | Fasting glycemia (mmol/L) | | Body weight (g) | | Food intake (g/100g bw/day) | Water intake (ml/100g bw/day) |
|---|---|---|---|---|---|---|
| | Day 1 | Day7 | Day 1 | Day7 | | |
| Tween-80 suspension (10ml/kg/ day) | 19.44 ± 1.71 | 16.81 ± 2.25 | 243.81± 20.42 | 225.18 ±18.45 | 10.29 ±2 | 53.27 ±13.15 |
| Aqueous extract (200mg/kg/day) | 17.14 ± 1.01 | 16.26 ± 1.93 | 223.46 ± 8.84 | 190.2 ± 6.58* | 10.48 ± 1.80 | 64.74 ±6.33 |
| Methanol extract (300mg/kg/day) | 17.07±0.63 | 19.32±0.90 | 278,25±9,86 | 226,5±10,61* | 13.70 ± 1.45 | 68.21 ±6.55 |

The values are expressed as mean ± SEM, (n=7), *$p < 0.05$.

## Further studies on methanol extract of *H. scoparia* aerial parts

The effect of *H. Scoparia* detected in previous section was obvious only with methanol extract, and this was effective only in OGTT, so we underwent the methanol extract to further studies.

## Sub-chronic toxicity test of methanol extract

When methanol extract was given orally and daily to normal rats at dose level of 300 mg/kg, during 21 days, no toxicity signs or death were recorded, the macroscopic examination performed on the end of the treatment showed intact organs without any visible damage, the levels of biochemical parameters urea, creatinine and ALTwere the same in both control and treated groups, but AST level was significantly reduced in treated group when compared with control group($p<0.05$) (Table3).

**Table 3. Body weight and biochemical parameters recorded in normal rats.**

| Daily treatment | Body weight (g) | | Biochemical parameters (21th day) | | | |
|---|---|---|---|---|---|---|
| | 1st day | 21th day | Urea (mmol/L) | Creatinine (µmol/L) | AST (IU/L) | ALT(IU/L) |
| Tween-80 suspension (10ml/kg) | 288.33 ±15.53 | 320.4±8.61 | 10.71 ± 0.29 | 53.92 ± 1.77 | 106.12 ± 7.54 | 39.98 ± 6,71 |
| Methanol extract (300mg/kg/day) | 303.17±10.02 | 315.4± 2.69 | 11.70 ± 0.48 | 54.80 ± 1.77 | 88.24 ± 0.84* | 42.88 ± 1.61 |

The values are expressed as mean ± SEM, (n=7), *$p < 0.05$.

## Effect of methanol extract on postprandial glycemia, and on intrahepatic and intramuscular glycogen concentrations

The methanol extract effect was also evaluated on postprandial glycemia in normal rats, the results showed a similar glycemic profile in both control and treated groups over 24 hours (Figure 3), furthermore the amount of glycogen in hepatic and skeletal muscle tissues remained the same in the two groups (Table 4).

