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The mechanisms of action of St. John's wort: an update

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Summary Pharmacological research confirms and supports the clinically observed antidepressant efficacy of St. John's wort (*Hypericum perforatum* L., SJW). This contribution is an update of a former review by the authors in 2007. Positive evidence of antidepressant effects has been found with SJW preparations, extract fractions, and single constituents. The efficacy of SJW is obviously defined by a range of parallel mechanisms of action, triggered by different constituents. *In vitro* research showed, among other tests, positive effects in neurotransmitter regulation (in beta adrenergic systems and glutamate receptors) and ion channel conductance. Antidepressant effects were confirmed in typical *in vivo* models such as the forced swimming test, the open field test, the tail suspension test, or a model of stress-impaired memory. The overall effect cannot be attributed to a single constituent or fraction. SJW is therefore an outstanding example of the total extract being defined as the active constituent of herbal medicines.

Keywords *Hypericum perforatum* · Active constituents · Pharmacology

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Die Wirkmechanismen von Johanniskraut – Ein Update

Zusammenfassung Die pharmakologische Forschung bestätigt und unterstützt die klinisch beobachtete antidepressive Wirksamkeit von Johanniskraut (*Hypericum perforatum* L.). Dieser Beitrag ist ein Update eines früheren Reviews der Autoren aus dem Jahr 2007. Positive Evidenz für antidepressive Effekte wurde für Johanniskrautzubereitungen, Extraktfraktionen und isolierte Inhaltstoffe gefunden. Die Wirksamkeit von Johanniskraut beruht offenbar auf einer Reihe paralleler Wirkmechanismen, die ihrerseits von verschiedenen Inhaltstoffen ausgelöst werden. Die *in vitro*-Forschung ergab unter Anderem positive Effekte in der Regulation von Neurotransmitters (betraadrenerge Systeme und Glutamat-Rezeptor) sowie von Ionenkanälen. Eine Bestätigung antidepressiver Effekte fand sich auch *in vivo* in typischen Modellen wie Forced Swimming Test, Open Field Test, Tail Suspension Test oder einem Modell Stress-bedingt eingeschränkter Gedächtnisleistung. Der Gesamteffekt kann nicht einem einzelnen Inhaltstoff oder einer einzelnen Fraktion zugeordnet werden. Johanniskraut ist damit ein besonders anschauliches Beispiel dafür, dass bei Phytopharmaka der Gesamtextrakt als Wirkstoff zu betrachten ist.

Schlüsselwörter *Hypericum perforatum* · Wirkstoffe · Pharmakologie

Introduction

The flowering herb of *Hypericum perforatum* L. (Clusiaceae), commonly referred to as Saint John's wort (SJW), is the starting material for well-established medicinal products for the treatment of mild to moderate depression. This use has been accepted by a monograph of the Herbal Medicinal Product Committee (HMPC) of the European

Medicines Agency (EMA), thus validating the positive results of a multitude of clinical trials, as evaluated in the corresponding assessment report [1, 2]. With the antidepressant activity solidly confirmed through double-blind trials and metaanalyses, the question of the mechanisms of action is, like for the chemically defined antidepressants, still not fully answered. Preparations from SJW have been shown to exert antidepressant-like effects in standard models of depression *in vitro* and *in vivo*. Although none of the potential mechanisms discovered to date would account for the overall clinical efficacy by itself, the combination of different effects is generally thought to result in a total effect much stronger than might be expected from the assessment of single mechanisms of action [3–6].

The role of individual SJW constituents has been reviewed. As a result, the total extract of SJW had to be regarded as the active constituent, and no single constituent could be considered the most important contributor to the clinical antidepressant activity of SJW preparations [7]. Research on SJW has lately shifted to new areas such as anti-cancer effects [8–11], effects in the context of Morbus Alzheimer [12–14], or antibacterial or antiviral applications [15–18]. The update of the literature review presented herein focuses on depression. The findings are presented in Tables 1 and 2.

The literature search strategy was kept broad on purpose, with a search in PubMed for the keywords "hypericum," "St. John's wort," "hypericin," "hyperforin," "hyperoside," and "amentoflavone," with a restriction to the years 2006 to date (data lock point October 31, 2014). This search produced 1745 hits, which were then handsearched and examined for citations with importance to the examined issue not yet mentioned in our previous review [7].