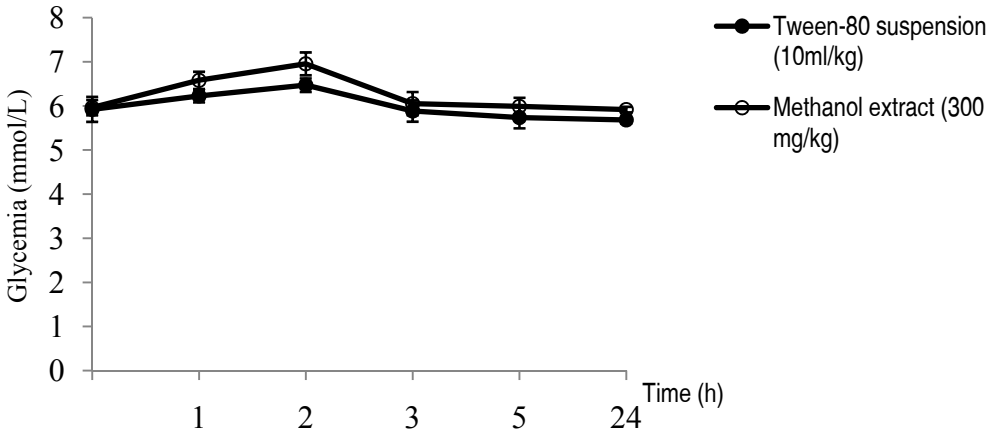


**Figure3. Postprandial glycemia recorded in normal rats treated by methanol extract, (n=8).**

**Table4. The concentrations of hepatic and skeletal muscle glycogen recorded in no fasting normal rats.**

| Rats treatment | Hepatic glycogen % (g/100g of tissue ) | Muscle glycogen % (g/ 100g of tissue ) |
|---|---|---|
| Tween-80 suspension (10ml/kg) x33) | 4.13 ± 0.49 | 0.23 ± 0.01 |
| Methanol extract (300mg/kg) | 3,40 ± 0.36 | 0.31 ± 0.04 |

The values are expressed as mean ± SEM, (n=8), *p < 0.05.

## Evaluation of antihyperglycemic effect of methanol fractions

Oral glucose tolerance test performed in normal rats previously treated by each fraction showed that, fraction A (containing flavonoids), Fraction B (alkaloids and saponins), fraction C (alkaloids) and D (saponins) had no notable effect on hyperglycemia over 150 min observation time. However the effect of methanol extract remained the same as previously recorded with a p-value less than 0.05, when compared with control group effect (Figure 4).