Pharmacology *in vitro*

SJW and its constituent fractions of hypericin, hyperforin, and polyphenols have been shown effective in different *in vitro* models of depression [7]. The accepted hypothesis on the mechanism of action is that of an impact on neurotransmitters in the brain, achieved either by regulation of their re-uptake from the synaptic cleft or by direct binding to neurotransmitter receptors. More recent research examined the effect on ion channels by an influence on intracellular concentrations of sodium or calcium, which may explain the fact that the synaptosomal re-uptake or the preceding release of neurotransmitters into the synaptic cleft seems to be influenced

Table 1 *In vitro* studies on antidepressant mechanisms of action of St. John's wort (SJW) published since 2006

Targets	Models	Comparators	Results	References
Number of β-adrenoreceptors	Rat C6 cells, 7 days of incubation	3.78 mg/mL SJW extract Ze 117 (< 0.02 % hyperforin, 0.036% hypericin, 0.176% pseudohypericin, 0.93% rutin, 0.69% hyperosid, 0.13% quercetin, 0.11% quercitrin, 0.048% biapigenin) 5 µM desipramine	Reduction in receptors: Desipramine: 65 ± 6 % SJW extract: 56±9%	[19]
Binding of Alexa532-NA to β-adrenoreceptors	Rat C6 glioblastoma cells, 3 days of incubation	1 µM Hyperforin 1 µM Hyperoside 1 µM Desipramine	Total agonist receptor binding: Desipramine: 62.0±12.6% (p<0.05) Hyperforin: 53.1±9.3% (p<0.001) Hyperoside: 59.4±8.8% (p<0.01)	[20]
Receptor internalization	HEK293 cells expressing a β_2-AR-GFP protein, 6 days of incubation	1 µM Hyperforin 1 µM Hyperoside 1 µM Desipramine 10 µM Isoprotenerol (positive control)	No internalisation with desipramine, hyperforin and hyperoside	[20]
Downregulation of β1 adrenoreceptors	Rat C6 glioblastoma cells	1 µM Hyperforin 1 µM Hyperoside	Reduced receptor density in plasma membrane Reduced downstream signalling	[21]
4-Aminopyridine-evoked release of glutamate	Nerve terminals from rat cerebral cortex	Hypericin	Dose-dependent inhibition of glutamate release, possibly caused by suppression of voltage-dependent Ca^{2+} channels and MAPK activity	[22]
Intracellular Ca^{2+} elevation	PC12 cells (mice)	0.3–10 µM Hyperforin	Intracellular calcium elevation only in cells expressing TRPC6	[23]
Expression of CREB and TrKB	Cortical neurons	Hyperforin 1 µM, 72 h	Expression of CREB, p-CREB and TrKB increased by 70, 109 and 71% (p<0.05) Effect on p-CREB blocked by calcium channel blockers Hyperforin acts through TRPC6 channels	[24]
LPS-induced NO release	Microglia	SJW (laboratory extract with 50 % ethanol v/v) Hyperforin Rutin, quercetin, quercitrin, hyperoside, hypericin, psydohyopericin, hyperforin	Suppression of NO release with SJW; hyperforin active between 0.25 and 0.75 µM No effect with other SWJ constituents	[28]
Zymosan phagocytosis	Microglia	Hyperforin	Inhibition by 20–40 %	[28]
NFκB and p-CREB	Microglia	Hyperforin	Suppression of activated state	[28]

Table 2 *In vivo* studies on antidepressant effects of SJW published since 2006

Targets	Models	Applications	Results	Reference
Nerve cell proliferation	Adult mice	Hyperforin, 4 weeks, 4 mg/kg daily i.p.	No effect on neurogenesis	[24]
Expression of TrKB in cortex and hippocampus	Adult mice	Hyperforin, 4 weeks, 4 mg/kg daily i.p.	Cortex: TrKB+124%, p-TrKB+200%; No significant effect on CREB and p-CREB. Hippocampus: No effects	[24]
Expression of p-CREB	Aged rat	SJW extract	Increase of p-CREB in the hippocampus ($p < 0.01$)	[26]
Forced swim test	Rat	SJW extract (0.3 % hypericin), 30–90 mg/kg i.p. Fluoxetine, imipramine: 30.70 mg/kg i.p.	SJW: 53% reduction of immobility time at 90 mg/kg Fluoxetine, imipramine: similar effect size	[29]
Locomotor activity test	Mice	SJW extract (0.3% hypericin), 1–30 mg/kg i.p. Fluoxetine: 30 mg/kg i.p. Dothiepin 10 and 50 mg/kg i.p. Venlafaxine 50 mg/kg i.p.	SJW extract: −80% at 30 mg/kg Antidepressants: −62 to −89%	[29]
Tail suspension test	Mice	Intragastrically SJW extract Paroxetine (10 mg/kg) Quercetin (5–20 mg/kg) Quercetin+SJW wort	5 mg quercetin+5 mg SJW: better effects on immobility time than with SJW 10 mg/kg alone and with quercetin 10 mg/kg	[30]
Caffeine-induced locomotor activity	Mice	SJW laboratory extract (50 % ethanol v/v); 6–48 mg/kg i.p.	Suppression of caffeine-induced locomotor activity at 6–24 mg/kg, reversible with L-arginine (NO donor)	[31]
Open field test	Mice	Oral administration: SJW extract (STW 3-VI, extraction solvent: 80 % ethanol); 250–500 mg/kg Hypericin: 0.1 mg/kg Hyperforin: 1–10 mg/kg Hyperoside, isoquercitrin, quercitrin: 0.6 mg/kg Rutin: 1 mg/kg Miquelianin: 1.2 mg/kg Amentoflavone: 0.1 mg/kg Imipramine 20 mg/kg Fluoxetine 10 mg/kg	SJW and hypericin: Reduction of stress-related increase of body temperature Flavonoids: partial effects Hyperforin, Imipramine, fluoxetine: no effect	[32]
Open field test	Mice with corticosterone-induced stress	SJW (not further specified) 30 mg/kg i.p. daily, 3 weeks	Significant anxiolytic effects by SJW ($p < 0.05$)	[33]
Forced swim test	Mice with corticosterone-induced stress	SJW (not further specified) 30 mg/kg i.p. daily, 3 weeks	Reduction of immobility time ($p<0.05$)	[33]
Elevated maze plus test	Mice	Kaempferol, quercetin, myricetin: 0.1–2.0 mg/kg p.o. and i.p. Metabolites p-HPAA and DOPAC i.p.	Anxiolytic effects shown for kaempferol and quercetin with oral, but not with i.p. administration p-HPAA and DOPAC active after i.p. administration	[34]
Stress-impaired memory	Rats	SJW extract (0.05–0.3 % hypericins, 2–4.5 % hyperforin, 2–4 % flavonoids, 8–12 % procyanidins): 350 mg/kg, 21 days	Significant improvement of processing of spatial information under corticosterone-induced stress conditions ($p<0.001$)	[37, 38]

for different neurotransmitters in a rather nonselective manner.

New studies have been published further enlarging the knowledge in these fields.

Effect of SJW on the beta adrenergic system

An extract devoid of hyperforin reduced the number of β-adrenoreceptors in cultured rat C6 cells to a similar extent as desipramine. Seven-day incubation with desipramine resulted in a reduction of β-adrenoreceptor numbers to 65±6%. Under the same conditions, SJW extract led to a downregulation of β-adrenoreceptors of 56±9% compared with untreated controls (100±4.7%) [19].

Preincubation of rat C6 glioblastoma cells with hyperforin and hyperoside and with the antidepressant desmethylimipramine for 3 days led to a significant reduction of beta adrenergic receptor ligand binding, and in turn to a reduced formation of cAMP. Closer examinations with a 6-day preincubation in HEK 293 cells stably transfected with GFP-tagged β2-adrenergic receptors led to the conclusion that the reduction of receptor binding was not caused by receptor internalization. Both SJW constituents, hyperoside and hyperforin, reduced β2-adrenergic sensitivity in rat glioblastoma cells like the antidepressant desmethylimipramine. This observation emphasizes the potential usefulness of hyperoside and hyperforin for the treatment of depressive symptoms [20].