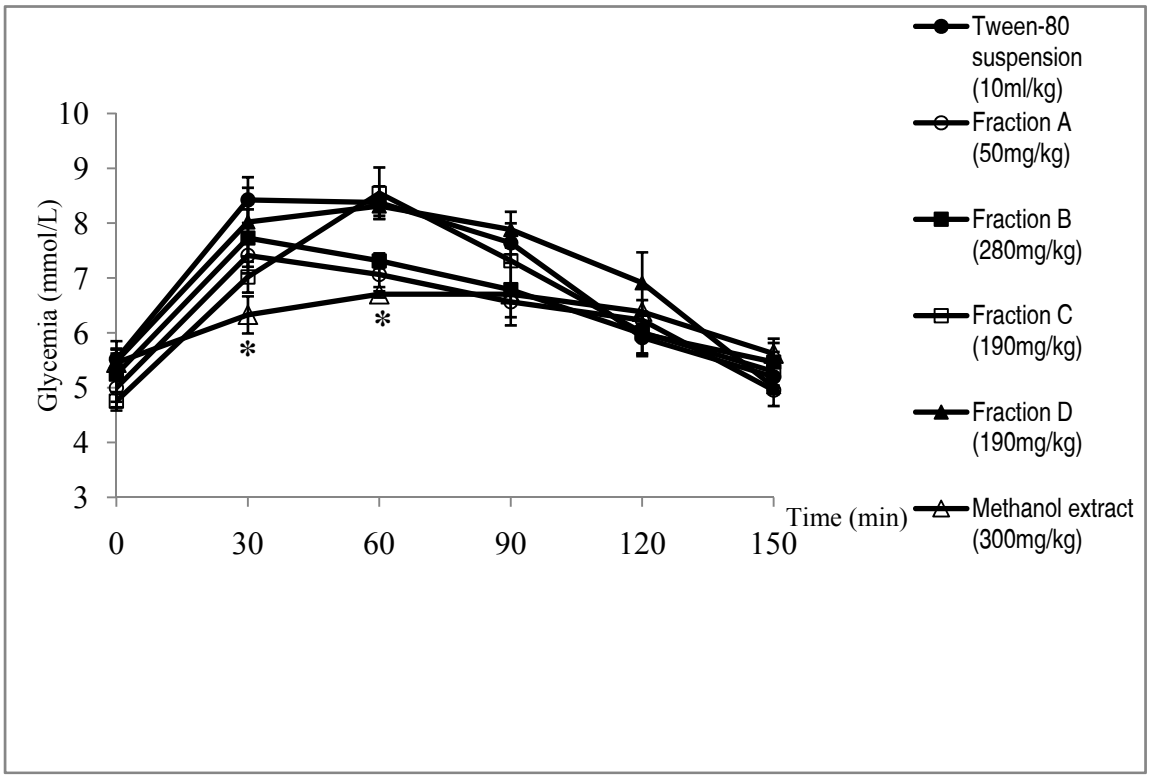


**Figure4. Glycemia recorded in normal rats treated by methanol extract and its fractions A,B,C,D and subjected to OGTT (2g/kg), (n=5).**

## Discussion

The present study was conducted on two extracts of *H. scoparia*; aqueous extract was apparently not effective on the glycemia of experimental animals, whereas methanol extract seemed to be effective, so our discussion will be outlined on results provided from methanol extract experiments.

The daily oral administration of methanol extract at dose of 300mg/kg to rats wasn't accompanied by any harmful trouble or death. In addition to this result, an extract of *H. scoparia* prepared by maceration in methanol (80% in water), showed no toxic effect up to oral unique dose of 2000 mg/kg in rats, furthermore $LD_{50}$ of the same extract given to rats by intraperitoneal injection was estimated to be 533.84 mg/kg (data not shown).Little is known about herbal toxicity in the literature [26];further studies are needed to define more *H. scoparia* toxicity.

Methanol extract was effective only in normal rats when hyperglycemia was induced by glucose charge. This antihyperglycemic action could be explained by three major possible mechanisms, lowering intestinal glucose absorption, enhancing insulin secretion, or enhancing peripheral glucose uptake in adipose tissue (since no effect was detected on intramuscular glycogen concentration). The non efficiency of methanol extract in diabetic rats, could be explained by a severe STZ-induced hyperglycemia, which needs insulin or mimic insulin molecule to be corrected. We suggest that methanol extract could be effective in mild diabetes mellitus. This experimental condition could be realized since STZ induced diabetes is dose response [27].

Alkaloids and flavonoids were very well studied in *H.scoparia*. Three alkaloidal classes have been isolated and identified from the aerial parts of the plant, tetrahydroisoquinolines, indoleand β-carboline [15,16].Tetrahydroisoquinolines are molecules that represent a promising source of potent antidiabetic molecules as no TZD agonist of PPARs[28].Indole alkaloids have been reported to stimulate glucose uptake in adipocyte [29],and β- carboline has shown also an effect on controlling PPARs expression and on improvement of insulin sensitivity [30].Three flavonols glycosides have been isolated from *H.scoparia* [16, 17]. This class of molecules could be effective in reducing hyperglycemia by it glycosidic moiety, that could interact with digestive carbohydrates by inhibiting their hydrolysis or could inhibit intestinal glucose transport [31]. Saponins are very common in chenopodiaceae family [32]. Saponins and especially triterpenoids generally show antidiabetic activity [33].The chemical nature of *H.scoparia* saponins has not been yet identified.

## Conclusions

*H. scoparia* is much known by its anti-cancer, antioxidant, antimalarial and molluscicidal activities [12-14], so all the interest was focused on this area of research, and till now no study was directed on its antidiabetic effect. Nevertheless our findings suggest that *H. Scoparia* could be a promising source of new antidiabetic drugs; a screening is needed to study more enriched fractions and individual molecules, and to compare their possible antidiabetic effect to the effect of their related structures previously studied.

### Authors' contributions

SM she is the director of the research, she chose "*Hammada scoparia*" as plant material, she believed strongly in its cure properties and she directed me on all the work, especially the phytochemistry part (chromatography column).

RD, he is the co- director, his contribution was carried out on the animal experimental protocol (postprandial glycemia and glycogen dosage part), and he has made a concise critical advice on the manuscript writing.

SC, he carried out the phytochemical screening of the plant.

FZS, she identified the plant, and gave its voucher number.

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