Another study examined the downregulation of β1-adrenergic receptors in rat C6 glioblastoma cells by hyperforin and hyperoside. Treatment with 1 µM hyperforin and hyperoside resulted in a reduced β1 adrenoreceptor density in the plasma membrane, and a subsequent reduced downstream signalling [21].

Effect of SJW on glutamate turnover

The effect of SJW on glutamate turnover has been confirmed and attributed to hypericin. Hypericin inhibited the release of glutamate evoked by 4-aminopyridine in a concentration-dependent manner in nerve terminals purified from rat cerebral cortex. A closer examination suggests that hypericin suppresses voltage-dependent Ca^{2+} channel and mitogen-activated protein kinase activity, and in so doing inhibits evoked glutamate release [22].

Effect on ion channels

Studies on the effect of SJW extracts on voltage and ligand-dependent ion conductances put an emphasis on hyperforin. Hyperforin turned out to be a relatively potent inhibitor of such conductances. However, its effect was shown to be irreversible, which casts doubts on the importance of the effect as an antidepressant mechanism of action. Reversible effects on ion conductances could be demonstrated for the SJW flavonoids. The concentration attainable *in vivo* at the central neuronal structures after intake of hyperforin-rich SJW preparations might not be sufficient to trigger inhibitions of ion conductances [6].

Interestingly, transient receptor potential channels of C type (TRPC) isoforms (TRPC1, TRPC3, TRPC4, TRPC5 and TRPC7) other than TRPC6 are insensitive to hyperforin. Hyperforin evoked intracellular Ca^{2+} transients and depolarizing inward currents sensitive to the TRPC channel blocker La^{3+}, thus resembling the actions of the neurotrophin brain-derived neurotrophic factor (BDNF) in hippocampal pyramidal neurons. These results suggest that the antidepressant actions of SJW might be mediated by a mechanism similar to that engaged by BDNF [23].

Gibon et al. [24] examined the consequences of a chronic hyperforin treatment on cortical neurons in culture. Hyperforin stimulated the expression of TRPC6 channels and TrkB via SKF-96365-sensitive channels controlling a downstream signalling cascade involving Ca^{2+}, protein kinase A and cyclic adenosine monophosphate response element binding protein (CREB). Similar effects are encountered *in vivo*: Daily injection (i.p.) of hyperforin (4 mg/kg) to adult mice for 4 weeks augmented the expression of TrkB in the cortex, but not in the hippocampus where hippocampal neurogenesis remained unchanged [24]. The hyperforin-induced cascade involving CREB and BDNF is controlled by Ca^{2+} ions and occurs specifically in the cortex but not in the hippocampus [25], a region where the effect would be expected for antidepressants.

The restriction to the cortex was not found with SJW. The extract increased the levels of pCREB in the aged rat's hippocampus ($p < 0.01$) as measured by western immunoblotting. Thus, a potential mechanism of action of SJW might be an increase of the CREB levels in hippocampus [26].

Effect of SJW on the hypothalamic–pituitary–adrenal axis

Recent investigations have indicated that SJW, like conventional antidepressants, is involved in the regulation of genes that control hypothalamic–pituitary–adrenal axis function and influence, at least in part, stress-induced effects on neuroplasticity and neurogenesis. Results from experiments carried out with extracts or pure compounds do, however, not always resemble biochemical and pharmacological profiles characteristic of synthetic antidepressants. In particular, the majority of findings in preclinical studies have been obtained with high doses of pure compounds and extracts that are not comparable to the concentrations of single active constituents after oral administration in humans [27].

Anti-inflammatory effects related to depression

Hyperforin reduces proinflammatory and immunological responses of microglia, processes involved in the progression of neuropathologic disorders. Upon activation, microglia, the immunocompetent cells in the brain, becomes highly phagocytic and release proinflammatory mediators like nitric oxide (NO). Excessive NO production is pivotal in neurodegenerative disorders, and there is evidence that abnormalities in NO production and inflammatory responses may at least support a range of neuropsychiatric disorders, including depression. SJW (laboratory extract with 50 % ethanol v/v) was shown to efficiently suppress lipopolysaccharide-induced NO release, and hyperforin was identified as the responsible compound, being effective at concentrations between 0.25 and 0.75 µM. The reduced NO production was mediated by diminished inducible NO synthase expression on the mRNA and protein level. In addition, at similar concentrations, hyperforin reduced zymosan phagocytosis to 20–40 % and putatively acted by downregulating the CD206 macrophage mannose receptor and modulation of cell motility. The observed effects were found to correlate with a suppression of the activated state of NFκB and phospho-CREB, while c-JUN, STAT1, and HIF-1α activity and cyclooxygenase-2 (COX-2) expression remained unaffected by hyperforin [28]. These observations, should they be of clinical relevance, are potential facets of SJW effects. Hyperforin is, however, not an essential constituent of SJW with respect to the efficacy of SJW

preparation tested against depression, as preparations devoid of hyperforin have been proven efficacious.

Conclusions on in vitro effects

The published *in vitro* studies support the clinically confirmed antidepressant efficacy and add new facets to the already known mechanisms of action. They do, however, still not allow concluding on a major fraction of SJW constituents responsible for the efficacy.

Pharmacology *in vivo*

Forced swimming test

The forced swimming test is a standard model in the search for antidepressant effects. It has already been applied in the testing of SJW. In accordance with previous studies, a recent comparison of effects of SJW and standard antidepressants found a dose-dependent reduction in immobility time in rats with a maximal effect of 53 % at 90 mg/kg of SJW extract (30–90 mg/kg i.p.). This effect was reversed at higher doses (100 mg/kg) showing a U-shaped dose response curve. Fluoxetine and imipramine (30–70 mg/kg i.p.) produced similar reduction in the immobility time in rats [29].

Tail suspension test

Liu et al. [30] demonstrated synergistic effects between quercetin and SJW in the tail suspension test. Mice were divided into nine groups: blank control, positive control (Paroxetine, 10 mg/kg), quercetin (A: 5 mg/kg, B: 10 mg/kg, C: 20 mg/kg), *Hypericum perforatum* extract (SJW 10 mg/kg), combination groups (A: quercetin 2.5 mg/ kg + SJW 5 mg/kg, B: quercetin 5 mg/kg + SJW 5 mg/kg, C: quercetin 10 mg/kg + SJW 5 mg/kg). All substances were administered intragastrically. Combination group B showed no significant difference ($p > 0.05$) with respect to immobility time compared with combination group C. However, its body temperature reversal effect was significantly higher ($p < 0.01$) than that of quercetin group B, and its effect in shortening immobility time was stronger than that of SJW 10 mg/kg group ($p < 0.05$) and quercetin group B ($p < 0.01$) [30].

Locomotor activity

SJW decreased locomotor activity counts of mice similar to standard antidepressants [29]. SJW (extraction solvent: 50 % ethanol, 6–24 mg/kg i.p.) significantly blocked caffeine-induced locomotor hyperactivity. Pretreatment with L-arginine (1 g/kg) reversed this inhibitory effect of SJW (6 mg/kg i.p.) without producing any significant effect on locomotor activity of the mice when administered alone. The inhibitory effect of SJW on caffeine-induced locomotor activity may therefore be related to an inhibition of the enzyme NO-synthetase [31].

Open field test

Grundmann et al. [32] used exposure to an open field as inescapable stressor. Exposure of mice to open field stress significantly increased body temperature by 1.8±0.13 °C ($p < 0.05$). Diazepam at 5 mg/kg, and the $5HT_{1A}$ receptor agonist buspirone at 10 mg/kg significantly reduced this increase of body temperature, whereas imipramine and fluoxetine had no effect. Oral administration of SJW extract (STW 3-VI; solvent: 80 % ethanol) significantly reduced the increase of body temperature at doses of 250 and 500 mg/kg. Higher (750 and 1000 mg/kg) as well as a lower doses (125 mg/kg) did not affect the increase of body temperature after stress, indicating a U-shaped dose-response curve. Hypericin (0.1 mg/kg, p.o.)—administered 60 min prior to testing—significantly decreased the increase in body temperature ($p < 0.05$) whereas hyperforin (1–10 mg/kg, p.o.) had no effect. The flavonoids hyperoside, isoquercitrin and quercitrin (all at 0.6 mg/kg, p.o.), and rutin (1 mg/kg, p.o.) only par- tially blocked open field-induced hyperthermia. Mique- lianin (1.2 mg/kg, p.o.) was the most potent compound tested in this experimental design. From the biflavonoids in SJW, only amentoflavone decreased stress-induced hyperthermia in a dosage of 0.1 mg/kg. The results point to anxiolytic effects of SJW extract and single constituents, which may contribute to the antidepressant efficacy [32].

These findings are confirmed by more recent findings where mice were submitted to 7 weeks of corticosterone administration. They then underwent behavioural tests as the open field test or the forced swimming test, with findings supporting anxiety/depression-like effects, which can be reversed by a 3-week application of SJW extract (30 mg/kg)[33].

Elevated plus maze

In the elevated plus maze, another model for the determination of anxiolytic activity, the action of the flavo- nols kaempferol, quercetin and myricetin, constituents of hypericum extracts, after oral and intraperitoneal administration to mice in a dose range of 0.1–2.0 mg/kg was compared. In addition, their corresponding metabolites p-hydroxyphenylacetic acid (p-HPAA) and 3,4-dihydroxyphenylacetic acid (DOPAC) which are generated by the intestinal microflora were tested after intraperitoneal administration. Anxiolytic activity was detected for kaempferol and quercetin only after oral administration. No anxiolytic effects were observed when kaempferol and quercetin were given via the intraperitoneal administration route. p-HPAA and DOPAC showed anxiolytic effects after intraperitoneal application. After antibi-

otic treatment, the anxiolytic effect of kaempferol and quercetin disappeared, whereas it was still present for the positive control diazepam [34]. Results support the hypothesis that the flavonoids are not only absorbed as aglyca, as is known from pharmacokinetic studies [35, 36], but also as their hydroxyphenylacetic acid metabolites, and act therefore also as prodrugs.

Memory impaired by stress

SJW (0.05–0.3% hypericins, 2–4.5% hyperforin, 2–4% flavonoids, 8–12% procyanidins; 350 mg/kg for 21 days) potently and significantly improved processing of spatial information in stressed and corticosterone-injected rats ($p<0.001$). SJW prevented the deleterious effects of both chronic restraint stress and prolonged corticosterone administration on working memory. SJW significantly ($p < 0.01$) improved hippocampus-dependent spatial working memory in comparison with control and alleviated some other negative effects of stress on cognitive functions [37, 38].

Conclusions on in vivo effects

SJW pharmacology continues producing positive support for the clinical observations. The contribution of the fraction of polyphenols, which may additionally also act as prodrugs activated by the intestinal microflora, is still underestimated, whereas the contribution of hyperforin still occurs overrated.

Overall discussion and conclusions

No studies on clinical pharmacology have been identified in the reviewed period. With the antidepressant efficacy of SJW demonstrated in clinical trials for different types of extracts, there is no doubt about the efficacy as such. Correspondingly, clinical pharmacology studies designed for the elucidation of the importance of potential mechanisms of action are not the primary focus of research. The pharmacological studies try to retrospectively add mechanistic plausibility to an already confirmed clinical efficacy, as in contrast to the development of new active pharmaceutical ingredients, where the development would normally be from bench to bedside.

The pharmacological studies performed with SJW regularly confirm effects in models generally used in depression research. Such models do, however, only highlight certain aspects of a complex clinical picture—even with chemically defined antidepressants the potential mode of action cannot be explained in a single model, with most models suffering from a poor transferability to the situation in depressed patients [39]. The effects confirmed with SJW—or generally with antidepressants—in depression models are as such no proof of efficacy, but they give plausibility to the clinical observations. Possibly the most interesting new findings since our previous review are the effects of SJW constituents on calcium ion conductance. The importance of these observations for the clinical overall picture of depression still needs to be addressed through further research.

With respect to the attribution of effect to single SJW constituents the overall conclusion remains as in the previous review [7]: Known bioactive compounds in SJW represent approximately 50–70 % of the known phytochemical constituents. About 30–50 % of the compounds present in SJW extracts are not yet structurally assigned—among them could be further constituents directly or indirectly contributing to the overall clinical efficacy. The total extract must still be regarded as the active constituent of SJW.

Conflict of interest

Veronika Butterweck and Mathias Schmidt declare that they have no conflict of interest.

Dedication

This article is dedicated to Prof. Dr. Dr. h.c. mult. Adolf Nahrstedt on the occasion of his 75th birthday for his achievements in SJW research.

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